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THE UNIVERSITY OF ALBERTA

Glomus dimorphicum Boyetchko & Tewari sp. nov. Mycorrhizal with Barley in Alberta: Taxonomy, Host Colonization, and Hyperparasitism

by

Susan Mary Boyetchko

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled *Glomus dimorphicum* Boyetchko & Tewari sp. nov. Mycorrhizal with Barley in Alberta: Taxonomy, Host Colonization, and Hyperparasitism submitted by Susan Mary Boyetchko in partial fulfilment of the requirements for the degree of Master of Science in Plant Pathology.

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Supervisor

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Date.....

Abstract

A new species of endogonaceous vesicular-arbuscular mycorrhizal fungus, *Glomus dimorphicum* Boyetchko & Tewari sp. nov., isolated from a field of barley under monoculture, has been described. This new species, along with two other almost concurrently described new species from Florida, are the only species of vesicular-arbuscular mycorrhizal fungi known to have dimorphic spores. This newly recognized feature of dimorphism warranted the emendment of the characteristics of the genus *Glomus* Tul. & Tul. Scanning electron microscopy in conjunction with energy dispersive X-ray microanalysis were used to study spore surface morphology and elemental composition.

Field studies were conducted for three years to compare the extent of indigenous vesicular-arbuscular mycorrhizal colonization in four barley cultivars:'Bonanza', 'Klondike', 'Gateway 63', and 'Olli'. Results indicated that there was no significant difference among the four cultivars except at one site in 1983 where 'Olli' exhibited significantly higher root colonization levels than the other three cultivars.

Greenhouse studies were also conducted to compare the extent and pattern of root colonization by *G. dimorphicum* in the four barley cultivars, in addition to beans, alfalfa, onions, red clover, and corn. The barley cultivars showed very low levels of mycorrhizal colonization. Beans, alfalfa, and onions exhibited intermediate levels of mycorrhizal colonization. The infection patterns of *G. dimorphicum* also

varied from host to host. All hosts, except for the barley cultivars, showed formation of arbuscules. Coiling of the intracellular hyphae occurred in the roots of corn, alfalfa, and red clover. The roots of red clover and beans contained appreciable numbers of intraradical vesicles. The production of vesicles in the host is important for raising root-borne inoculum. Variations in colonization patterns of *G. dimorphicum* among different hosts indicated that they are regulated by the host and are not diagnostic for the fungal species, as had been generally believed for VA mycorrhizal fungi.

Since vesicular-arbuscular mycorrhizal fungi cannot be grown in pure culture, researchers must rely on spores in the soil and/or in root pieces for inoculum. It is, therefore, vital to ensure that any contaminants, in the form of hyperparasites, are absent. The spores of *G. dimorphicum* showed signs and symptoms of hyperparasitism by some still unidentified, non-filamentous soil microbiota. Perforations in the spore wall were observed and the formation of papillae proved that some of the spores were parasitized while still viable. Amoeba-like cysts were observed inside some of the spores and a free living amoeba-like organism was isolated on hay-infusion agar.

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Chapter I

Literature Review

A. Introduction

Mycorrhiza is a term, first used by A.B. Frank in 1885, to describe a mutually beneficial root fungus association (Schenck, 1982; Harley, 1984). As a plant physiologist and forester, Frank initially set out to develop methods of increasing truffle production, and observed the ubiquity of mycorrhizae in tree species. The rootlets of many plants were completely covered by a mantle of fungal hyphae; it was to the external morphology of such infected roots that the term mycorrhiza was applied. Frank later recognized that other plants (Orchidaceae and Ericaceae) were also infected by fungi but no external signs of the mycorrhizal association were evident. In 1894, Frank established that the mycorrhizal symbiosis was beneficial to the plant (Harley, 1984). A. Schlict (1889) later described the endotrophic vesicular-arbuscular (VA) mycorrhizal associations in angiosperms (Harley, 1984). During the early 1900's, many reports documented mycorrhizal associations.

One of the first reports on such fungi from Canada described phycomycetous mycorrhizal fungi in stems, leaves, and roots of mosses, liverworts, and tobacco (Koch, 1935). It was not until 1950-1960, however, that greater emphasis was placed on the VA mycorrhizal fungi (Schenck, 1981). The relative neglect of the VA mycorrhizal fungi prior to this

period may be attributed to the lack of external signs of symbiosis (Gerdemann, 1968; Sanders and Tinker, 1973) and to the fact that the symbiont could not be grown in pure culture. The research on VA mycorrhizal symbiosis accelerated when workers realized the ability of these fungi to promote plant growth by increasing the uptake of nutrients, particularly those elements which are relatively immobile in soil.

Relative to some other countries such as U.S.A., U.K., Australia, and New Zealand, little work on VA mycorrhizae has been done in Canada. The ecology of *Endogone* Link ex Fries (Sutton, 1973; Koske *et al.*, 1975; Sutton and Sheppard, 1976) and morphology and development of some VA mycorrhizal fungi (Brundrett *et al.*, 1984; Brundrett *et al.*, 198[redacted] have been studied at Guelph. Physiological studies of VA mycorrhizal fungi are currently underway by researchers at Waterloo (Jabaji-Hare *et al.*, 1984; Jabaji-Hare and Kendrick, 1985). Researchers from Laval have concentrated on taxonomy and morphology (Berch and Fortin, 1983a,b) and on the interaction of VA mycorrhizal fungi with root pathogens (Caron *et al.*, 1985). Very little research on VA mycorrhizal fungi has been conducted in western Canada. Mycorrhizal species have been isolated from soils in Alberta (Kucey and McCready, 1982; Zak *et al.*, 1983; Zak and Parkinson, 1984; Boyetchko and Tewari, 1985), Saskatchewan (Kucey and Paul, 1983) and British Columbia (Molina *et al.*, 1978). The effects of various soil factors on VA mycorrhizal fungi are

being studied at Lethbridge, Alberta (Kucey and Diab, 1984).

The inability to grow these fungi in the absence of a host makes the study of VA mycorrhizal fungi difficult. This prevents *in vitro* physiological and biochemical studies as well as studies on fungal development (Trappe and Schenck, 1982; Cooper, 1984). The inability to grow VA mycorrhizal fungi in pure culture also hinders mass production of pure inoculum. Root pieces and soil containing spores of VA mycorrhizal fungi must be utilized as sources of inoculum but the presence of contaminants and the need for large quantities of these inoculum sources makes storage, handling and transportation difficult. Some strain variation among VA mycorrhizal fungi also exists. Different species may vary in their ability to induce plant growth responses (Schenck, 1984). Other complications result because extensive VA mycorrhizal infection usually occurs when soil phosphorus (P) is relatively low. This complicates the study of other factors affecting the plant-fungus symbiosis separately from the influence of improved P nutrition (Cooper, 1984).

B. Types of Mycorrhizal Associations

Three types of mycorrhizal associations are recognized: ectomycorrhizae, ectendomycorrhizae, and endomycorrhizae. Ectomycorrhizae are formed by fungi of the groups Basidiomycotina, Ascomycotina, and Zygomycotina associated with higher plants, the Basidiomycotina comprising most of the ectomycorrhizal associations (Miller, 1982).

Ectomycorrhizal fungi characteristically produce a mantle of hyphae externally around the feeder roots and produce a network of intercellular hyphae, known as the Hartig net, in the root cortex (Miller, 1982; Wilcox, 1982). Ectendomycorrhizal infections are morphologically similar to those described for ectomycorrhizae but differ in that they also form intracellular hyphae in the root cortex (Wilcox, 1982). Endomycorrhizal fungi develop inter- and intra-cellular hyphae but do not show external signs of their symbioses. Endomycorrhizal fungi are of two types: septate and aseptate fungi. Arbutoid, ericoid, and orchid mycorrhizae are examples of septate mycorrhizal fungi. VA mycorrhizal fungi are aseptate fungi but develop septa upon aging and during spore formation. They develop endomycorrhizal associations characterized by the presence of vesicles and arbuscules in the roots and will be the subject matter of research reported in this thesis.

C. Taxonomy

At present, the VA mycorrhizal fungi are placed in the family Endogonaceae, subdivision Zygomycotina (Gerdemann and Trappe, 1974). Morphological and biochemical studies have provided evidence for the placement of this family as indicated above. However, whether or not this family should be placed in the order Mucorales, or in its own order Endogonales, is still being debated (Hall, 1984). Members of this family are ubiquitous in soil and may very well be the

most common types of soil fungi.

The first comprehensive monographic treatment of this family was carried out by Thaxter (1922) who included the four genera, *Endogone*, *Sphaerocreas* Sacc. & Ellis, *Sclerocystis* Berk. & Broome, and *Glaziella* Berk., in this family. The monotypic genus *Sphaerocreas* was transferred to *Endogone* by Zycha (1935), and finally to *Glomus* Tul. & Tul. by Gerdemann and Trappe (1974). Gerdemann and Trappe (1974) subdivided the species of *Endogone sensu lato* into five genera including *Endogone sensu stricto*, *Glomus*, the new genus *Gigaspora* Gerd. & Trappe, *Modicella* Kanouse, and *Sclerocystis*. They also described another new genus, *Acaulospora* Gerd. & Trappe.

Presently, the family Endogonaceae consists of nine genera including *Acaulospora*, *Complexipes* Walker, *Endogone*, *Entrophospora* Ames & Schneider, *Gigaspora*, *Glaziella*, *Glomus*, *Modicella*, and *Sclerocystis* (Ames and Schneider, 1979; Walker, 1979; Trappe, 1982; Hall, 1984). Of these, only *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis* are known to form endomycorrhizal associations of the the vesicular-arbuscular type. The mycorrhizal relationships of the other five genera are either unknown or are of the ectomycorrhizal type.

This review deals only with the genera that form VA mycorrhizal associations and more specifically with the genus *Glomus*. Trappe (1982) included 43 species in the genus *Glomus*. Since then, approximately 20 more new species

belonging to this genus have been described.

1. Spore types

The VA mycorrhizal fungi produce two spore types: azygospores (parthenogenic zygospores) and chlamydospores (asexual resting spores) (Gerdemann and Trappe, 1974; Trappe and Schenck, 1982). The criteria used to identify the VA mycorrhizal fungi rest largely on the morphology of the spores of which there are only a few available characteristics. *Glomus* forms chlamydospores borne on a hypha (or hyphae) which is cylindric or flared, the spore contents being separated from the hypha by an occlusion or a septum. *Gigaspora* is characterized as forming azygospores singly in soil and having a bulbous hyphal attachment (Gerdemann and Trappe, 1974). *Acaulospora* develops azygospores singly in soil. The azygospores bud laterally from a terminal thin-walled vesicle which eventually collapses at maturity. *Sclerocystis* characteristically forms a single layer of chlamydospores in a sporocarp around a central plexus of interwoven hyphae. The sporocarps, in most species, are surrounded by a peridium of interwoven hyphae (Gerdemann and Trappe, 1974). So far, each of these genera has been known to produce only one morphological spore type. This thesis and another concurrent study from Florida (Smith and Schenck, 1985), however, report on a total of 3 species of *Glomus*, each of which have two morphological spore types. This spore dimorphism has so far been unknown in the genus

Glomus and also in the VA mycorrhizal fungi at large.

2. Spore arrangement in the genus *Glomus*

The aggregation of spores in this genus is quite variable. They may develop in sporocarps, singly in soil, and/or in the root cortex (Gerdemann and Trappe, 1974; Schenck and Smith, 1982; Trappe and Schenck, 1982; Hall, 1984). Sporocarps consist of two or more spores produced from one to many hyphae (Berch and Fortin, 1983). Arrangements of the spores in the sporocarps vary from radial, typical of *G. radiatum* (Thaxter) Trappe & Gerd., to loose variable aggregates, as in *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe and *G. macrocarpum* Tul. & Tul. The spores may also be arranged in compact clusters, as in *G. fasciculatum* and *G. fuegianum* (Speg.) Trappe & Gerd. (Gerdemann and Trappe, 1974). Most species also form chlamydospores inside the roots in addition to those formed in the soil. Some species, such as *G. intraradices* Schenck & Smith (Schenck and Smith, 1982) and *G. diaphanum* Morton & Walker (Morton and Walker, 1984), preferentially form chlamydospores inside the roots.

3. Spore size and color in the genus *Glomus*

Most species have a wide range of spore sizes, some of which overlap with the size ranges of the other species. The spores of *G. tenue* (Greenall) Hall are typically very small (less than 15 μm) (Hall, 1977, 1984) while those of *G.*

manihotis Howeler, Sieverding & Schenck (Schenck *et al.*, 1984) may be as large as 450 µm in diameter. Spore color also varies among species. Most spores are hyaline when young (Trappe, 1982) and become darker with age. The walls of mature spores may be hyaline to yellow as in *G. claroideum* Schenck & Smith, white as in *G. lacteus* Rose & Trappe, or black as in *G. fecundisporum* Schenck & Smith (Rose and Trappe, 1980; Schenck and Smith, 1982).

The wall colors in cross-section under tungsten light illumination also vary. All walls may be the same color as in *G. clarum* Nicol. & Schenck and *G. fecundisporum* or each wall may be different in color as in *G. aggregatum* and *G. hoi* Berch & Trappe (Nicolson and Schenck, 1979; Schenck and Smith, 1982; Trappe, 1982; Berch and Trappe, 1985).

The spore contents of most *Glomus* spp. are colorless, but *G. convolutum* Gerd. & Trappe spores contain deep yellow oil globules and *G. radiatum* spores may be filled with hyphae (Gerdemann and Trappe, 1974). Spores of *G. monosporum* Gerd. & Trappe may also be occasionally filled with hyaline thin-walled hyphae (Gerdemann and Trappe, 1974). Spores of other fungi do not contain such hyphal inclusions. Their exact nature in the VA mycorrhizal fungal spores is not known. However, the possibility exists that they may be signs of autoparasitism or parasitism by another fungus.

4. Spore wall structure in the genus *Glomus*

Spore wall ornamentation is an important taxonomic feature of *Glomus* spp. The surface of most spores are smooth to dull-roughened but some spore walls may be warty, as in *G. scintillans* Rose & Trappe (Rose and Trappe, 1980) or reticulate as in *G. leptotichum* Schenck & Smith (Schenck and Smith, 1982) and *G. ambisporum* Smith & Schenck (Smith and Schenck, 1985). The inner wall of *G. monosporum* contains minute echinulations which protrude into its outer wall (Gerdemann and Trappe, 1974). A mantle of hyphae may also be present around the spores of *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *G. convolutum* (Gerdemann and Trappe, 1974).

The number of spore walls in *Glomus* spp. varies from 1 to 5 (Trappe, 1982). Also, the thickness of the walls relative to their positions may differ. In *G. claroideum*, the inner and outer walls are of equal thickness. The outer wall of *G. aggregatum* is thicker than the inner wall while the inner wall is thicker than the outer wall in *G. etunicatum* Becker & Gerd. and *G. gerdemannii* Rose, Daniels, & Trappe (Becker and Gerdemann, 1977; Rose *et al.*, 1979; Schenck and Smith, 1982).

The structure of the spore wall can be easily observed by light microscopy. The various species have walls which can be categorized as unit walls, laminated walls, evanescent walls, and membranous walls (Walker, 1983) but not all mycorrhizal species contain all wall types.

A unit wall is rigid, single-layered and varies in thickness (Walker, 1983). A unit wall may either separate readily from the other walls when the spore is crushed or it may adhere to the other walls in a group. The unit wall can be seen in the spores of *G. caledonicum* (Nicol. & Gerd.) Trappe & Gerd. (Gerdemann and Trappe, 1974).

Laminated walls are fused layers of wall material resulting from deposition of laminae as the spore matures (Berch and Fortin, 1983; Walker, 1983). Spores of *G. intraradices* may contain up to four laminated walls (Schenck and Smith, 1982). The laminae of some species may be difficult to observe and they may separate when the spores are crushed resulting in the misinterpretation of spore wall characteristics (Walker, 1983).

The evanescent wall is one which sloughs off as the spore matures. Spores exhibiting this type of wall are *G. gerdemannii*, *G. etunicatum*, grouped spores of *G. ambisporum* (Becker and Gerdemann, 1977; Rose *et al.*, 1979; Smith and Schenck, 1985), and the single spores of *G. dimorphicum* Boyetchko & Tewari (Boyetchko and Tewari, 1985).

The membranous wall is often a thin, inner, flexible wall which usually shrinks upon plasmolysis and therefore does not usually break when the spore is crushed (Walker, 1983). The various species possessing this type of wall are *G. hoi*, *G. diaphanum* (Morton and Walker, 1984; Berch and Trappe, 1985), and single and grouped spores of *G. dimorphicum* (Boyetchko and Tewari, 1985).

Walker (1983) suggested that researchers should adopt the use of graphical representations (murographs) of wall structures of VA mycorrhizal fungi which include the four wall types previously described. He suggested that the use of standardized diagrams would facilitate the studies on spore wall characteristics of VA mycorrhizal spores.

5. Sporogenous hyphae in the genus *Glomus*

One other morphological feature used to the describe species of *Glomus* is the sporogenous hypha. The number of sporogenous hyphae per spore may range from one to several and may vary at the point of attachment of the spore. They may be cylindric or flared as in *G. aggregatum* Schenck & Smith (Schenck and Smith, 1982), *G. etunicatum* (Becker and Gerdemann, 1977) and *G. mosseae* (Gerdemann and Trappe, 1974). Sporogenous hyphae of *G. constrictum* Trappe (Trappe, 1977) and *G. halonatum* Rose & Trappe (Rose and Trappe, 1980) are bulbous and constricted at the point of attachment.

The pore in the sporogenous hypha at the point of attachment may be open as in *G. reticulatum* Bhatt. & Muker. (Bhattacharjee and Mukerji, 1980) or it may be closed by an occlusion or septum. An occlusion results through thickening of the inner wall (Trappe and Schenck, 1982; Berch and Fortin, 1983) in many species including *G. aggregatum* (Schenck and Smith, 1982) and *G. fasciculatum* (Gerdemann and Trappe, 1974). The pore may also be distinctly closed by a septum which is usually very thin and which may be straight

or slightly curved. *G. caledonicum* and *G. mosseae* show this type of pore closure (Gerdemann and Trappe, 1974).

6. Techniques of spore observation

Most species descriptions of VA mycorrhizal fungi are prepared by examining spores by light microscopy. Spores may be mounted in water, lactoglycerine, or lactophenol on microscope slides, covered with a cover slip, and observed (Trappe and Schenck, 1982). Melzer's reagent (1.5 g potassium iodide, 0.5 g iodine, 20.0 ml water, 22.0 g chloral hydrate) may also be used as a mounting medium. This reagent may react positively with the spore wall to produce a black or grayish color. However, it is becoming more popular to use scanning electron microscopy (SEM) to study spore morphology. SEM is a useful technique for examining the surface details of biological materials at higher magnifications than can be obtained by light microscopy. Morphological features and finer details can be observed because of the high resolving power and the three dimensional image reconstruction of the object (Goldstein *et al.*, 1981; Brown and White, 1982).

An electron beam, with a resolving power a thousand times greater than that of visible light (Brown and White, 1982), is passed through a number of electromagnetic lenses which reduce the size of the beam and produce a coherent, focused spot. The electron beam is scanned, line by line, over the surface of the object producing secondary

electrons. These secondary electrons are collected and converted to electrical pulses which are photographed on a cathode ray tube and appear as spots of visible light. The intensity of these spots indicate the amount of secondary electrons emitted (Goldstein *et al.*, 1981; Brown and White, 1982).

The samples in SEM are kept in a vacuum; therefore, the samples must be dried. Any free or bound water may result in alterations in morphology (Brown and White, 1982) and deterioration of the vacuum. Dry samples are not very good conductors; therefore, treating the specimens with osmium tetroxide and coating the sample with a layer of carbon, gold, or gold-alloy is necessary to increase the conductivity of the sample (Goldstein *et al.*, 1981; Brown and White, 1982). Osmium tetroxide serves a dual purpose since it also acts as a fixative.

Chemical analysis of the sample may also be conducted by means of energy dispersive X-ray analysis (Goldstein *et al.*, 1981). X-rays of a particular wavelength and energy characteristic for a particular element are emitted when atoms are excited by a high-energy electron beam (Brown and White, 1982). By use of static probe analysis, the electron beam is held in one spot of the sample, and X-rays emitted from that location are analyzed. All elements between sodium and uranium in atomic weight can be detected by this technique (Goldstein *et al.*, 1981).

D. Anatomy

1. The Extraradical Phase

The extraradical phase of VA mycorrhizae is continuous with the intraradical phase (Bonfante-Fasolo, 1984). The network of external hyphae can extend from the root upto a distance of about 1 cm (Gerdemann, 1968). The VA mycorrhizal fungi do not alter the general root morphology as is typical of the ectomycorrhizal fungi (Gerdemann, 1968; Carling and Brown, 1982). Some roots of maize, pea, onion, and various members of Solanaceae colonized by VA mycorrhizal species, however, do exhibit a bright yellow color (Gerdemann, 1968) but generally, microscopic examination of the roots, which have been cleared and stained, is required to confirm the presence of these fungi (Phillips and Hayman, 1970; Brundrett *et al.*, 1984). The amount of external hyphae produced by various VA mycorrhizal fungi differ among species and isolates (Graham, *et al.*, 1982; Abbott and Robson, 1985). Researchers have measured the amount of soil bound by VA mycorrhizal mycelium associated with the roots to indirectly quantify the the external mycelium (Graham *et al.*, 1982). Although this is not an effective method for evaluating the influence of VA mycorrhizal fungi on plant growth, it is a simple method of monitoring the amount of extraradical hyphae.

The extraradical hyphae are of two types: 1) thick-walled hyphae which are aseptate and coarse and

comprise the major part of the external phase and, 2) thin-walled hyphae which are fine and become septate when mature (Mosse, 1959; Brown and King, 1982; Bonfante -Fasolo, 1984).

Although, ultrastructural studies of the extraradical phase are scarce, Bonfante-Fasolo and Grippiolo (1982) have shown that the external hyphae do possess nuclei, mitochondria and an endoplasmic reticulum. The extraradical hyphal wall of *G. fasciculatum* is 2-layered and 0.3 to 0.5 μm thick. The outer layer is electron-translucent and fibrillar. The inner layer which is electron-dense and 0.15 to 0.40 μm thick, has a lamellar structure with alternating light and dark lamellae. Scannerini and Bonfante-Fasolo (1979) have ultrastructurally and cytochemically demonstrated that the wall of the extraradical hyphae is composed of proteins and polysaccharides.

2. The Penetration Process

Just prior to penetration of the root epidermis, the external hyphae swell at their tips producing appressoria and penetrate through or between epidermal cells (Gerdemann, 1968; Brown and King, 1982; Carling and Brown, 1982; Bonfante-Fasolo; 1984). Direct penetration without an appressorium is also known to occur (Carling and Brown, 1982). Penetration between epidermal cells is most common. Penetration of root hairs may also occur (Gerdemann, 1968). When penetrating the host wall, the hyphae constrict as they

push back the host plasmalemma (Scannerini and Bonfante-Fasolo, 1983), thereby producing an interfacial region between the fungal wall and the host plasmalemma. The interfacial region contains matrix which is continuous with the host wall at the point of penetration (Scannerini and Bonfante-Fasolo, 1983). The hyphae immediately return to their previous diameter after penetration (Kinden and Brown, 1975; Bonfante-Fasolo, 1984). Kinden and Brown (1975) observed that the middle lamella of yellow poplar is modified upon penetration suggesting that enzymatic activity as well as mechanical forces are involved in the penetration process. However, no evidence was presented to indicate the involvement of mechanical forces during this process.

3. The Intraradical Phase

VA mycorrhizal fungi infect root tissue which is actively growing, usually in areas of differentiation and elongation (Gerdemann, 1968; Cox and Sanders, 1974; Carling and Brown, 1982). Fungal development is usually restricted to the root cortex, but penetration of the stele also rarely occurs (Gerdemann, 1968; Hayman, 1981; Carling and Brown, 1982). Mycorrhizal colonization of the xylem in ginger and common spiderwort has been observed (Taber and Strong, 1982; Taber and Trappe, 1982). Inter- and/or intracellular hyphae develop in the cortex following penetration of the root. Cox and Sanders (1974) described the "infection unit" as a sequential development of hyphae, the oldest originating at

the entry point and the youngest following closely the development of the root. As roots mature, they lose the cortex which would normally support young infection. Older roots which are often pigmented and have secondary growth do not support VAM infection. There are basically four intraradical structures: 1) intracellular hyphae which may or may not form coils, 2) intercellular hyphae, 3) arbuscules and, 4) vesicles.

a. Intracellular hyphae

Linear or coiled intracellular hyphae are often found in the outer layers of the root cortex (Bonfante-Fasolo, 1984). The formation of coils in roots is common in many host plants. (Kinden and Brown, 1975). The behavior of the hyphae (e.g. coiling) appears to be influenced by the host rather than the VA mycorrhizal species (Hetrick *et al.*, 1985). Invagination of the host plasmalemma and tonoplast is typical throughout intracellular colonization (Bonfante-Fasolo, 1984). Carling and Brown (1982) found that the intracellular hyphae, oriented longitudinally in the root, are often appressed to the inner surfaces of the host cell wall. As the intracellular hyphae progress from cell to cell, constriction of the hyphae occurs at the points of penetration, similar to that exhibited by the extraradical hyphae (Kinden and Brown, 1975; Bonfante-Fasolo, 1984). A thin sheath of host cytoplasm surrounds the intracellular hyphae. The surrounding host

material consists of a compact zone similar to the host cell wall, plasmalemma and cytoplasm (Cox and Sanders, 1974; Kinden and Brown, 1975). As infection gets older, the cytoplasm of the intracellular hyphae deteriorates and the hyphal cell walls eventually collapse (Kinden and Brown, 1975).

b. Intercellular hyphae

The intercellular hyphae are usually found in the intermediate layers of the root cortex. When active, these hyphae are aseptate (Bonfante-Fasolo, 1984). Hyphae which are aligned parallel to each other may form H-bridges (Abbott and Robson, 1979) but only certain VA mycorrhizal species seem to exhibit this characteristic. This raises the possibility of the formation of heterokaryons in these fungi. Ultrastructural studies indicate that intercellular hyphae contain nuclei, mitochondria, and α-glycogen granules (Cox and Sanders, 1974; Scannerini and Bonfante-Fasolo, 1983) as well as lipid droplets rich in triglycerides (Nemec, 1981). Through TEM studies, Lim *et al.* (1984) found that intercellular hyphae in old roots of white clover are thick-walled with their primary and secondary walls staining cytochemically for polysaccharides. The function of these thick-walled hyphae is unclear but it is suggested that they may function as propagules upon disintegration of the root cortex. An alternative role could be that these structures furnish an apoplastic

route through which nutrients can be transported. These thick-walled intercellular hyphae are, however, rare in species such as *G. fasciculatum*.

Lim *et al.* (1983) reported intrahyphal hyphae within the hyphae of *G. fasciculatum*. Cox and Sanders (1974) also observed hyphae growing inside collapsed intercellular hyphae of *G. mosseae* in onion roots. The significance of these hyphae is not understood. These may be cases of autoparasitism or simply a utilization of space by another fungus or themselves.

c. The arbuscule

As infection by the intercellular hyphae progresses towards the inner layers of the cortical tissue closer to the stele, highly branched, haustoria-like structures called arbuscules develop (Gerdemann, 1968; Brown and King, 1982; Carling and Brown, 1982; Bonfante-Fasolo, 1984). Researchers now agree that the arbuscule is the most significant structure in the VA mycorrhizal fungus-host relationship and that it is the most probable site of nutrient exchange between the host and the symbiont (Scannerini and Bonfante-Fasolo, 1983; Toth and Miller, 1984).

Arbuscules successively develop in the inner cortical cells behind the hyphal tips (Brown and King, 1982). The arbuscule arises from the penetration hypha which becomes the arbuscule trunk and grows into the cortical cell causing invagination of the host

plasmalemma. The arbuscule trunk is similar to the intercellular hypha and has a diameter of about 4 μm (Cox and Sanders, 1974). The arbuscule trunk branches dichotomously and terminates in a series of bifurcate branches which may be less than 1 μm in diameter (Gerdemann, 1968; Brown and King, 1982; Carling and Brown, 1982; Bonfante-Fasolo, 1984). The individual arbuscule branches are always surrounded by the host plasmalemma which is usually appressed to the fungal cell wall (Cox and Sanders, 1974; Bonfante-Fasolo, 1982). In places, the arbuscule and plasmalemma are separated by an electron-transluscent zone (Cox and Sanders, 1974). It is possible that this material is deposited by the host rather than the symbiont. The interfacial zone has attracted much attention since it is assumed to be the site of nutrient transfer.

During the early stages of arbuscule development, the cytoplasm is dense and nonvacuolated. During the later stages, the cytoplasm contains many nuclei, mitochondria, glycogen particles, lipid droplets and small vacuoles (Brown and King, 1982; Bonfante-Fasolo, 1984). X-ray microanalysis revealed that the electron-dense inclusions in the vacuoles of *Glycine max* (L.) Merr. and *Allium cepa* L. are rich in P and calcium (Ca) suggesting that the vacuoles contain polyphosphates (Cox, *et al.*, 1980). Proteins and polysaccharides are present in the wall of the arbustule (Nemec, 1981;

Gianinazzi *et al.*, 1981; Bonfante-Fasolo and Grippiolo, 1982).

Using cryoultramicrotomy, Bonfante-Fasolo (1982) has shown that the texture of the arbuscule wall is amorphous in *Ornithogalum umbellatum* L./*G. fasciculatum* combination. N-acetylglucosamine is localized in the fungal wall but chitin fibrils are not formed. Although the chemical composition of the fungal wall is not positively known, it is suggested that the polymerization of chitin during the arbuscule phase is not completed.

The estimated longevity of the arbuscule ranges from 4 to 15 days (Cox and Tinker, 1976). Toth and Miller (1984) determined that in corn, a mature arbuscule occupies 42% of the cell; 24% of this constitutes the 1 μm branches and the remaining 18% the trunk.

In the early stages of arbuscule development, host cytoplasm increases in volume, host nuclei increase in size (Carling and Brown, 1982; Brown and King, 1982; Toth and Miller, 1984) and the number of host organelles increases (Cox and Sanders, 1974; Brown and King, 1982). At the end of the arbuscule phase, the characterisitcs of the uninfected cortical cells are reinstated.

Deterioration of the arbuscule advances towards the trunk and is characterized by a rapid reduction in arbuscule branches and host cytoplasm (Cox and Sanders,

1974; Toth and Miller, 1984). Cross walls may form within the arbuscule isolating the degrading components from the still viable contents (Kinden and Brown, 1976). The branches collapse and aggregate into an irregular mass or clump near the entry point. The arbuscule trunk deteriorates more slowly than the branches and then the host plasmalemma encases the entire clump. The senescent arbuscule trunks can be observed as empty, collapsed intercellular hyphae (Cox and Sanders, 1974; Kinden and Brown, 1976; Brown and King, 1982; Toth and Miller, 1984). It is not known whether degradation of the arbuscule is due to the autolytic activity or to the physiological activity of the host cell.

Researchers believe that the arbuscule is most probably the site of nutrient exchange. Two popular hypotheses exist regarding nutrient transfer: 1) P is transferred while the arbuscule is still functional and, 2) P is transferred passively upon digestion of the arbuscule. Since the large surface areas of the host and fungus (i.e., arbuscule) are in contact with each other, it is believed that nutrient transfer probably occurs prior to clump formation, while the arbuscule is still active (Cox and Sanders, 1974; Cox and Tinker, 1976; Toth and Miller, 1984).

d. Vesicles

Structures which are globose and form as terminal or intercalary swellings of a fungal hypha are referred

to as vesicles. They vary in size and may be thick- or thin-walled (Gerdemann, 1968; Brown and King, 1982; Carling and Brown, 1982; Bonfante-Fasolo, 1984). Vesicles are not formed by all VA mycorrhizal fungi. *Gigaspora margarita* does not produce these structures (Gerdemann and Trappe, 1974) and a few species of *Glomus* are known not to form "typical" vesicles (Schenck and Smith, 1982).

Vesicles may be found in the inner and outer layers of the cortex in older infections and may function either as storage organs or reproductive structures (Gerdemann, 1968; Brown and King, 1982; Carling and Brown, 1982). Because of the latter function, they are also often termed as chlamydospores in the genus *Glomus*.

The cytology of vesicles has not been studied extensively but it is known that the cytoplasm of vesicles in the early stages of development contains numerous nuclei, glycogen particles and small lipid droplets (Brown and King, 1982; Bonfante-Fasolo, 1984). Mature vesicles are almost completely filled with large lipid globules (Kinden and Brown, 1975a). The intracellular vesicles are enclosed by a condensed layer of host cytoplasm (Kinden and Brown, 1975a; Scannerini and Bonfante-Fasolo, 1983).

Histochemical studies by Nemec (1981) revealed that vesicles of *G. etunicatum* are filled with neutral lipids, particularly triglycerides. Cooper and Lösel

(1978) have also demonstrated that the mycorrhizal roots of onions, ryegrass and clover contain more total lipids than the uninfected roots. Lipid content of the vesicles was also determined by Jabaji-Hare *et al.* (1984). The vesicles of *Glomus* spp. contained 58% lipid, the glycolipid-containing fraction being quite high. The presence of high amounts of lipids suggests that the function of vesicles is that of storage.

Biermann and Linderman (1983) have studied the possibility of using vesicles as sources of inoculum. They found that intraradical vesicles had a high inoculum potential whereas roots, infected with VA mycorrhizal fungi that produce extraradical vesicles only, were not infective. It is suggested that any nutrients stored in the vesicles could increase the infectivity of the mycorrhizal fungus. Further research is required to investigate the possible use of vesicles as sources of inoculum since roots heavily colonized with vesicles could provide an inoculum source which may be economically feasible for storage and handling.

E. Occurrence of VA Mycorrhizal Fungi

VA mycorrhizal fungi are cosmopolitan fungi which infect approximately 95% of all the plants including most angiosperms, numerous gymnosperms, pteridophytes, and bryophytes establishing a mutually beneficial symbiotic relationship (Hayman, 1981; Laferriere and Koske, 1981;

Bonfante-Fasolo, 1984). Members of certain plant families may become infected with VA mycorrhizal fungi to a limited extent but do not appear to derive any benefit from them. These plant families include Cruciferae, Chenopodiaceae, Fumariaceae, Caryophyllaceae, Cyperaceae, Commelinaceae, Urticaceae, Polygonaceae, and Proteaceae (Hayman, 1981; Linderman and Hendrix, 1982; Glenn *et al.*, 1985). Most members of the Pinaceae, Betulaceae, and Fagaceae do not become infected by VA mycorrhizal fungi but do form ectomycorrhizal associations with other fungi. Members of Myrtaceae, Salicaceae, and some *Quercus* and *Alnus* species may form both ectomycorrhizal and VA mycorrhizal associations (Linderman and Hendrix, 1982). Earlier investigations have indicated that members of the Chenopodiaceae and Cruciferae are not hosts to VA mycorrhizal fungi but limited infection by *G. fasciculatum* has been observed in four species of Chenopodiaceae and two species of Cruciferae (Hirrel *et al.*, 1978). Infection of these plant species was observed when grown in association with mycorrhizal host plants (citrus and onion). The infection of these plants occurred in older root tissues, including the vascular tissue, which is considered by many researchers to be atypical of VA mycorrhizae. There was no arbuscule formation. Glenn *et al.* (1985) also reported VA mycorrhizal fungi infecting *Brassica* spp. where the hyphae penetrated older, thicker roots and arbuscules did not develop. Hirrel *et al.* (1978) proposed that arbuscule

formation is an important determinant of a VA mycorrhizal association and indicated the need for further work to determine whether a true VA mycorrhizal association develops in the Chenopodiaceae and Cruciferae. Allen (1983) observed arbuscule formation in the roots of *Atriplex gardneri* (Moq.) D. Dietr. (Chenopodiaceae). Therefore, according to the criteria proposed by Hirrel *et al.* (1978), Allen (1983) contended that *A. gardneri* forms VA mycorrhizae.

The mycorrhizal status of members of the Chenopodiaceae and Cruciferae remains controversial. Whether the mycorrhizal status of a plant is dependent on arbuscule formation or not should be determined. It is possible that nutrient exchange could occur in the absence of arbuscules via other modified fungal structures. Growth responses should be measured in infected Chenopodiaceae and Cruciferae to determine whether a nutritional benefit to the plant has accrued.

F. Physiology of VA Mycorrhizal Fungi

1. Phosphate Uptake

One of the major benefits of VA mycorrhizal association to the host is increased uptake of nutrients such as P, zinc (Zn), copper (Cu), and sulfur(S).

Experiments have revealed a close correlation between VA mycorrhizal fungi and P nutrition of the host (Daft and Nicolson, 1969; Sanders and Tinker, 1973; Menge *et al.*,

1978). Only 20-30% of P applied as fertilizer is recovered by the crop in the first year and the remaining amount becomes fixed in the soil (Daft and Nicolson, 1969). The residual P may be continually used by the plant for up to 10-15 years after application. Because P is a highly immobile element, the VA mycorrhizal fungi play an important role in the recovery of fixed P which may be largely unavailable to the plant.

VA mycorrhizal fungi are able to increase P uptake from soil and improve plant growth generally in P deficient soils (Mosse, 1973; Abbott and Robson, 1982). Generally, VA mycorrhizal infection is greater in soils low in P with infection decreasing with increasing P fertilization rates (Jensen and Jakobsen, 1980; Stribley *et al.*, 1980; Safir and Duniway, 1982; Hoefner *et al.*, 1983). Many researchers agree that plant growth responses to VA mycorrhizal infection are greater when plants are supplied with lower P rates than with higher P rates (Lambert *et al.*, 1980; Hayman, 1983). Studies by Hall *et al.* (1984) have demonstrated, however, in increasing rates of N and P over a wide range resulted in greater plant growth responses due to VA mycorrhizal infection. They suggested that by supplying a plant with a nutrient which is limiting, a greater growth response to the mycorrhiza may result. It is possible that at least some mycorrhizal fungal species or strains can tolerate higher soil P levels. Hayman (1983) has stressed the need for caution when making general conclusions based on the effect

of mycorrhizal fungi in a single soil, since variability occurs between different soils.

VA mycorrhizae increase P uptake by extending their hyphae beyond the zone of P depletion, thereby, exploring a greater volume of soil not readily accessible to the plant root (Sanders and Tinker, 1971; Hayman and Mosse, 1972). The extraradical mycelium has been observed at a distance of at least 1 cm beyond the root (Sanders and Tinker, 1971). Graham *et al.* (1982) reported that the amount and distribution of external mycelium reflects the extent of host growth response better than the amount of mycorrhizal root colonization and that the amount of root colonization does not always correlate with the magnitude of increased plant growth.

Many researchers cannot agree whether VA mycorrhizal plants obtain P from similar or different sources than the nonmycorrhizal plants. Sodium bicarbonate-extractable P was determined to be the chief source of P for both the mycorrhizal and nonmycorrhizal plants (Sanders and Tinker, 1971; Hayman and Mosse, 1972). Supplying poorly available P sources such as rock P or apatite has resulted in improved plant growth in the mycorrhizal plants (Murdoch *et al.*, 1967; Azcon *et al.*, 1976; Powell and Daniel, 1978). The mycorrhizal hyphae are in close proximity to the dissociating P ions from rock P which form an equilibrium with the soil solution. It was concluded that VA mycorrhizal plants can utilize rock P whereas there was no marked

improvement in plant growth of the nonmycorrhizal plants. Other researchers believe that if the nonmycorrhizal plants are supplied with sufficient amounts of rock P, they will grow as well as the mycorrhizal plants (Pairunan *et al.*, 1980; Biermann and Linderman, 1983).

2. Translocation of Phosphate

Translocation of P through VA mycorrhizal fungus hyphae has been demonstrated in onions and subterranean clover (Sanders and Tinker, 1973; Cooper and Tinker, 1978; Smith, 1982). Mass inflow was thought to be the most likely transport mechanism involved (Sanders and Tinker, 1973). Transport of ^{32}P in VA mycorrhizal fungi has been measured over distances of 7 and 9 cm (Rhodes and Gerdemann, 1975; Alexander *et al.*, 1984). Translocation of ^{32}P from roots to shoots was demonstrated in both mycorrhizal and nonmycorrhial plants, but mycorrhizal plants showed 160 times more radioactivity (Gray and Gerdemann, 1969). Phosphate translocation in the mycorrhizal hyphae may occur in both directions but autoradiographic studies have shown that P translocation is 10 times greater towards the host than away from it (Skinner and Bowen, 1974). Due to the strong directional movement of P, it may be concluded that translocation is occurring as a result of cytoplasmic streaming and not just by a process of simple diffusion.

Most researchers believe that P is translocated in the mycorrhizal fungus hyphae in the form of condensed

polyphosphate granules (Cox *et al.*, 1975; Callow *et al.*, 1978; Cox *et al.*, 1980). Polyphosphate comprises an appreciable amount (40%) of the total P in the fungal component of the mycorrhizal roots.

Alkaline phosphatase activity has been detected in the mycorrhizal fungi. Its activity decreases when the infected onion plants are supplied with available P (Gianinazzi-Pearson and Gianinazzi, 1976). It has been proposed that alkaline phosphatase may, therefore, be involved in active P transport in the hyphae with the maximum enzymatic activity occurring during early stages of infection. This correlates with mycorrhizal development and host growth response (Gianinazzi-Pearson and Gianinazzi, 1976, 1978; Cooper, 1984). As plant development and infection progresses, the specific activity of alkaline phosphatase decreases. This implies that the enzyme is involved in the establishment of mycorrhizal infection. Acid phosphatase, on the other hand, is found in actively growing germ tubes and hyphae indicating that the enzyme may be significant in the initiation of infection (i.e. elongation and growth of hyphae) (MacDonald and Lewis, 1978). The polyphosphate transported into the arbuscules is translocated into the host.

3. Transfer of Phosphate

Two theories regarding P transfer from VA mycorrhizal fungi to the host have been proposed. Phosphorus is either

released into the host upon digestion of the arbuscule (Kinden and Brown, 1975c) or is translocated across the host-arbuscule interface in the living arbuscule system (Cox and Tinker, 1976). The walls of the arbuscules are composed primarily of glycolipids while those of vesicles and hyphae are predominantly made of chitin (Nemec, 1981). It is believed that the glycolipid component facilitates the bi-directional flow of nutrients more readily than the chitinous component. The activity of various enzymes at the host-fungus interface also supports the theory that nutrient transfer occurs in a living system. There are conflicting reports, however, on the composition of the cell wall in nearly all these parts of the symbiont. Bonfante-Fasolo (1982) observed that the arbuscule wall did not contain chitin but was composed of N-acetylglucosamine which is a precursor of chitin. Scannerini and Bonfante-Fasolo (1979) observed that the cell wall of extraradical hyphae contained polysaccharides and proteins whereas Lim *et al.* (1984) found only polysaccharides in the primary and secondary walls of intercellular hyphae. Obviously, further work is necessary to confirm the presence of these components in the hyphae, vesicles, and arbuscules of the VA mycorrhizal fungi.

Inorganic P is released upon degradation of the polyphosphate by a polyphosphatase enzyme as it reaches the arbuscule (Callow *et al.*, 1978). Enzymes involved in polyphosphate degradation, such as endopolyphosphatase and exopolyphosphatase, have shown greater activity in

mycorrhizal than in nonmycorrhizal roots (Capaccio and Callow, 1982).

Polyphosphate glucokinase activity was detected in the external hyphae of the mycorrhizal roots, glucose-6-phosphate being the intermediate product. It is believed that polyphosphate glucokinase has a potential role in the transfer of glucose from the host to the fungus, but because its activity was detected in external hyphae, its complete role is not understood (Capaccio and Callow, 1982).

It has been suggested that a polyphosphate kinase enzyme liberates ATP, which can be used in the translocation of inorganic P across the host-arbuscule interface.

Marx *et al.* (1982) support the hypothesis that P is transferred across the host-arbuscule interface via an active transport mechanism. Plasmalemma bound ATPase activity of the host was concentrated around the arbuscule branches when VA mycorrhizae developed in the root cells. The redistribution of ATPase activity was not detected in very young or degenerating arbuscules. It was therefore suggested that nutrient exchange occurs across the interface in a living system.

4. Uptake of Other Nutrients

Very few papers relate to the effect of nitrogen (N) nutrition on mycorrhizal development. Also, these studies contradict each other as to whether N increases, decreases, or does not alter VA mycorrhizal infection (Hayman, 1982).

VA mycorrhizae infected plants have a lower concentration of N in the tissues than the nonmycorrhizal plants (Cooper, 1984). In some cases, mycorrhizal plants have a higher N content but whether this is a result of increased N uptake or a consequence of stimulated root growth or due to increased P uptake by the mycorrhizal fungus is not clearly understood. Hayman (1983) has suggested that VA mycorrhizal fungi do not increase the uptake of ions which are mobile (e.g. nitrate-nitrogen). Recent work by Ames *et al.* (1983) has indicated that VA mycorrhizal fungi affect N nutrition of plants directly. They discovered that mycorrhizal celery plants obtained more ^{15}N-labelled N from inorganic (ammonium sulfate) and organic sources than did the control plants. Ames *et al.* (1983) suggested that VA mycorrhizal fungi may play a more important role in acquiring ammonium-nitrogen than nitrate-nitrogen in a natural ecosystem, the latter being the more mobile form of N.

VA mycorrhizal fungi have the ability to improve the uptake of other elements besides P. Pairunan *et al.* (1980) reported that VA mycorrhizal fungi increased Zn uptake in subterranean clover at low rates of P application. Cooper and Tinker (1978) have demonstrated that Zn is translocated through mycorrhizal hyphae from the soil to the plant. Similarly, enhanced Cu uptake has been reported (Gildon and Tinker, 1983b; Hall *et al.*, 1984).

Some researchers believe that improved uptake of microelements is related to the improved uptake of P

(Hayman, 1983; Cooper, 1984). However, Gildon and Tinker (1983b) have shown that Cu is absorbed and translocated by mycorrhizal hyphae in conditions where a response to P had not occurred. Heavy applications of Zn, Cu, nickel (Ni), or cadmium(Cd) can however reduce the extent of infection by *G. mosseae* (Gildon and Tinker, 1983a).

It has also been demonstrated that uptake of S in plants is enhanced by VA mycorrhizal fungi and that the S is translocated through the hyphae (Gray and Gerdemann, 1973; Cooper and Tinker, 1978; Rhodes and Gerdemann, 1978a). Mycorrhizal onions took up more ^{35}S than the nonmycorrhizal plants (Rhodes and Gerdemann, 1978a). Improved S uptake is most likely to occur in S-deficient soils. It is also suggested that the enhanced S uptake by mycorrhizal roots is influenced by the increased uptake of P (Cooper, 1984).

Calcium (Ca) has also been shown to be translocated by external hyphae of VA mycorrhizal fungi (Rhodes and Gerdemann, 1978b). Schoknecht and Hattingh (1976) have reported, through the use X-ray microanalysis, that Ca is a secondary constituent of the polyphosphate granules. Calcium has also been implicated in P transfer from the fungus to the host as it could stimulate alkaline phosphatase activity (Gianinazzi-Pearson and Gianinazzi, 1978).

There are reports that VA mycorrhizal fungi increase plant tolerance to drought (Nelson and Safir, 1982; Allen and Boosalis, 1983). Allen *et al.* (1981) showed that resistance to water transport was reduced when mycorrhizal

plants were exposed to drought stress and suggested that the reduced water resistance may, in part, have resulted from improved water uptake by the mycorrhizal fungi. Hetrick *et al.* (1984), however, observed that mycorrhizal plants showed no distinct improvement over nonmycorrhizal plants when grown under drought stress. There were increases in VA mycorrhizal root colonization without corresponding benefits to the plant. These results may indicate that the fungi had been growing parasitically in the plant roots. It, therefore, appears that VA mycorrhizal fungi may have a role in increasing water uptake of plants, under certain conditions, but the means by which this is achieved is unclear.

5. Carbon Physiology

VA mycorrhizal fungi are obligate symbionts, and therefore, how costly this symbiosis is in terms of plant photosynthates, and how the host supports the mycorrhizal infection, are of great interest.

Carbon (C) obtained by the mycorrhizal fungus is most probably from host carbohydrates (e.g. sugars) (Cooper, 1984). The major soluble sugars in the carbohydrate pools of nonmycorrhizal roots, mycorrhizal roots and fungal mycelium are sucrose and glucose but it is not known whether sucrose is the sugar transported from the root to the fungal hyphae. It is believed that the transported photosynthate may be stored as lipids in the fungal structures and can accumulate

in spores, hyphae, vesicles, and arbuscules (Cox *et al.*, 1975; Cooper, 1984). Carbohydrates may also be stored in the hyphae as glycogen particles (Kinden and Brown, 19[illegible]). Snellgrove *et al.* (1982) calculated that 7% more total [illegible] C was translocated from shoots to roots in the mycorrhizal plants. Both mycorrhizal and nonmycorrhizal plants had similar C assimilation rates per unit leaf area. Ocampo and Azcon (1985) also found that the amount of sugar in the mycorrhizal roots increased once the fungus was established in the host. However, the level of mycorrhizal infection did not determine the magnitude of increase of sugar in the root.

Although little is known about the mechanism by which C is transferred between the host and the symbiont, it is assumed that the arbuscule, the site of P transfer, is also the likely site of C transfer (Cooper, 1984). Some researchers believe that polyphosphate glucokinase activity in mycorrhizal roots and ATPase activity located in the host plasmalemma would facilitate the transfer of sugar at the host-arbuscule interface (Callow *et al.*, 1978; Marx *et al.*, 1982).

G. Role of Exudates

VA mycorrhizal fungi must rely on nutrients and fixed C from the living roots for survival (Graham, 1984). Some researchers believe that mycorrhizal infection is regulated by exudates released by the root in the form of soluble

amino acids and reducing sugars (Ratnayake *et al.*, 1978; Graham *et al.*, 1981). A correlation was found between the increased amount of root exudate with a corresponding reduction in the root phospholipid levels in P-deficient soils. Increases in root exudates have been related to changes in root membrane permeability (Ratnayake *et al.*, 1978; Graham *et al.*, 1981). Snellgrove *et al.* (1982) have suggested that carbon losses from the root (as root exudates) are sufficient to support VA mycorrhizal development. It was also found that membrane permeability decreased with increasing levels of phospholipids in the root tissue (Ratnayake *et al.*, 1978). Graham *et al.* (1981) also observed that as the VA mycorrhizal root infection increased, plant P nutrition improved, resulting in reduced membrane permeability and exudation. The effect of mycorrhizal fungi on root exudation could affect the activities of microorganisms which may also respond to root exudates in the rhizosphere (Graham, 1984).

Azcon and Ocampo (1981), while studying various wheat cultivars for their mycorrhizal dependency, discovered that the cultivars with the least amount of VA mycorrhizal infection had the lowest rate of exudation. Further work is needed to study and compare the composition of root exudates between mycorrhizal and nonmycorrhizal plant species as well as differences among the various cultivars of a crop species.

Some other researchers feel that VA mycorrhizal fungi may be attracted to the host by volatile root exudates (Vancura and Stotzky, 1976; Koske, 1982). Germ tubes of *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe grew towards germinating bean and corn seedlings (Koske, 1982). This may indicate the ability of VA mycorrhizal fungi to locate hosts by a chemotropic response. The nature of the volatile attractant(s), however, is still unknown.

H. Mycorrhizal Fungi and Biocontrol of Plant Pathogens

Several reviews describe the interactions between VA mycorrhizal fungi and plant pathogens (Schenck and Kellam, 1978; Schenck, 1981; Dehne, 1982). Since VA mycorrhizal colonization occurs in the roots, efforts to study disease interactions have focused largely on the diseases caused by soil-borne pathogens.

Some research has shown that VA mycorrhizal fungi may increase the disease severity (Gerdemann, 1968; Mosse, 1973; Rhodes, 1980). Verticillium wilt of cotton was more severe when plants were inoculated with *G. fasciculatum* (Davis *et al.*, 1979), particularly when P nutrition was adequate. Increased levels of P resulting from VA mycorrhizal infection or P fertilization resulted in increased Verticillium wilt.

Some other reports conclude that VA mycorrhizal fungi do not affect disease severity (Zambolin and Schenck, 1983; Baath and Hayman, 1984). Tolerance to disease may be due to

increased mineral uptake by mycorrhizal fungi (Davis and Menge, 1981). Studies on the Verticillium wilt of tomato demonstrated that infection by VA mycorrhizal fungi did not influence disease severity (Baath and Hayman, 1983).

Generally, the incidence of disease is lower in plants colonized by mycorrhizal fungi than in the nonmycorrhizal plants (Davis and Menge, 1980; Graham and Menge, 1982; Krishna and Bagyaraj, 1983). Most greenhouse studies have demonstrated that mycorrhizal plants show less disease severity than nonmycorrhizal plants (Schonbeck and Dehne, 1977; Schenck and Kellam, 1978). Dehne and Schonbeck (1975) found a reduction in the wilting of tomato caused by *Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc.) Snyder & Hansen when plants were preinfected with a mycorrhizal fungus. Mycorrhizal poinsettia plants tolerated infection by *Pythium ultimum* Trow, and the tolerance was thought to be distinct from improved plant nutrition, particularly since the inoculum density of *P. ultimum* was reduced in the rhizosphere of mycorrhizal plants relative to that in the nonmycorrhizal plants (Kaye *et al.*, 1984).

Improved plant P nutrition is, however, one of the popular theories used to explain the mechanism by which VA mycorrhizal fungi show reduced severity of certain diseases caused by soil-borne plant pathogens (Davis and Menge, 1980; Graham and Menge, 1982). Take-all disease was reduced when wheat infected with VA mycorrhizal fungi was grown in P-deficient soils (Graham and Menge, 1982). As plant P

increased, there was a corresponding decrease in exudation of reducing sugars and amino acids. Levels of P in plants appear to regulate the amount of root exudation through its effect on the phospholipid content of cells and associated changes in root membrane permeability (Ratnayake *et al.*, 1978; Graham *et al.*, 1981). Root exudation also influences the activity of soil-borne pathogens in that P-induced decreases in root exudates may result in decreases disease severity. The application of either P or VA mycorrhizal fungi resulted in improvement of P nutrition of the host, ultimately resulting in reduced severity of the take-all disease of wheat (Graham and Menge, 1982).

Other researchers believe that alterations in host physiology affect plant pathogens. The mycorrhizal roots are more lignified than the nonmycorrhizal roots and the lignification is thought to restrict the growth of the soil-borne pathogens in the host tissue (Dehne, 1982). Baltruschat and Schonbeck (1975) observed that mycorrhizal plants had higher levels of arginine. Arginine, or root extracts from mycorrhizal plants, when added to extracts from the nonmycorrhizal roots, inhibited the formation of the chlamydospores of *Thielaviopsis basicola* (Berk. & Broome) Ferraris. Studies have also shown that phenols accumulate in the mycorrhizal roots and inhibit the growth of *Sclerotium rolfsii* Sacc. (Krishna and Bagyaraj, 1983).

While many VA mycorrhizal species reduce disease severity caused by soil-borne pathogens, they do not

necessarily react in the same manner under different soil conditions. One particular mycorrhizal fungus may only be adapted to certain soil conditions (temperature, salinity, pH, etc.). Variability in controlling disease, may be attributed to differences in cultivar and host reaction, aggressiveness of pathogen biotypes or races, soil types and experimental procedures (Schenck, 1981). Differences in substrates will influence the interaction between VA mycorrhizal fungi and plant pathogens (Caron *et al.*, 1985). It has been suggested that one type of soil medium be used as a standard when experimentally studying the interaction between the VA mycorrhizal fungi and the plant pathogens. Furthermore, extensive field trial studies are needed since most experimental work has so far been conducted under greenhouse conditions.

I. Objectives of the Thesis

The objectives of this thesis were to study the taxonomy, morphology, and hyperparasitism of a new species of *Glomus* (*G. dimorphicum*) found to be associated with barley in Alberta and to study the reactions of this VA mycorrhizal fungus to different cultivars and species of barley and to some other hosts.

Chapter II

Taxonomy[1]

A. Introduction

Interest in VA mycorrhizal research has increased considerably over the last two decades; however, very little work has been conducted in western Canada. Only a limited number of studies have been conducted in British Columbia, Alberta, and Saskatchewan (Molina *et al.*, 1978; Zak *et al.*, 1982; Kucey and Paul, 1983; Zak and Parkinson, 1983).

Most of the work on VA mycorrhizae has been done on hosts such as citrus (Hattingh and Gerdemann, 1975; Kleinschmidt and Gerdemann, 1972; Graham *et al.*, 1982), onions (Owusu-Bennoah and Mosse, 1979; Berch and Fortin, 1983), and various legumes (Azcon *et al.*, 1979; Abbott, 1982; Lim *et al.*, 1983) while cereals have so far received only minor attention (Khan, 1977; Saif and Khan, 1977; Owusu-Bennoah and Mosse, 1979; Jensen and Jakobsen, 1980; Powell *et al.*, 1980; Azcon and Ocampo, 1981; Jensen, 1982; Kucey and Paul, 1983; Hetrick and Bloom, 1984; Hetrick *et al.*, 1984).

The purpose of this study was to identify and classify a new VA mycorrhizal fungus of the genus *Glomus* Tul. & Tul. which forms a mycorrhizal association with barley grown in a

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[1] A portion of this chapter has been accepted for publication as follows: Boyetchko, S.M. and Tewari, J.P. 1985. A new species of *Glomus* (Endogonaceae, Zygomycotina) mycorrhizal with barley in Alberta. Can. J. Bot., in press, accepted on Sept 19, 1985.

field in north-central Alberta. The unique features of this new species, and two others recently described (Smith and Schenck, 1985), have also necessitated the emendment of the characteristics of the genus *Glomus*.

B. Materials and Methods

Soil samples were first collected on July 21, 1983 from a field of barley under monoculture for 15 years at Neerlandia, Alberta (Sec 3 Tsp 34 Rge 61 W of 5). Subsequent collections were made from the same field in May, July, and August of 1984.

The spores were collected by wet-sieving and decanting the soil according to the method of Gerdemann and Nicolson (1963). The soil (100 g) was suspended in tapwater (1 litre), stirred for about 5 minutes and the heavier particles were allowed to settle. The soil suspension was decanted through a series of sieves (1 mm, 710 μm, 425 μm, 250 μm, 150 μm, 106 μm, 75 μm, and 45 μm). The pores in the coarse sieves were small enough to retain the larger particles of organic matter but large enough to allow spores of the VA mycorrhizal fungi to pass through. The spores were large enough to be retained on the finer-meshed sieves. The material retained on the sieves was washed and collected onto Whatman 1 filter paper under suction using a Millipore filtration apparatus. The VA mycorrhizal spores were then examined under a stereoscopic microscope and picked up with dissecting needles.

Spores were observed by light and scanning electron microscopy (SEM). For the former, the spores were mounted in a drop of lactophenol, covered with a cover slip and examined under a Leitz light microscope.

Spores were prepared for SEM by placing them on a filter paper and fixing with osmium tetroxide vapour overnight. Spores were then mounted onto stubs, coated with gold and examined in a Cambridge Stereoscan 150 SEM. Energy dispersive X-ray microanalysis was carried out in the same instrument using a Kevex Micro-X 7000 analytical spectrometer.

The spores isolated from the field soil were multiplied in pot culture by inoculating the seeds of *Hordeum vulgare* L., cultivar 'Olli', before sowing. Barley was inoculated with single and clustered spores in separate pot cultures. The spores and seeds were surface sterilized in 0.5% sodium hypochlorite for 20 seconds and 20 minutes, respectively, and rinsed in two changes of sterile distilled water. Two morphologically similar spores were placed on the seed which was then placed in a shallow depression in the potting medium and subsequently covered by the potting medium. Three seeds were sown in each 15 cm diameter pot and the potting experiment was repeated 5 times. The potting medium consisted of autoclaved sand and loam (3:1). The pots were watered regularly with tapwater, in addition to weekly watering with Hoagland's solution, lacking phosphorus and with the pH adjusted to 6.0. The night temperature was

maintained at 18°C. To avoid any cross-contamination, pots were kept well-spaced on the greenhouse bench. The spores were isolated from the soil after the barley had grown for 2 months, just as heading was occurring. The spores were isolated as described earlier.

The morphology of the spores was compared to those of a few species of *Glomus* obtained from the herbarium at Oregon State University (Table 1). Specimens of *G. aggregatum* were obtained from the Department of Biology at the University of Calgary.

C. Results and Discussion

1. Description of the new species

Glomus dimorphicum Boyetchko & Tewari sp. nov.

Sporae dimorphicae, singillatim et in glomeribus raris in solo formatae. Sporae singulae luteae ad rubro-brunneae, globosae ad sub-globosae, 90-300 μm, cum tribus; tunica externa hyalina, cum laminis, 2-8 μm crassa, in maturioribus disiuncta et deposita; tunica media pallide lutea, in maturioribus rubro-brunnea, cum laminis, 2-8 μm crassa, cum premitur facile a tunica externa separans; tunica interna pallide lutea, in maturioribus rubro-brunea, cum mambranis, saepe in quibusdam locis exigue rugosa, circa 1 μm crassa. Singularum sporarum hyphae sporas gignentes rectae vel exigue curvatae, pallide luteae ad pallide brunneae, tunica 1.5-9.0 μm crassa et similis sporae e tribus tunicis

Table 1. *Glomus* spp. from the Oregon State University Herbarium.

Species	Accession Number
G. clarum	OSC #37,519
G. intraradices	OSC #40,255
G. fasciculatum	OSC #30,894
	OSC #34,801
	OSC #30,831
	OSC #34,593
	OSC #30,829
G. mosseae	OSC #32,358
	OSC #34,378
	OSC #34,313
	OSC #32,914
	OSC #32,899

consistens; punctum colligationis aliquando septatum, saepe occlusum, forma coni vel cylindri, 10-34 μm crassum, septum alterum saepe prope punctum colligationis. Sporae in glomeribus radiantibus ad 15 vel plures quam 15 continentibus compositae, 50-130 μm diametro, globosae ad sub globosae, cum duabus tunicis; tunica extern cum laminis, 2-5 μm crassa, lutea, in maturioribus rubro-brunnea; tunica interna lutea, cum membranis, saepe in quibusdam locis exigue rugosa, circa 1 μm crassa. Sporarum glomeratarum hyphae sporas gignentes rectae, in centro glomeris reticulum rarum hypharum exigue septatarum formantes, pallide luteae ad brunneae, tunica 2-6 μm crassa et similis sporae e duabus tunicis consistens; punctum colligationis forma cylindris vel coni, 7-12 μm crassum. Hyphae exigue septatae in radicibus contaminatis hordei inventae, vesiculae rarae, arbusculae non inventae.

Spores dimorphic, forming singly (Figures 1A, 1D) and in loose clusters in soil; contents hyaline; not reacting

diagnostically with Melzer's reagent. Single spores yellow to reddish-brown, globose to subglobose, 90-300 μm. Wall of single spores of 3 walls organized in 2 wall groups (Figure 6); wall 1 (Figures 1A-1C, 1E, 1F, 6) hyaline, laminate, 2-8 μm thick, sloughing-off with age; wall 2 (Figures 1A-1C, 1E, 1F, 6) light yellow becoming reddish-brown at maturity, laminate, 2-8 μm thick, separating readily from wall 1 under pressure (Figures 1A, 1B, 1E, 1F); wall 3 (Figures 1A-1C, 1E, 1F, 6) light yellow to reddish-brown, membranous, often incipiently wrinkled in certain places, about 1 μm thick. Sporogenous hypha of single spores (Figures 1D, 2A-2D) straight or slightly curved, light yellow to light brown, wall 1.5-9.0 μm thick and consisting of walls 1-3; point of attachment occasionally possessing a septum, often occluded, flared or cylindric, 10-34 μm thick, another septum often present a little distance from the point of attachment. Spores in clusters (Figures 3A, 3B, 4A) often arranged radiately in groups of up to 15 or more, 50-130 μm in diameter, globose to subglobose. Wall of clustered spores of 2 distinct walls organized in 1 wall group (Figure 6); wall 1 (Figures 3C, 6) laminate, 2-5 μm thick, yellow, becoming reddish-brown when mature; wall 2 (Figures 3C, 3D, 6) yellow, membranous, often incipiently wrinkled in certain places, about 1 μm thick. Sporogenous hypha of clustered spores (Figures 3C, 3D) straight, forming a loose network of sparsely septate hyphae in the central part of the cluster, light yellow to brown, wall 2-6 μm thick and consisting of

walls 1 and 2; point of attachment cylindric to flared, 7-12 µm thick. Sparsely septate hyphae present in the infected roots of barley, vesicles rare, arbuscules not seen.

The latin etymology of *G. dimorphicum* refers to the dimorphic nature of the spores in this species; *di* from the Greek duo meaning two and *morphicum* from the Greek noun, morphe, meaning form or shape.

Holotype: From pot culture in association with *Hordeum vulgare* 'Olli' started with spores isolated from barley field soil collected on July 21, 1983 from Neerlandia (Lat. 54° 25'N, Long. 114° 20'W), Alberta, deposited at the Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario as DAOM 192997. The isotype is being maintained in pot cultures, with various hosts, in the greenhouse of the Plant Science Department, University of Alberta, Edmonton.

The outer hyaline wall is present in young single spores of *G. dimorphicum* isolated from pot cultures but older spores and spores isolated from field soil do not show this wall layer. The outer wall layer, besides being naturally evanescent, may therefore be also sensitive to microbial degradation and/or weathering. The wall surface of the clustered spores, which do not possess an outer evanescent wall layer, was found to be smooth.

2. **Distribution of** *G. dimorphicum* spores in the field

Glomus dimorphicum was the only species of VA mycorrhizal fungus isolated from the barley field soil collected. This species was originally isolated from this soil on July 21, 1983. Further collections of soil made in May, July, and August of 1984, also yielded spores of this fungus. During the isolation procedure, the spores were retained on 150 and 106 μm mesh sieves. Quantification of *G. dimorphicum* spores from the soil was conducted and the spore density averaged 1153 spores/100 g dry soil (Table 2). There was considerable variation in spore densities among the samples. The overall density may be affected by annual tillage practices which resulted in site disturbance. As the field is intensively farmed, high levels of nitrogen and phosphorus also may have affected the spore population (Hayman, 1982). However, the spore population of *G. dimorphicum* in Neerlandia soil was higher than that found for VA mycorrhizal fungi (326/100 g dry soil) in a barley field in Saskatchewan sampled during July, 1977 (Kucey and Paul, 1983). Unfortunately, the specific identity of the spores reported from Saskatchewan is not reported to enable any direct comparisons. Also, the prevailing soil factors and any possible barley cultivar interactions must be taken into account. Kucey and Paul (1983) have reported that mycorrhizal spore populations are greater in undisturbed soils than in the cultivated soils. Mycorrhizal spore numbers may also be affected by low soil temperatures in the

Table 2. Spore Density of *G. dimorphicum* in Barley Field Soil at Neerlandia, Alberta.

Sample	Spore Density (spores/100g dry soil)
1	850
2	1790
3	2210
4	621
5	694
6	756
Average	1153

spring and fall. Soil temperatures of 10°C reduced mycorrhizal infection in winter wheat (Hetrick *et al.*, 1984; Hetrick and Bloom, 1984). It appears that fall sown crops may show low levels of mycorrhizal colonization in the spring, particularly since low fall soil temperatures may not be conducive to spore germination and infection (Hetrick and Bloom, 1984). Zak *et al.* (1982) suggested that the ability of a VA mycorrhizal fungus to re-establish its mycorrhizal associations after disturbance may partially determine the success of the fungus in a disturbed site. *G. dimorphicum* may be adapted for establishment in a disturbed site as it was the only mycorrhizal fungus present in this particular field under barley monoculture. If future work reveals that *G. dimorphicum* is able to enhance plant growth significantly, the aforesaid ecological characteristic may become useful in application of *G. dimorphicum* in furthering barley production and perhaps that of some other crop species as well.

3. Pot culture experiments

The spores of *G. dimorphicum* are dimorphic, occurring singly and in loose, often radiate clusters in the soil. The dimorphism of this fungus was determined by inoculating barley with single and clustered spores in separate pot cultures. After allowing the barley plants to grow for 2 months in the greenhouse, the pot cultures were harvested. Inoculation with either spore type resulted in the production of both the single and clustered spores. Similar results were obtained in all 5 repeats. Control pot cultures containing uninoculated barley plants maintained in a manner similar to the inoculated pot cultures, never showed any spores of *G. dimorphicum* nor any mycorrhizal colonization of the roots. Initially, the two spore types were believed to be two distinct *Glomus* spp., however, results from the pot culture studies indicated that they belong to the same species. Many taxonomic studies have been conducted where species descriptions were based on spores isolated directly from field soil. Concurrently with the present study, Smith and Schenck (1985) also described two new dimorphic species, *G. ambisporum* Smith & Schenck and *G. heterosporum* Smith & Schenck. These researchers also conducted extensive pot culture studies before describing the dimorphic VA mycorrhizal species. Results from these studies re-inforce the need for extensive pot culture studies before describing new species of VA mycorrhizal fungi.

4. Emendment of the characteristics of the genus *Glomus*

The two spore types of *G. dimorphicum* differ in size and also in the structure of their cell walls. The general morphology of the single spores is typical of the genus *Glomus*. The features of the clustered spores are reminiscent of the genus *Sclerocystis* Berk. & Broome, particularly the characteristics of *S. rubiformis* Gerd. & Trappe (Gerdemann and Trappe, 1974; Dalpe, 1984). In *Sclerocystis*, spores are formed in a compact single layer around a central plexus of hyphae. The individual spores are usually elongate and the whole sporocarp may be covered by a peridium of hyphae (Gerdemann and Trappe, 1974; Hall, 1977). The clustered spores of *G. dimorphicum* lack a peridium, form loosely around a loose network of hyphae, are not elongate, and possess two wall layers. The spore clusters of *S. rubiformis* are similar to those of *G. dimorphicum* in that they do not have a peridium. However, the individual spores of *S. rubiformis* differ from those of *G. dimorphicum* in having one laminate wall layer.

It is, therefore, obvious that the grouped spores of *G. dimorphicum* are structurally very close to those of *S. rubiformis*. These characteristics, along with the single spore stage of *G. dimorphicum*, indicate that this species is a form intermediate between these two genera. However it shows greater resemblance to the genus *Glomus* and is herein disposed of as such. Species of the genus *Glomus* have so far been known to have only one type of spore. Almost concurrent

discovery of three dimorphic species (*G. ambisporum*, *G. heterosporum*, and *G. dimorphicum*) necessitates a change in the generic description of *Glomus*. Therefore, the characteristics of this genus are emended as follows.

Glomus Tulasne & Tulasne emend. Boyetchko & Tewari[2]

Chlamydospores hypogeous or epigeous, naked or surrounded by an envelop of hyphae, borne terminally on a single (rarely two or more) undifferentiated, nongametangial hypha in sporocarps and/or individually in soil or in roots. Sporocarpic and single chlamydospores in soil morphologically similar or dimorphic. Sporocarps without peridium and generally with irregularly dispersed, rarely radiately arranged chlamydospores. Spore contents continuous with the sporogenous hypha or separated by a septum or an occlusion which is a thickening of the innermost wall layer.

5. Comparison of *G. dimorphicum* with other species

The grouped spores of both *G. dimorphicum* and *G. radiatum* (Thaxter) Trappe & Gerd. (Gerdemann and Trappe, 1974) are radially arranged but the spores of the latter species are grouped or dispersed in a matrix of hyphae. The sporocarps of *G. radiatum* are very large, develop acrogenously and the individual spores possess only one wall layer. The clustered spores of *G. dimorphicum*, however, possess two wall layers. The spores of *G. aggregatum* Schenck & Smith emend. Koske (Figure 7) (Schenck and Smith, 1982;

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[2] The description is partly adapted from Thaxter (1922) and Gerdemann and Trappe (1974).

Koske, 1985) are also arranged in loose clusters but the radial arrangement of the spores often seen in *G. dimorphicum* is not evident in *G. aggregatum*. The spores of *G. aggregatum* also differ from the grouped spores of *G. dimorphicum* in having one or two separable wall layers and in having the sporogenous hypha separated by a curved septum or spore wall thickening (Schenck and Smith, 1982; Koske, 1985).

The presence of septa in the sporogenous hyphae is characteristic for both *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (Gerdemann and Trappe, 1974) and single spores of *G. dimorphicum*. In *G. dimorphicum*, unlike in *G. mosseae*, an additional septum is present a little distance away from the point of attachment and the point of attachment is often occluded. The spore walls of *G. mosseae* consist of a thick inner wall layer and a barely perceptible outer hyaline wall layer. Some spores may also be surrounded by a hyphal mantle (Figure 8). Walls of the single spores of *G. dimorphicum* are characterized by a thin membranous inner wall 3, a thick middle laminate wall 2, and a relatively thick outer hyaline laminate wall 1.

The single spores of *G. dimorphicum* also resemble those of *G. aggregatum* and *G. fasciculatum* Gerd. & Trappe (Figures 7, 9A, 9B) (Gerdemann and Trappe, 1974) but differ in being much larger and in having an outer hyaline ephemeral wall 1. *G. etunicatum* Becker & Gerd. (Becker and Gerdemann, 1977) and *G. clarum* Nicol. & Schenck (Figure 10) (Nicolson and

Schenck, 1979) also possess an outer hyaline wall layer in their spores. In *G. clarum*, this wall layer is persistent while in *G. etunicatum* it is ephemeral like that in *G. dimorphicum*. The spores of *G. etunicatum* have one additional inner laminate wall layer whereas the single spores of *G. dimorphicum* have two additional wall layers. In contrast to this, the spore walls of *G. clarum* are complex, consisting of an inner wall and outer wall, the inner wall being comprised of two to five layers.

Many species of *Glomus* are reported to have one type of spore which may occur singly and in variable groupings. *G. dimorphicum* differs from all the reported species of *Glomus*, with the exception of a recent report by Smith and Schenck (1985), in having two morphologically different spore types present separately as single spores and organized into discrete spore clusters. Smith and Schenck (1985), almost concurrently with the recognition of *G. dimorphicum*, have reported two new dimorphic species of *Glomus* from Florida. These species are *G. ambisporum* Smith & Schenck and *G. heterosporum* Smith & Schenck. The spores of *G. ambisporum*, produced in sporocarps, are dark brown to black in color and possess three walls; an inner membranous wall, a middle laminate wall and an outer, subhyaline, faintly reticulate, ephemeral wall. The grouped spores of *G. heterosporum* contain only two walls; an inner laminate wall and an outer hyaline evanescent wall. The grouped spores of *G. dimorphicum* do not possess an outer hyaline wall which is

characteristic of its single spores. The single spores of *G. dimorphicum* do, however, resemble the second spore stages of both *G. ambisporum* and *G. heterosporum* in having similar 3 walled spores, the outer wall being hyaline and evanescent. However, in the latter two species, this spore stage ranges from hyaline to subhyaline and the spores are formed singly or in unordered sporocarps in roots or dead host tissue. In *G. dimorphicum*, the mature single spores are reddish-brown and are mostly formed dispersed in the soil.

A summary of the morphological features of some VA mycorrhizal fungi is outlined in Table 3.

6. A technique for study of the spore surface

The spores of Endogonaceae isolated from soil are often associated with soil particles which obscure a clear view of the spore surface. The agglutination of soil particles prevents study of the spore wall surface and makes it difficult to distinguish them from the real structures on the spore surface. Energy dispersive X-ray microanalysis was a method used to study the characteristics of the outer wall layer of single spores of *G. dimorphicum*. X-ray spectra of this wall layer free from soil particles (Figure 5A) revealed the presence of calcium only (Figure 5B) but areas containing soil particles (Figure 5A) revealed the presence of silicon, aluminum and potassium in addition to calcium (Figure 5C). This technique was used in a similar manner to study the outer wall layer of the grouped spores of *G.*

TABLE 3 COMPARISON OF MORPHOLOGICAL FEATURES OF SOME VA MYCORRHIZAL FUNGI

Species	Spore Arrangement	Spore Size (um)	Spore Color	Number of Walls	Wall Characteristics	Reference
Glomus dimorphicum	singly	90-300	yellow to reddish brown	3	inner membranous middle laminate outer hyaline and evanescent	Boyetchko and Tewari 1985
	discrete clusters	50-130	yellow to reddish brown	2	inner membranous outer laminate	
G aggregatum	sporocarps	40-85 x 40-85	yellow to orange-brown	1-2	inner thin outer thick and laminate	Schenck and Smith 1982
G ambisporum	singly	54-197 x 44-163	subhyaline to hyaline	2-3	inner membranous middle laminate outer hyaline and evanescent	Schenck and Smith 1985
	sporocarps	85-157	dark brown to black	3	inner membranous middle laminate outer faint, reticulate and ephemeral	
G clarum	singly	68-290	hyaline to yellow	2	inner wall 2-5 layers outer hyaline	Nicolson and Schenck 1979
G etunicatum	singly	68-144	yellow to brown	2	inner laminate outer ephemeral	Becker and Gerdemann 1977

continued next page

TABLE 3 CONTINUED

Species	Spore Arrangement	Spore Size (um)	Spore Color	Number of Walls	Wall Characteristics	Reference
G fasciculatum	sporocarps or small compact clusters	35-105 or 75-150 x 35-100	hyaline to yellow-brown			Gerdemann and Trappe 1974
G heterosporum	singly	31-102 x 27-68	hyaline	3	inner membranous middle thick, outer evanescent	Smith and Schenck 1980
	sporocarps	99-206 x 61-201	light to dark brown	2	inner laminate outer hyaline and evanescent	
G mosseae	singly or in sporocarps	105-310 x 110-305	yellow to brown	2	inner thick outer thin and bare, persistent	Gerdemann and Trappe 1974
G radiatum	sporocarps	60-110 x 48-75	yellow	1	laminate	Gerdemann and Trappe 1974
Sclerocystis rubiformis	sporocarps	37-125 x 29-86	dark brown	1	laminate	Gerdemann and Trappe 1974

dimorphicum. Similar results were obtained as those of the single spores but iron was also found in the soil particles adhering to the walls of the grouped spores (Figures 4B, 4C). Presence of iron (Fe) is not thought to have any particular significance. It was probably, per chance, present in the samples analysed. The surface of the spore wall of the single spores, clear of any soil particles, appeared smooth to slightly roughened in some areas whereas other views showed fine reticulations on the surface (Figure 5A). These views may indicate different laminations of wall 1 of the single spore as they slough-off. The surface of the spore wall of the grouped spores appeared to be smooth (Figure 4B).

This study also indicates that the cell walls of the spores of *G. dimorphicum* are rich in calcium similar to those of the higher plants. In higher plants, calcium is largely present as calcium pectate. No information of this type is available for the VA mycorrhizal fungi.

7. Colonization of barley

Colonization of the roots of *Hordeum vulgare* by hyphae of *G. dimorphicum* was sparse with very few vesicles, and no arbuscules. Most species of *Glomus* form vesicles in the root cortex. *Glomus intraradices*, in particular, forms most of it's vesicles (chlamydospores) in the root cortex rather than singly in the soil (Figures 11A, 11B). Currently available evidence also indicates that, in other hosts, arbuscules are

the site of transference of nutrients (such as phosphorus and calcium, etc.) from the VA mycorrhizal fungus to the host (see Chapter 1). Whether any nutrient transference takes place in barley in the absence of the arbuscules, needs to be studied. Furthermore, it should also be studied whether barley accrues any growth benefit through an association with *G. dimorphicum*. It should be mentioned that some studies have reported growth benefits in barley as a result of VA mycorrhizal association (Owusu-Bennoah and Mosse, 1979; Jensen, 1982). However, it was not reported in these studies whether arbuscules were formed.

D. Conclusions

It was established that the two spore types isolated from the barley field soil from Neerlandia, Alberta belonged to one species of *Glomus* which was recognized as the new species *G. dimorphicum*. Extensive pot culture experiments were necessary to determine whether both spore types belonged to one or two distinct *Glomus* species. It is indicated by this study that new species of VA mycorrhizal fungi should not be described simply after being directly isolated from field soil. Many such reports in the literature may be subject to revision after pot culture experiments. The description of two other new dimorphic species by Smith and Schenck (1985) support the practice of extensive pot culture studies when describing a new species. Prior to these two almost concurrent studies, no dimorphic

VA mycorrhizal species had been described. The generic description of *Glomus* is emended to include the spore dimorphism. All the three dimorphic species appear to be morphologically intermediate between *Glomus* and *Sclerocystis*.

High spore numbers of *G. dimorphicum* recovered from field soil indicate that this VA mycorrhizal fungus may be adapted to soil disturbance that is a normal complement of the agricultural practices and also to higher levels of soil nutrients (such as phosphorus and nitrogen). If future studies reveal that growth benefits result from colonization by this symbiont, the aforesaid ecological attributes of *G. dimorphicum* may suggest its application at the field level to increase crop production.

Energy dispersive X-ray microanalysis in conjunction with SEM has proved to be useful for studying the morphology and elemental composition of the spore surface and should find application in future studies.

E. Figures and Legends

Figure 1.

Glomus dimorphicum, single spores; See Figure 6 for explanation of walls 1-3.

1A. Spore showing hyaline outer wall 1 (large arrowheads), laminate middle wall 2 (arrows), and thin inner wall 3 (small arrowheads). x 350 Note the separation of wall 1 from wall 2.

1B. Part of a single spore showing sloughing-off of wall 1 (large arrowhead). Arrow-wall 2; small arrowhead-wall 3. x 950

1C. Enlarged view of a single spore showing remains of wall 1 (large arrowhead), wall 2 (arrow), and wall 3 (small arrowhead). x630

1D. SEM photograph of a single spore with flared hyphal attachment (arrow). Part of the spore wall appears smooth indicating sloughing-off of the outer wall layer in that region (compare with Figure 5A). The remaining spore wall is covered with soil particles. x300

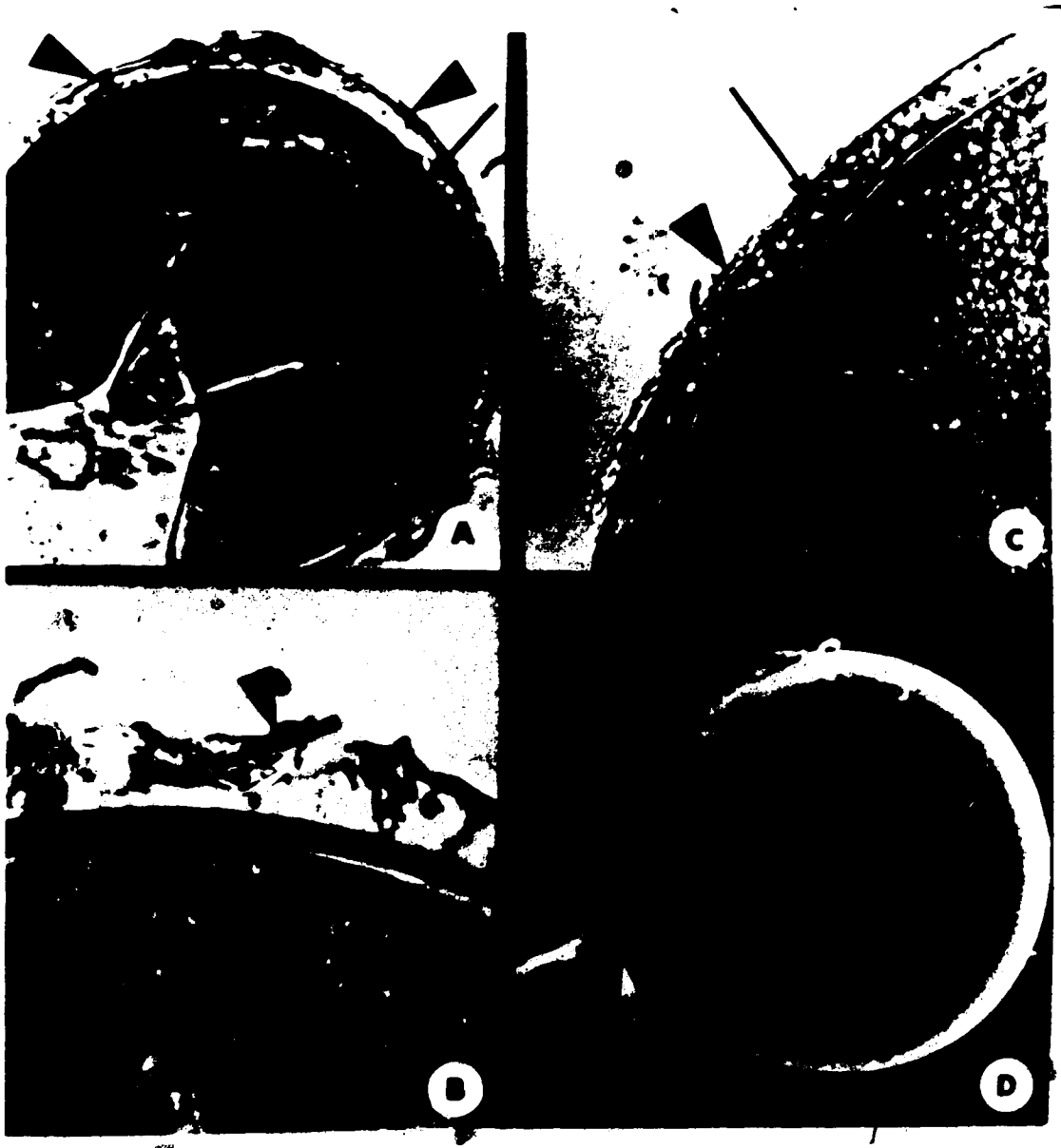

A
C
D
D

Figure 1.

Glomus dimorphicum, single spores..

1E. Lower spore showing hyaline outer wall 1 (large arrowhead), laminate middle wall 2 (arrow), and thin inner wall 3 (small arrowheads). Wall 1 has been largely sloughed-off in the upper spore. x300

1F. Single spore showing separation of outer wall 1 (large arrowhead) from middle laminate wall 2 (arrow). x290

In both Figures, almost the natural colors of the different walls are depicted.


E


F

Figure 2.

Glomus dimorphicum, single spores; enlarged views showing cylindric hyphal attachment.

2A. SEM photograph showing cylindric hyphal attachment. x4930

2B, 2C. Note the septum at the point of attachment (small arrows) and the septum a little distance from the point of attachment (small arrowheads). In Figure 2B, also note the continuation of wall 1 in the sporogenous hypha (large arrows). Figure 2B x1150; Figure 2C x750

2D. Occlusion of the pore as a result of spore wall thickenings (arrowhead). x800


A
B
C
D

Figure 3.

Glomus dimorphicum, grouped spores; see Figure 6 for explanation of walls 1 and 2.

3A, 3B: Radially arranged spores. Note the central plexus of hyphae (arrow). Figure 3A x200; Figure 3B x370

3C. A spore from a cluster showing laminate wall 1 (arrows on the right side), inner wall 2 (small arrowheads), and the sporogenous hypha (arrow) on the left side. Large arrowheads-hyphae of the central plexus. x600

3D. Spores from a cluster showing nearly occluded hyphal attachment (arrow) and hyphae of the central plexus. x650


A
C
B
D

Figure 4.

Glomus dimorphicum, grouped spores.

4A. SEM photograph of a group of radiately arranged spores. Arrow-central plexus of hyphae. x500

4B. SEM photograph of a spore from a group. The cell wall is relatively smooth (arrowhead). Parts of the wall are covered with agglutinated soil (asterisk). x890

4C. X-ray spectrum (shaded area) of wall area (encircled in Figure 4B) relatively clean of soil; note the presence of calcium. Background X-ray spectrum (shown in dotted line) of a soil particle agglutinated to the cell wall (indicated by arrow in Figure 4B); note that aluminum, silicon, potassium and iron are present in the soil particle in addition to calcium. Gold signals are derived from coating for SEM.


A
B
C
SI
AU
AL
MG
AU

Figure 5.

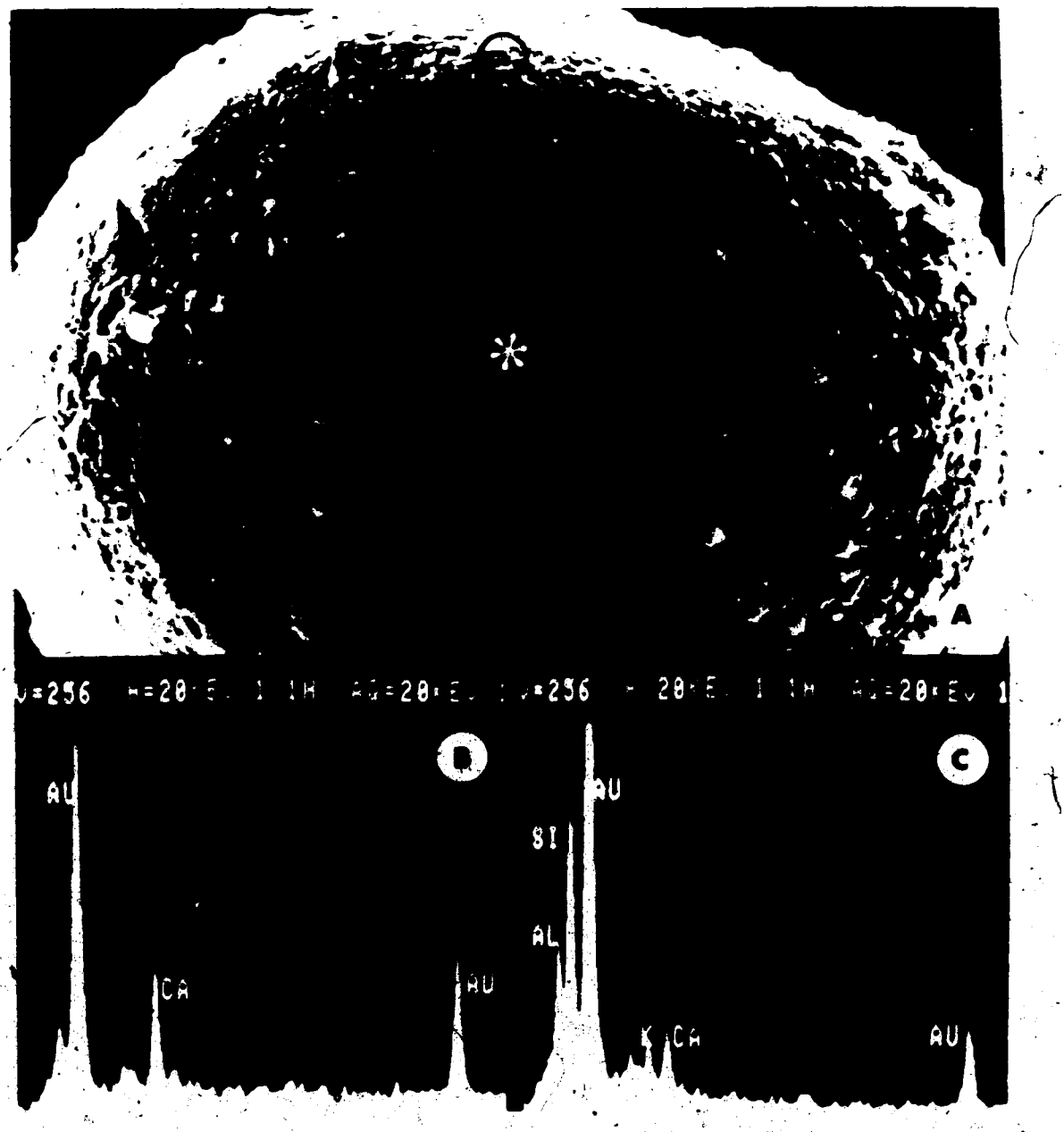
Glomus dimorphicum, single spore.

5A. SEM photograph of the single spore. Different parts of the cell wall are relatively smooth (arrowheads) or show minute reticulations (circle). One of the adhering soil particles is indicated by an arrow. The central part (asterisk) is almost completely covered with agglutinated soil. x1750

5B. X-ray spectrum of wall area (encircled in Figure 5A) relatively clean of soil. Note the presence of calcium.

5C. X-ray spectrum of a soil particle indicated by an arrow in Figure 5A. Note that aluminum, silicon, and potassium are present in addition to calcium.

Gold signals in Figures 5B and 5C are derived from the coating for SEM.


A
B
C
AU
CA
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SI
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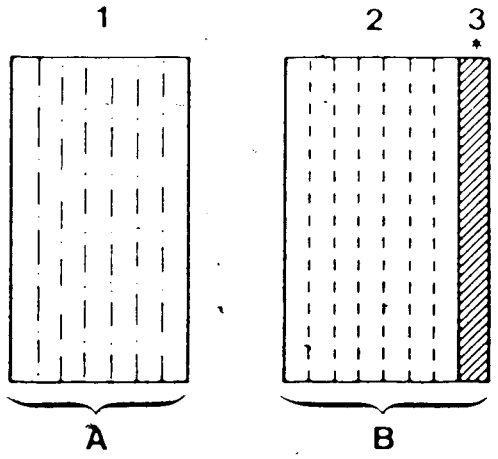
CA
AU

Figure 6.

Murographs (Walker, 1983) of *Glomus dimorphicum*.

Diagrammatic representation of wall structure observed in crushed spores by light microscopy. Upper diagram showing wall structure of single spore with two wall groups (A, B); wall 1 laminate and evanescent, easily separable from wall 2; wall 2 laminate; wall 3 membranous, may be difficult to observe as indicated by an asterisk. Lower diagram showing wall structure of spores in cluster consisting of one wall group (A); wall 1 laminate; wall 2 membranous, may be difficult to observed as indicated by an asterisk. Shading as per Walker (1983) to indicate the characteristics of the different walls.

Vertical dashed lines:Laminate wall.
Combination of dashed lines and dots:Laminate, evanescent wall.
Cross-hatching at 45°:Membranous wall.


1
2
3
A
B

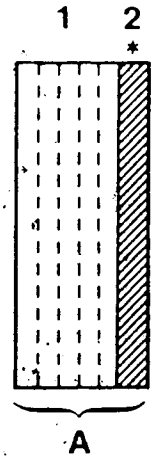

1
2
A

Figure 7.

Spores of *Glomus aggregatum*.

Note that the spores are arranged in a loose cluster. x520

Figure 8.

Chlamydospore of *Glomus mosseae*.

The spore is covered by a mantle of hyphae. x150


7

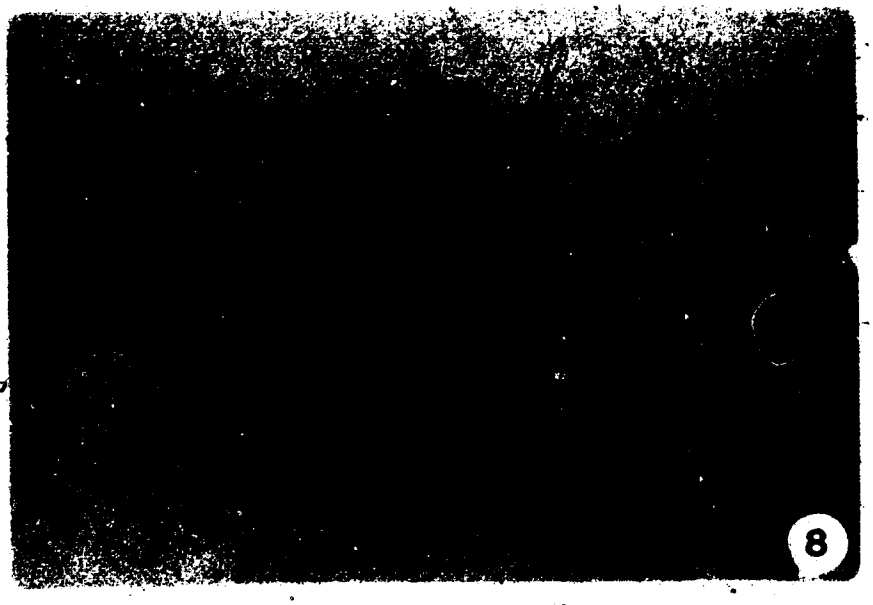
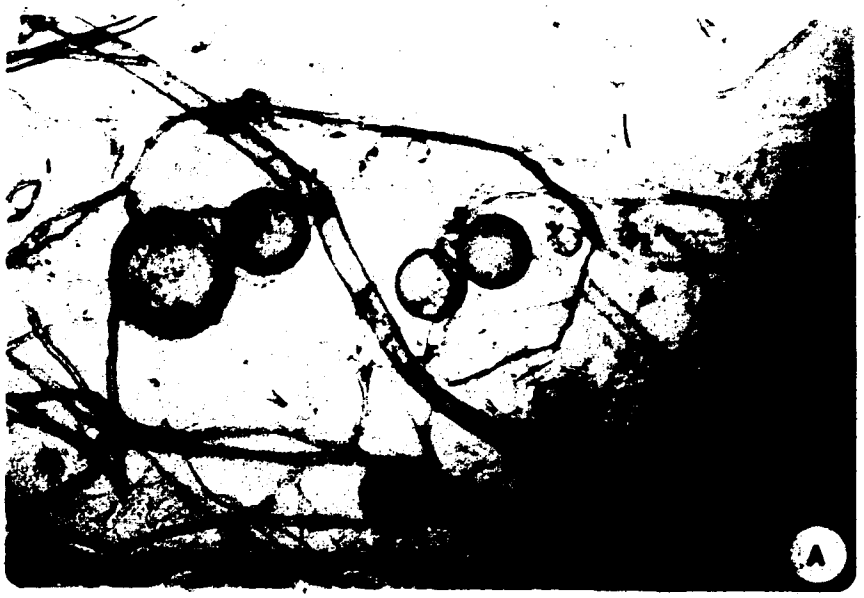

8

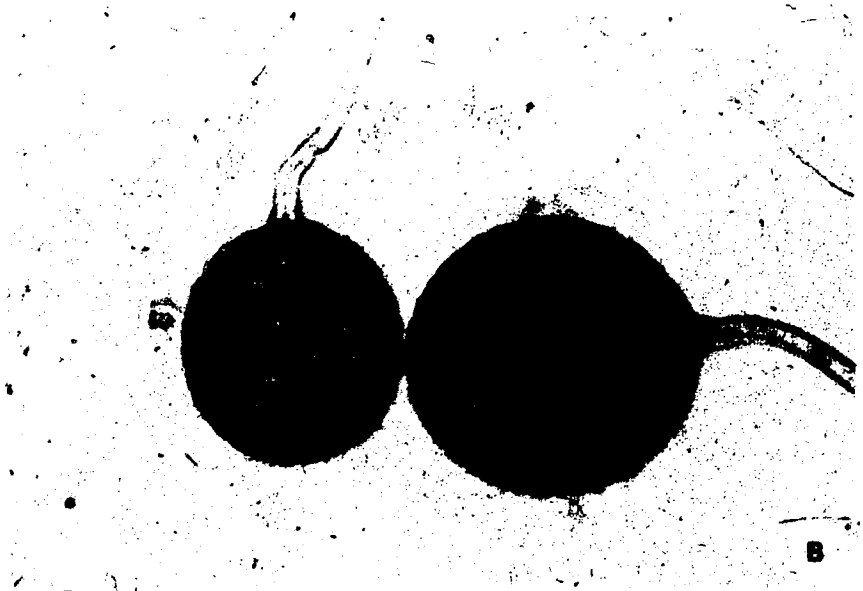
Figure 9.

Spores of *Glomus fasciculatum*.

9A. Spores are arranged in a loose cluster. x140

9B. Hyphal attachment is occluded by spore wall thickening. x380.


A


B

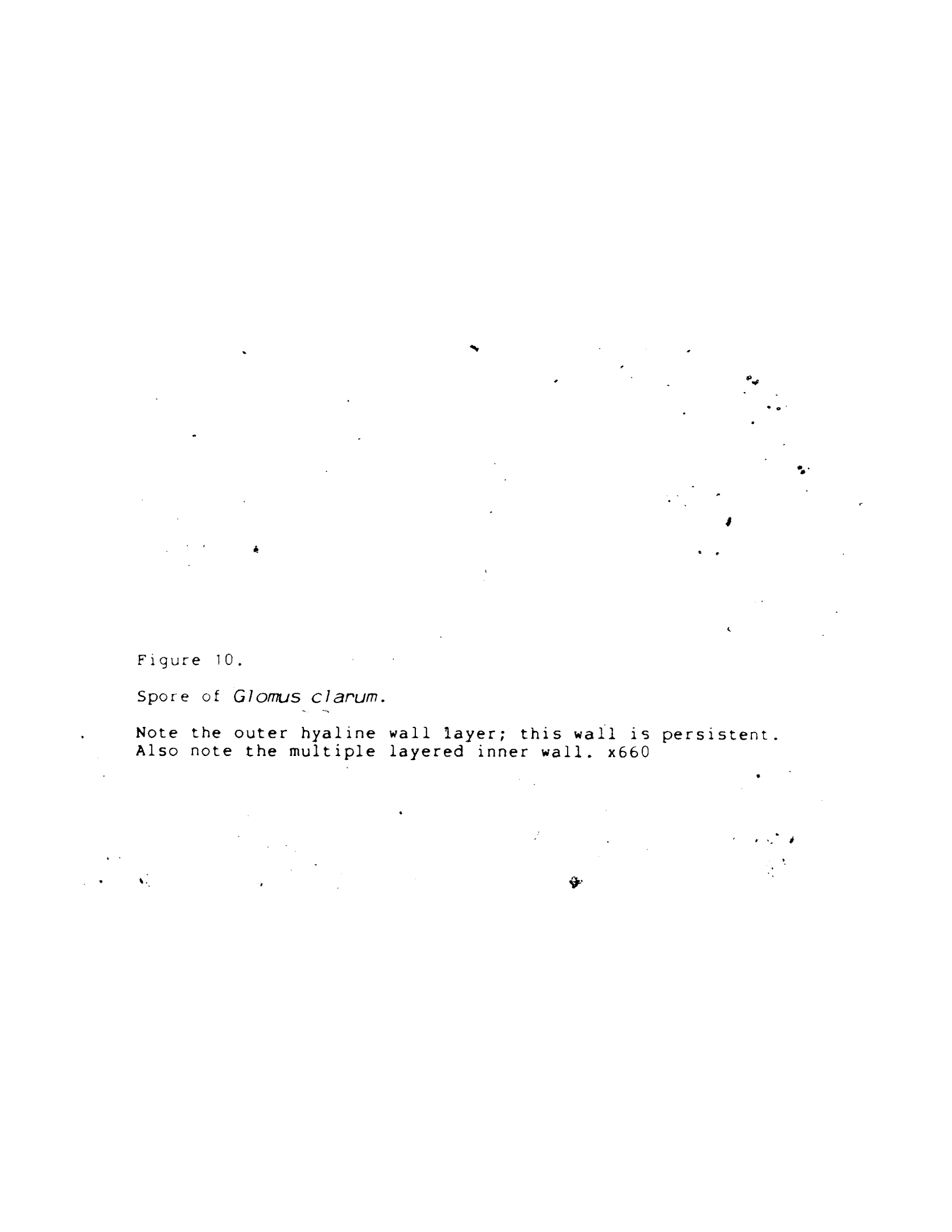


Figure 10.

Spore of *Glomus clarum*.

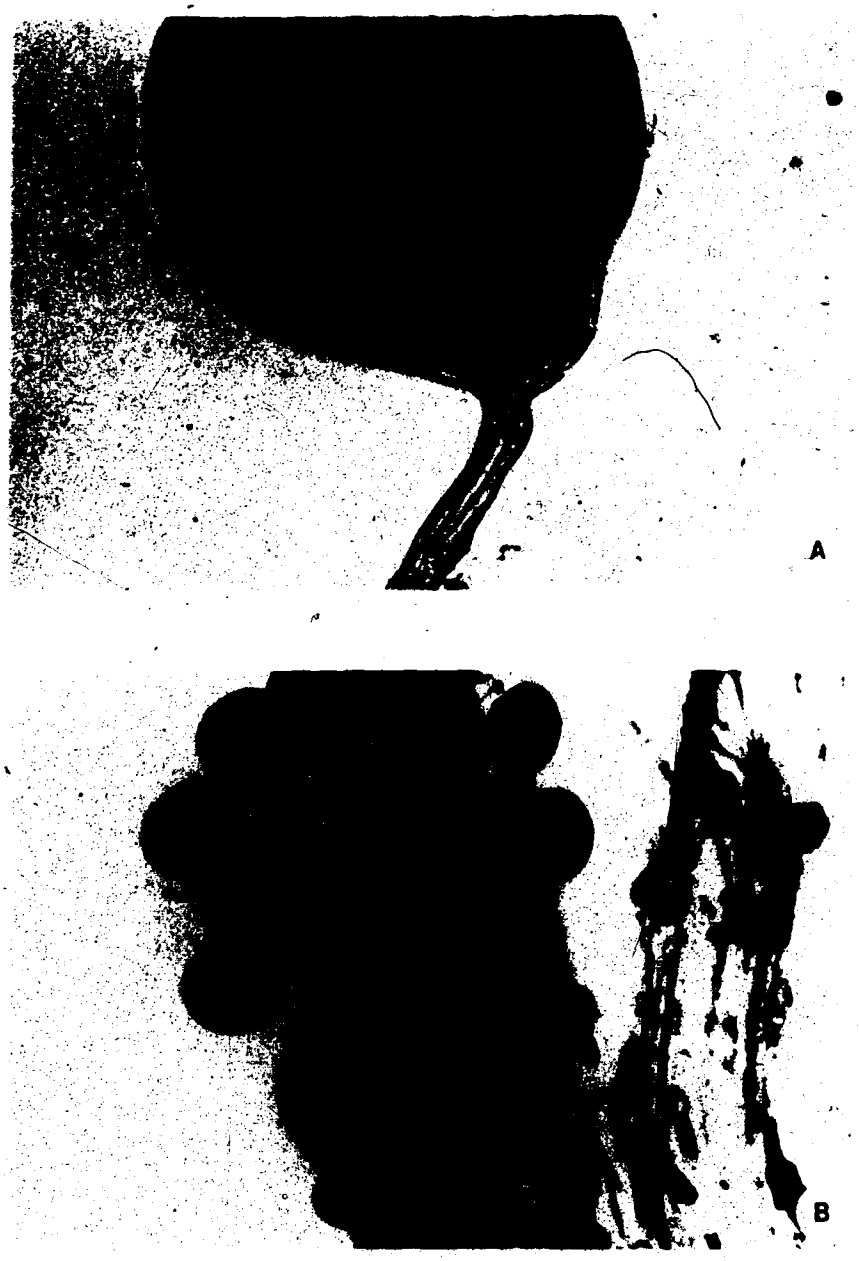
Note the outer hyaline wall layer; this wall is persistent. Also note the multiple layered inner wall. x660

Figure 11.

Chlamydospores of *Glomus intraradices*.

11A. Spore showing the complex multiple wall layers. x560

11B. Cleared root showing spores in the cortex. The spores are predominantly intraradical in this species. x150


A
B

Chapter III

Root Colonization of Different Hosts

A. Introduction

VA mycorrhizal fungi are widely occurring obligate symbionts infecting most families of plants, including those of economic importance. These fungi increase phosphate uptake (Daft and Nicolson, 1969; Sanders and Tinker, 1973; Menge *et al.*, 1978) and are capable of stimulating plant growth in soils generally of low to moderate fertility (Mosse, 1973; Abbott and Robson, 1982). It has been shown that root colonization of wheat and barley by various VA mycorrhizal fungi has led to increases in grain and straw yields (Khan, 1975; Saif and Khan, 1977; Powell *et al.*, 1980; Powell, 1981; Jensen, 1982). The increases in growth of barley were not correlated with the level of infection in the roots. This is consistent with the fact that given a minimal level of root colonization, the growth increase correlates with the extent of extraradical mycelium and not with the extent of root colonization (Owusu-Bennoah and Mosse, 1979; Jensen, 1982).

It has been suggested that the extent of root colonization may be determined by the host (Hetrick, 1984; Hetrick *et al.*, 1985). Although, VA mycorrhizal fungi are not host specific, some mycorrhizal species colonize certain hosts to a greater extent than others (Jensen, 1982; Hetrick, 1984). Also, the ability of VA mycorrhizal fungi to

improve yield and nutrient uptake is partially determined by their efficiency (Jensen, 1982). The host may also influence root colonization patterns by VA mycorrhizal fungi (Cox and Sanders, 1974; Abbott and Robson, 1978; Hetrick *et al.*, 1985) where the behavior of mycorrhizal hyphae (e.g. coiling) are probably influenced by the host (Abbott, 1982).

It is believed that some plants are more dependent on VA mycorrhizal fungi for growth enhancement than others (Menge *et al.*, 1978; Azcon and Ocampo, 1981). Mycorrhizal dependency is believed to be the extent to which a plant relies on mycorrhizal fungi to produce maximum growth under various soil conditions. Baylis (1970) believed that the number of root hairs in a plant determined the degree of mycorrhizal dependency; the greater the number of root hairs, the lower the dependency. Also, long root hairs is correlated with a low degree of mycorrhizal dependency. Menge *et al.* (1978) found that the length of the root hairs of different citrus cultivars was not a major factor of mycorrhizal dependency. They did, however, find that the major factor responsible for mycorrhizal dependency of citrus was the inability of the plants to take up phosphorus from P deficient soils.

Different wheat cultivars inoculated with *Glomus mosseae* differed in the extent of VA mycorrhizal infection and dependency (Azcon and Ocampo, 1981). The responses by the various wheat cultivars may depend on the nature of the root exudates, particularly on the amount and the specific

components of the exudates. Similar parameters may also regulate the responses of barley cultivars to root pathogens such as *Cochliobolus sativus* (Ito & Kurib) Drechsl. ex Dastur. Plant breeders have developed cultivars that are moderately resistant to such soil-borne pathogens and while breeding for resistance to the pathogens, they may have also selected for resistance to VA mycorrhizal fungi as well. Barley cultivars susceptible to *C. sativus* may also exhibit susceptibility to VA mycorrhizal infections. These considerations should help explain the selection of barley cultivars used during the present study which deals with the extent and pattern of infection by *G. dimorphicum* in barley and some other plants and by the indigenous VA mycorrhizal fungi in barley and wild barley (*Hordeum jubatum* L.).

B. Materials and Methods

Field plots were established in Edmonton at the University Farm (W240 in 1983, 1984, 1985 and Parkland in 1984, 1985). The sites were chosen because of their low available soil phosphorus levels. Soil analyses determined that the soil at W240 had 7 ppm extractable P and Parkland had 17 ppm extractable P. Phosphorus was analyzed by the method of Olson and Dean (1965) with the help of Mr. Y. Kalra, Northern Forest Research Center, Edmonton. Four cultivars of barley were chosen according to their relative resistance/susceptibility to *C. sativus*, the causal agent of the common root rot of barley: Bonanza (moderately

resistant), Klondike and Gateway 63 (intermediately resistant) and Olli (highly susceptible) (Cohen *et al.*, 1969; Mills and Tekauz, 1975; Anonymous, 1983; Anonymous, 1985). The four cultivars, considered as treatments, were randomized in four replicates with four rows per treatment. Only the middle two rows were sampled in each treatment; the outside rows were treated as guard rows.

Root samples were collected from the sites at the end of July or in the first week of August, just as heading occurred. The roots were placed in FAA (5 ml formalin:5 ml acetic acid:90 ml 50% alcohol), cleared and stained according to the method developed by Phillips and Hayman (1970), which will be described in detail later, and assessed for VA mycorrhizal root colonization.

Greenhouse experiments were conducted to compare VA mycorrhizal root colonization in the same four barley cultivars. Other hosts such as red clover, beans, alfalfa, onions and corn were also compared. Seeds were sown in autoclaved soil (3 sand:1 loam) and inoculated with soil containing the chlamydospores of *G. dimorphicum* (200 spores per 100 g soil). The plants were watered weekly with Hoagland's solution, lacking phosphorus, in addition to regular watering. After two months, the roots were collected, placed in FAA, and later cleared and stained prior to assessing for VA mycorrhizal root colonization. Each barley cultivar was grown in four pots and the experiment was replicated three times. The other hosts were

also replicated over time (3 times). Plants of wild barley (*H. jubatum*) were collected from the field at Wainwright, Viking, and Camrose in August, 1984. The roots were stored in FAA and later cleared, stained and assessed for VA mycorrhizal root colonization.

Roots stored in FAA were washed in tapwater and cleared and stained according to the method of Phillips and Hayman (1970). The roots were placed in beakers containing 10% potassium hydroxide and heated in an oven at 90°C for 1 hour. The potassium hydroxide was then poured off and the roots were rinsed in tapwater until no brown color appeared in the rinse water. Roots were then bleached in alkaline hydrogen peroxide (3 ml ammonium hydroxide, 30 ml 10% hydrogen peroxide, 567 ml tapwater) for ten minutes, rinsed in tapwater and acidified in 1% trypan blue-lactic acid staining solution (875 ml lactic acid, 63 ml glycerin, 63 ml tapwater, 0.1 g trypan blue) for 5 minutes. The staining solution was poured off and lactic acid destaining solution was added (same as above but lacking trypan blue) to remove the excess stain. Lactic acid staining solution was used instead of lactophenol because it is non-volatile and can be re-used. The destaining solution may be re-used upon removal of the stain with decolorizing charcoal. The roots were now ready for mycorrhizal assay. The grid-line intersect method was used to quantify the amount of VA mycorrhizal root infection. This method is a modification of the line transect method developed by Newman (1966) and modified by

Giovannetti and Mosse (1979).

Roots were placed in an area measuring 2.5 x 5.0 cm on a microscope slide, with the roots not overlapping each other and covering as much of this area as possible. This procedure was repeated until the roots in the sample were laid out onto 10 microscope slides. The slides containing the root samples were placed onto a Petri dish containing marked grid lines forming 0.5 inch squares. The grid lines thus marked corresponded to systematic observation points. An estimate of the root length was given by:

$L = \pi NA/2H$, where,

N=number of intersections,

A=area over which root segments are spread,

and H=total length of horizontal and vertical grid lines per slide.

At any particular position along the root segment, the intersections were scored for the presence and absence of VA mycorrhizal infections. If the roots were decayed or if the root cortex was missing, perhaps due to slight maceration during clearing, they were not included in the assessment. This may have led to slight underestimation of the infection levels. However, such cases were not too many and perhaps did not affect the counts appreciably. The % infection was determined by dividing the total mycorrhizal root length by the total length of root per sample. When more detailed observation of VA mycorrhizal structures was desired, the roots were examined under a compound light microscope.

Spores of *G. dimorphicum* were surface sterilized in 0.5% sodium hypochlorite for 20 seconds and placed on 2% bactoagar. The mode of germination of the spores was examined under the compound light microscope.

C. Results and Discussion

1. Levels of root colonization

Results from the field plots indicated that in 1983 at W240, Olli had statistically higher levels of VA mycorrhizal root infection than Bonanza, Klondike, and Gateway 63 (Table 4). However, there was no statistical difference among the barley cultivars in the extent of VA mycorrhizal root colonization in 1984 and 1985 at W240 and Parkland. Low soil moisture conditions in 1984 and 1985 may have affected the ability of VA mycorrhizal fungi to colonize the barley roots. A study of drought stressed corn (Hirrel *et al.*, 1984) revealed that although plants showed increased VA mycorrhizal root colonization over non-stressed plants, there were no differences in growth between VA mycorrhizal plants and nonmycorrhizal plants (Hetrick *et al.*, 1984). There was a lack of nutritional benefit from the symbiont under drought conditions and the increased root colonization under drought stress may suggest parasitism by the VA mycorrhizal fungi. It is evident, from the erratic results obtained from the field plots, that long term field experiments are needed before any conclusions can be drawn.

Table 4. VA Mycorrhizal Infection (%) in Barley Cultivars (Field).

Site	Cultivar (% Infection)*			
	Bonanza	Klondike	Gateway 63	Olli
W240 1983	4.2a	3.5a	2.5a	29.7b
W240 1984	4.0a	3.0a	5.6a	5.3a
W240 1985	2.7a	2.7a	2.4a	3.1a
Parkland 1984	2.4a	1.9a	1.9a	2.8a
Parkland 1985	2.3a	2.1a	1.6a	4.7a

* Duncan's Multiple Range Test was conducted within the sites and not between the sites from year to year.
a-b Means sharing the same letters are not statistically different at the 5% level.

The results from the 1983 data may suggest that the susceptibility of barley to *C. sativus* and VA mycorrhizal fungi are related. However, this data alone cannot be used to make any conclusions.

Azcon and Ocampo (1981) studied infection by VA mycorrhizal fungi of thirteen different wheat cultivars. They observed that the amount of sugar in the root exudates partially regulated the susceptibility of the cultivars to VA mycorrhizal infection. Infection was low when the amount of sugar in the root exudates was low.

It is also possible that the indigenous populations of the VA mycorrhizal fungi present at W240 and Parkland do not normally colonize barley to any great extent. Powell *et al.* (1980) also observed that levels of infection in barley by indigenous VA mycorrhizal fungi was relatively low (15%). Jensen (1982) observed that some species of *Glomus* colonize barley roots better than others. The infection of barley by

G. constrictum was 6% while the levels increased to 57% and 64% when barley was infected by *G. fasciculatum* isolate no. 185 and 0-1, respectively.

Results of root colonization of wild barley (*H. jubatum*) by indigenous VA mycorrhizal fungi showed that the levels of infection may vary from site to site (Table 5). Roots of wild barley had a level of infection of 55.6% at Wainwright, 37.1% at Viking, and 24.6% at Camrose. Although the same host was infected, different levels of infection were observed. The variations in soil factors could have affected the level of VA mycorrhizal root infection. It is also possible that the varying levels of colonization at the three sites actually reflects the presence of different species of VA mycorrhizal fungi.

The results of greenhouse experiments showed that there was no statistical difference in the levels of infection by *G. dimorphicum* in the barley cultivars (Table 6). Although *G. dimorphicum* was isolated from a barley field under monoculture, this mycorrhizal species, at least under certain conditions, may not necessarily infect barley roots to any great extent. In view of the high spore numbers of *G. dimorphicum* in this soil (see Chapter II), this species may be a mycorrhizal fungus well adapted to the field conditions such as cultivation and high fertility levels. *G. dimorphicum* was, however, observed to infect other hosts to a greater extent (Table 6). Beans, alfalfa, and onions had moderate levels of infection while red clover and corn roots

Table 5. VA Mycorrhizal Infection (%) in *H. jubatum*.

Site	% Hyphal Infection*	% Vesicular Infection*
Wainwright	55.6 ± 1.0	19.9 ± 1.9
Viking	37.1 ± 0.4	9.0 ± 0.5
Camrose	24.6 ± 0.5	5.7 ± 0.5

* Each number represents the mean of 3 replicates ± standard deviation.

Table 6. Infection (%) of *G. dimorphicum* in Different Hosts.

Host	% Hyphal Infection	% Vesicular Infection	Arbuscules**
'Bonanza'	0.7a	0.0	-
'Klondike'	1.2a	0.0	-
'Gateway 63'	1.2a	0.0	-
'Olli'	1.4a	0.0*	-
Beans	12.4b	3.7	+
Alfalfa	20.6c	0.0*	+
Onions	28.0c	0.0	+
Red Clover	59.8d	7.3	+
Corn	55.7d	0.0	+

a-d: Means sharing the same letters are not statistically different at the 5% level (Duncan's Multiple Range Test).
* The grid-line intersect method did not detect the presence of vesicles but a few vesicles were observed.
** plus (+) and minus (-) indicates presence or absence of arbuscules.

exhibited the highest levels of infection. Although *G. dimorphicum* infected the barley cultivars at low levels, it is obvious that this fungus is capable of infecting other hosts grown under similar greenhouse conditions.

2. Root colonization patterns

The germination of the spores of *G. dimorphicum*, was observed to occur through the old sporogenous hypha (Figures 12A, 12B). Most species of *Glomus* germinate in this manner with germination rarely occurring through the spore wall (Hall, 1984).

VA mycorrhizal fungi are known to be host non-specific but patterns of infection may vary from host to host. Abbott (1982) indicated that VA mycorrhizal species showed characteristic infection patterns and that these root infection patterns could be used as criteria to key out various species. Hetrick *et al.* (1985) showed that infection patterns by *G. epigaeum* Daniels and Trappe differed among the host plants. Results from this study have shown that infection patterns of *G. dimorphicum* differed among the host plants. Barley cultivars showed limited levels of hyphal development with few vesicles and no arbuscules forming. Extraradical hyphae (Figures 13A, 13B) and a limited amount of intercellular hyphae were observed associated with the barley roots (Figure 13E). Formation of the grouped and single spores of *G. dimorphicum* was also observed (Figures 13C, 13D).

Extraradical hyphae were associated with the roots of all the hosts studied. Penetration of the root via appressoria was observed in all the host roots (Figures 16A, 16B, 17A). Penetration may occur through or between the epidermal cells (Carling and Brown, 1982) but it did not

become clear, from this study, which mode of penetration was more common. The extraradical hyphae were often appressed to the root surface.

Aseptate intercellular hyphae were observed in all the hosts (Figure 14B). These hyphae were predominantly found in the intermediate layers of the root cortex and generally appeared to align themselves parallel to each other (Figures 14A, 14B).

Intracellular hyphae, occurring usually in the outer layers of the root cortex, were observed in roots of corn, beans, onions, alfalfa, and red clover. It was observed, in corn roots, that the intracellular hyphae passed from cell to cell by constricting their hyphae. The process is also common in the extraradical hyphae during penetration of the root (Kinden and Brown, 1975a; Bonfante-Fasolo, 1984). Coiling of the intracellular hyphae occurred in the roots of corn, alfalfa, and red clover (Figure 17B). Coiling of the hyphae was seen in the second or third layer of cells in the roots. The formation of coils in roots has been observed in many plants and it is believed that this behavior is influenced by the host and not the VA mycorrhizal species (Kinden and Brown, 1975a). It has been suggested that mycorrhizal species such as *G. fasciculatum* do not form coils in the roots of host plants such as subterranean clover (Bonfante-Fasolo, 1984).

Beans and red clover contained intraradical vesicles in the inner and outer layers of the root cortex in older

infections (Figures 14D, 15A, 15B), while the other hosts did not (Table 6). Although the grid-line intersect method did not measure the presence of vesicles in alfalfa and 'Olli' barley roots, a few vesicles were otherwise observed. Upon closer examination of the vesicles under light microscopy, lipid droplets could be seen (Figures 14D, 16D). Lipid droplets in vesicles of *G. etunicatum* have been found to be high in triglycerides (Nemec, 1981; Jabaji-Hare *et al.*, 1984). Vesicles are believed to function as storage organs but Biermann and Linderman (1983) have suggested that vesicles in roots may function as inoculum. Since VA mycorrhizal fungi cannot be successfully grown in pure culture, the presence of appreciable numbers of vesicles in the roots of various hosts may be important in raising root-borne inoculum.

Arbuscules, which are haustoria-like structures and considered to be involved in nutrient transfer between the host and the symbiont, were observed in all hosts (Figures 15C, 17C) except the barley cultivars (Table 6). These structures occurred in the inner layers of the root cortex. The arbuscule could be seen arising from an arbuscule trunk (Figure 15C), formerly the penetration hypha entering the cell. The arbuscule trunk branched dichotomously to form fine branches at the terminal ends of the arbuscules. In all hosts, the morphology of the arbuscule appeared to be similar. As infection became older, it was observed in bean roots that vesicles were present along with the arbuscules

and that the arbuscules appeared to exhibit clumping (Figure 15B), perhaps due to deterioration. It has been reported earlier that as the arbuscule get older, the branches characteristically collapse and aggregate into a clump near the penetration point (Cox and Sanders, 1974; Kinden and Brown, 1976; Toth and Miller, 1984).

The roots of wild barley from all the sites sampled showed high levels of extraradical and intraradical hyphae. Coiling of the intraradical hyphae occurred similar to that in corn, alfalfa, and red clover. Appreciable numbers of vesicles, as well as arbuscules, were seen in the root cortex (Figures 18A, 18C).

D. Conclusions

The extent and pattern of VA mycorrhizal infection varied from host to host. This study, along with a concurrently published study (Hetrick *et al.*, 1985), have revealed that the host may influence the pattern of VA mycorrhizal root colonization. Hetrick *et al.* (1985) reported that the effect of host on the infection patterns was unique to *G. epigaeum*. This study reports that such a behavior is also shown by *G. dimorphicum* and probably occurs with some other VA mycorrhizal species as well. It was suggested that infection patterns were specific to mycorrhizal species (Abbott and Robson, 1978; Abbott, 1982) and that infection morphology could be used as characteristics for certain VA mycorrhizal species. This

study would sound a caution against such a practice as *G. dimorphicum* has been found to show varying infection patterns in the different hosts.

Vesicles of *G. dimorphicum* are produced only in certain hosts. The vesicles are storage structures and, in *Glomus*, also serve as chlamydospores. Therefore, this study indicates that the infected roots of beans and red clover can be used as the inoculum of *G. dimorphicum*.

The level of infection by *G. dimorphicum* also varied among hosts. Barley cultivars showed very low levels of mycorrhizal colonization under greenhouse conditions. Jensen (1982) reported that *G. constrictum* colonized barley roots at very low levels while *G. fasciculatum* colonized it at much higher levels. It could, therefore, be suggested that, at least under certain conditions, *G. dimorphicum* does not heavily colonize the roots of barley cultivars while it colonizes other hosts such as red clover and corn to a much greater extent. Although low levels of root colonization were observed in barley, there may still have been growth-benefit effects since increases in growth are reportedly not correlated with the intensity of root colonization (Powell *et al.*, 1980; Jensen, 1982). Further experiments are therefore necessary to determine the growth responses of barley and other hosts as a result of infection by *G. dimorphicum*. The absence of arbuscules in barley roots is interesting as these structures are known to be the sites of exchange of materials between the VA mycorrhizal fungi

and the host. Whether this response is unique to *G. dimorphicum* or to the cultivars investigated should be studied further. Unfortunately, most of the available work on growth benefits in barley as a result of symbioses with VA mycorrhizal fungi does not indicate if arbuscules were formed in the roots or not (Powell *et al.*, 1980; Powell, 1981; Jensen, 1982). However, Saif and Khan (1977) mention the presence of arbuscules in barley. This, along with the formation of arbuscules in the roots of wild barley, indicates the potential of arbuscule development in the genus *Hordeum*.

E. Figures and Legends

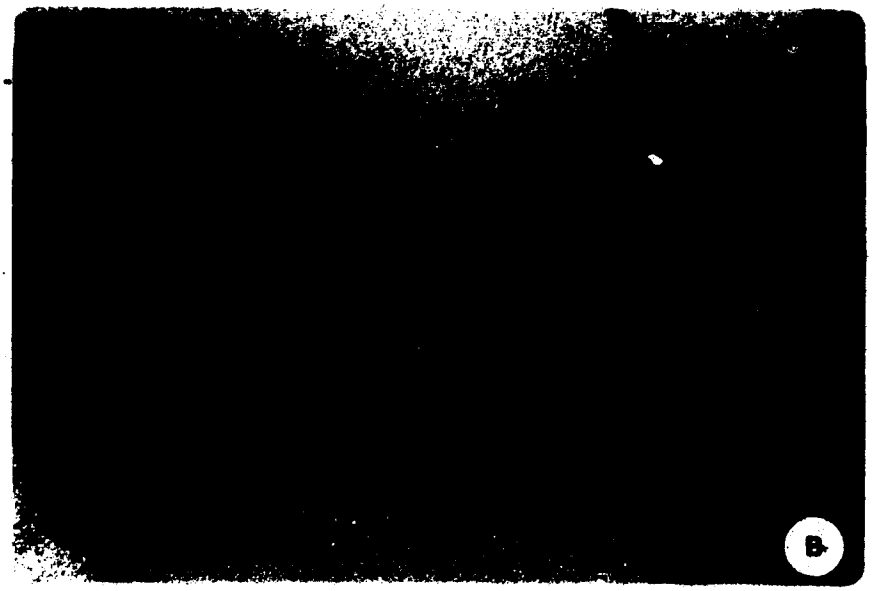
Figure 12.

Germinating single spore of *Glomus dimorphicum*.

12A. Germination of spore occurring through the subtending hypha (arrowhead). x140

12B. Enlarged view of part of the germinating spore shown in Figure 12A. x325


A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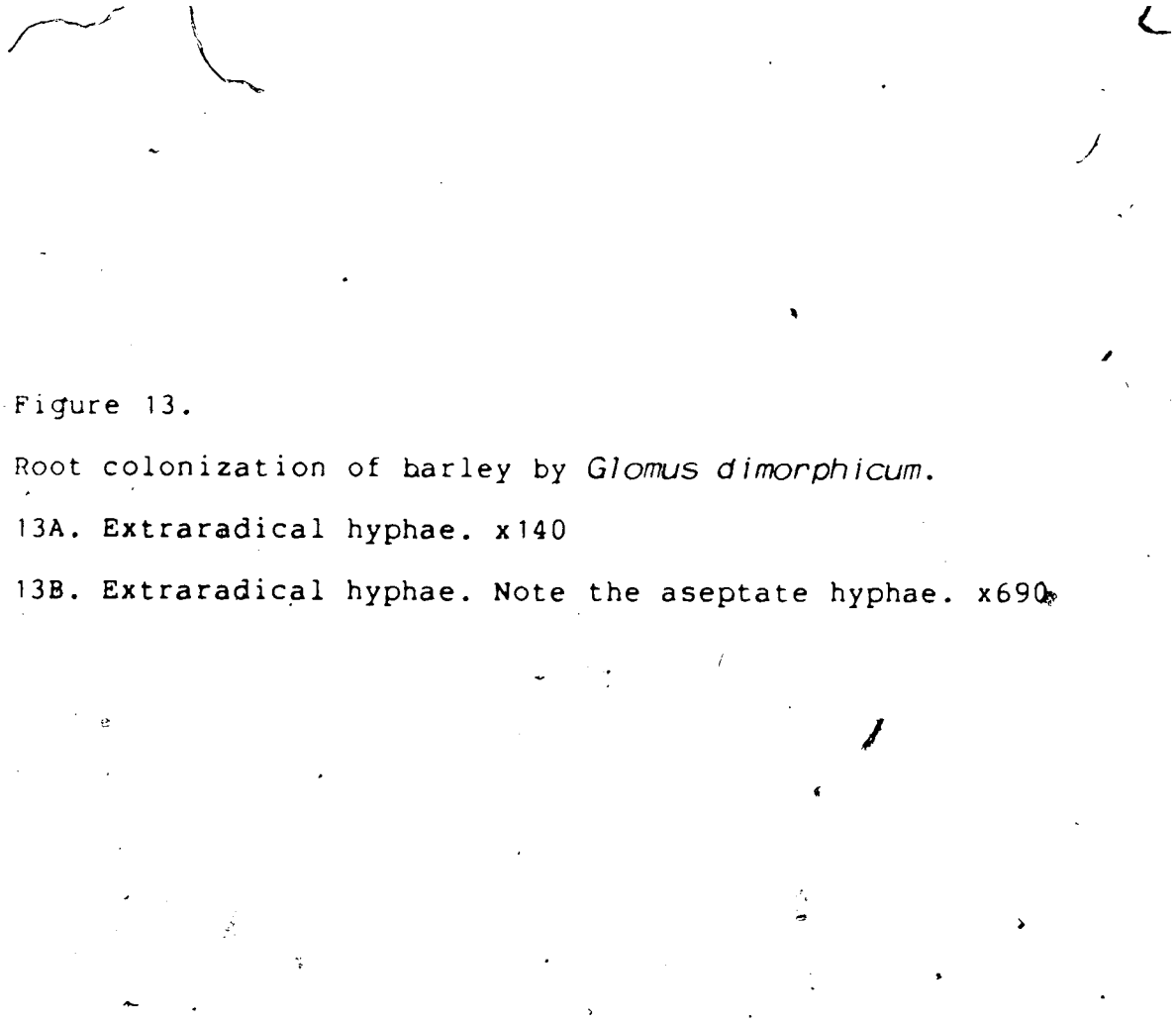


Figure 13.

Root colonization of barley by *Glomus dimorphicum*.

13A. Extraradical hyphae. x140

13B. Extraradical hyphae. Note the aseptate hyphae. x690


A

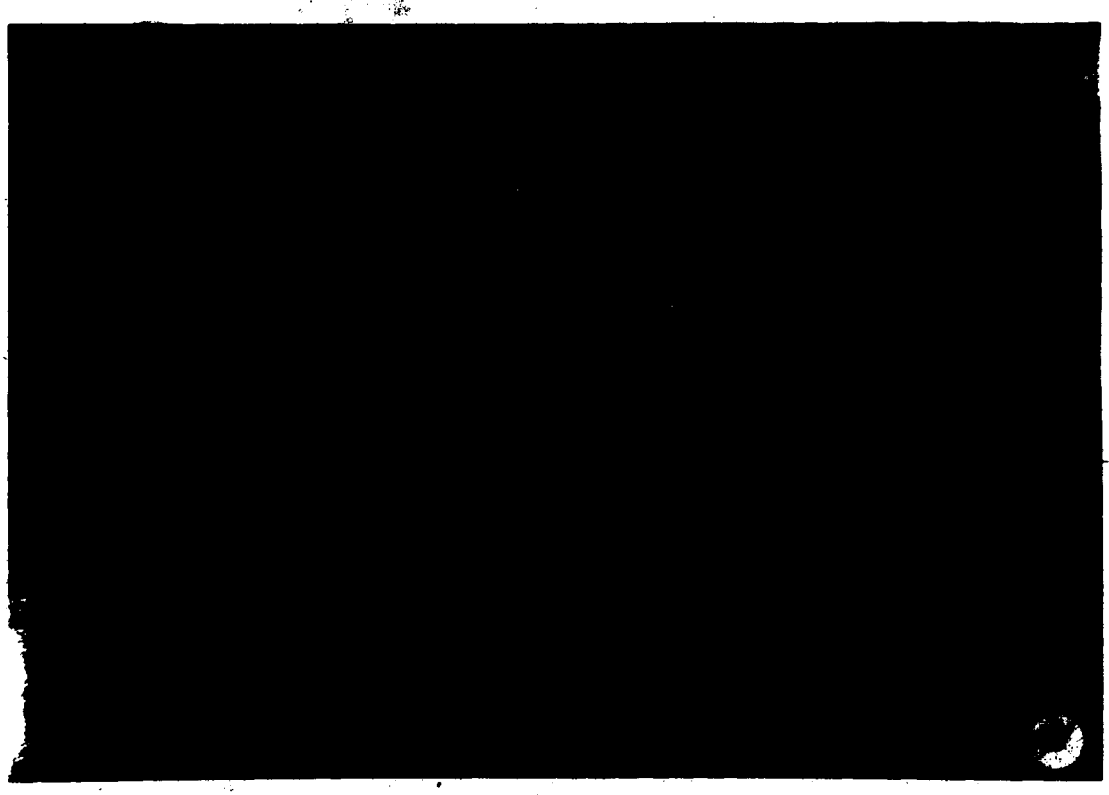
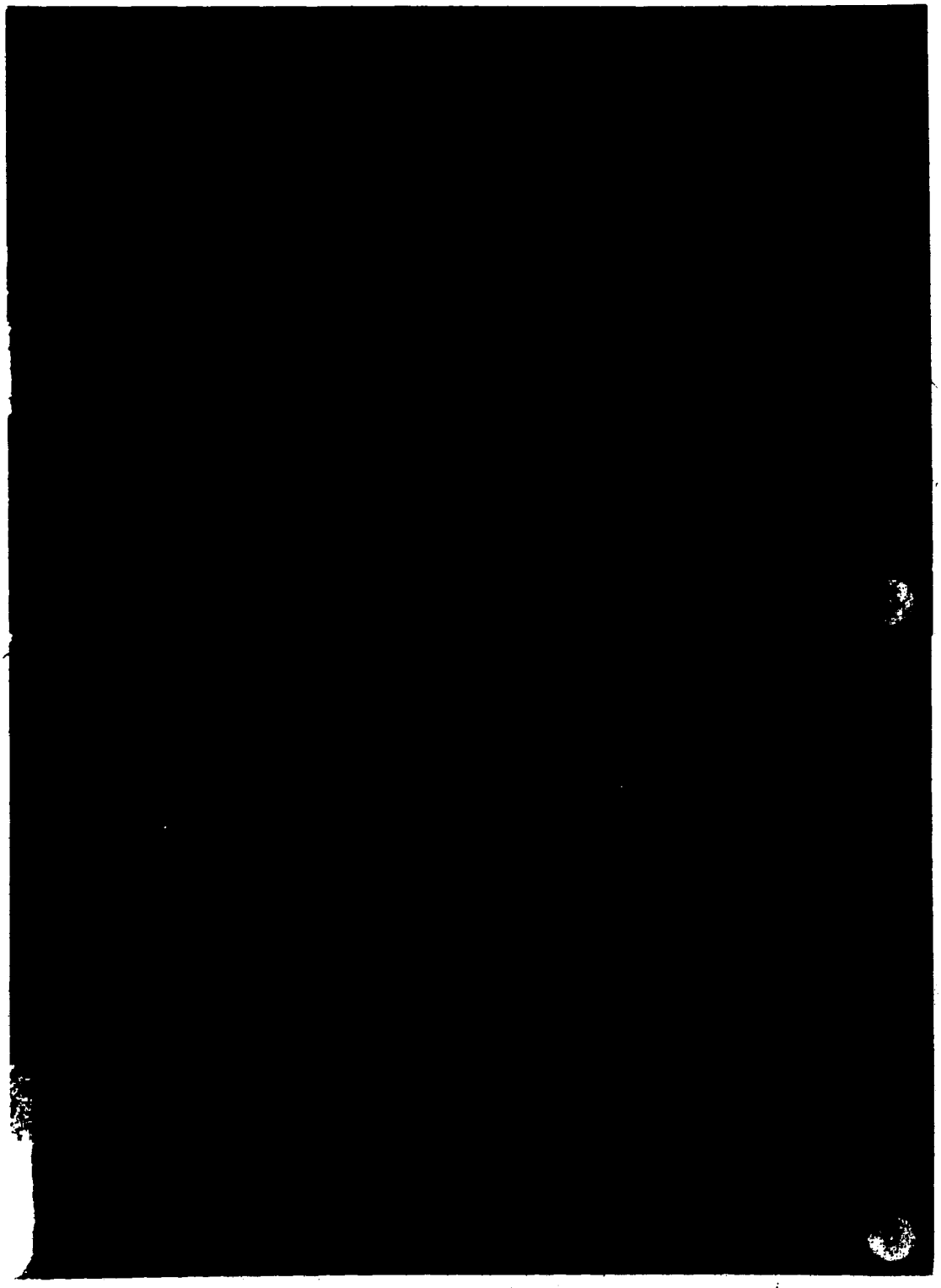


Figure 13.

Root colonization of barley by *Glomus dimorphicum*.

13C. Formation of grouped spores outside the root. x170

13D. Formation of single spores outside the root. x180



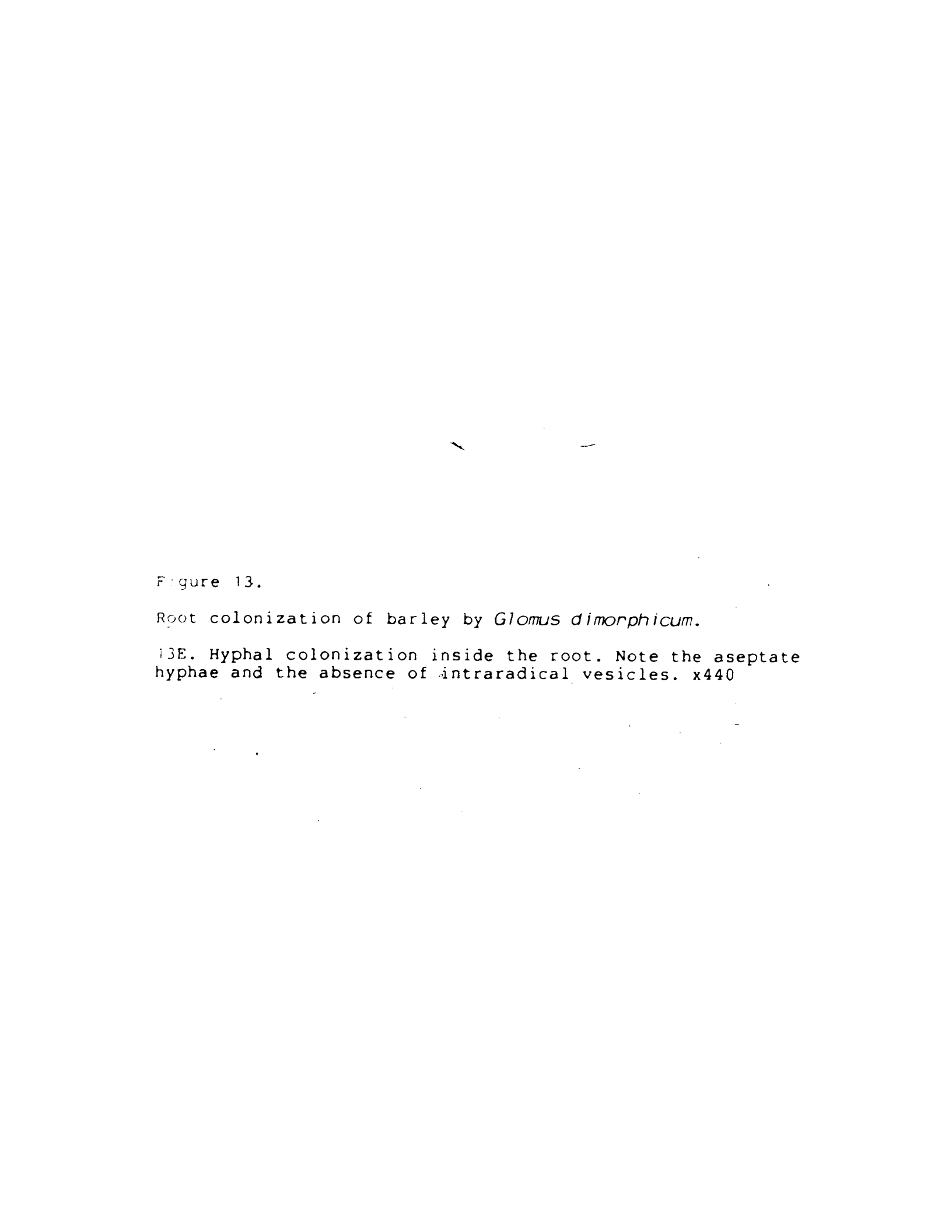


Figure 13.

Root colonization of barley by *Glomus dimorphicum*.

13E. Hyphal colonization inside the root. Note the aseptate hyphae and the absence of intraradical vesicles. x440

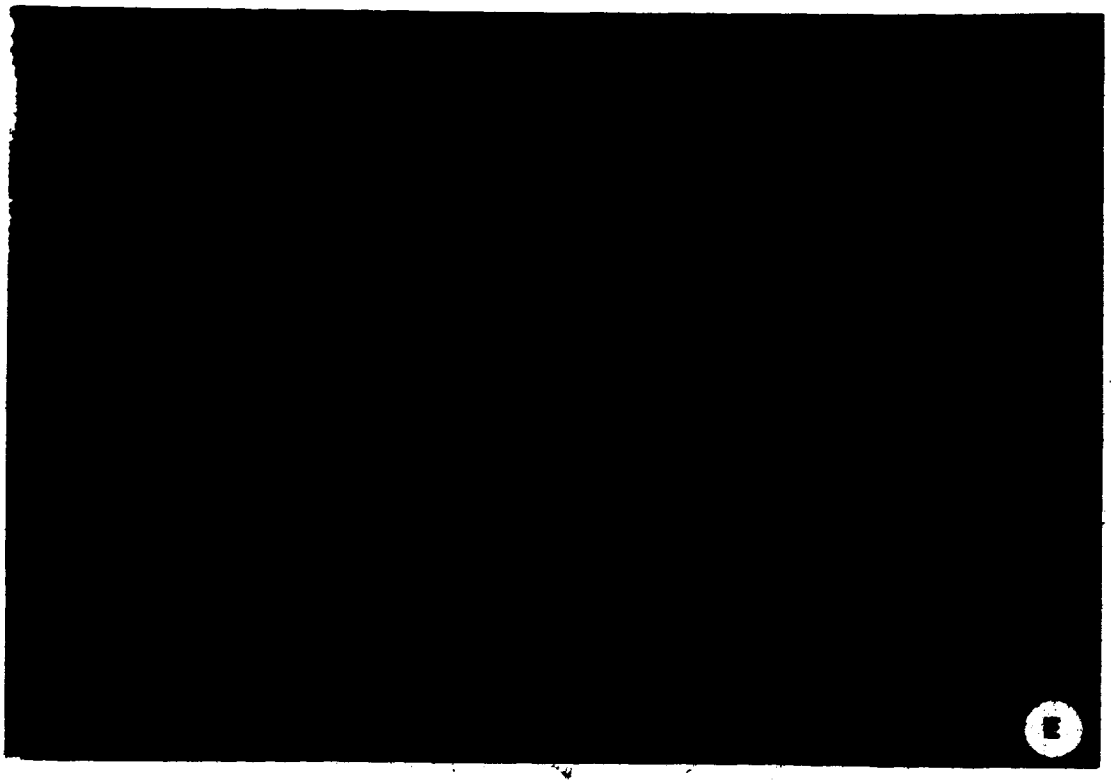


Figure 14.

Colonization of alfalfa roots by *Glomus dimorphicum*.

14A, 14B. Aseptate intercellular hyphae occurring in the intermediate layers of the root cortex. Note the almost parallel alignment of the hyphae relative to each other. Figure 14A x190; Figure 14B x350


A

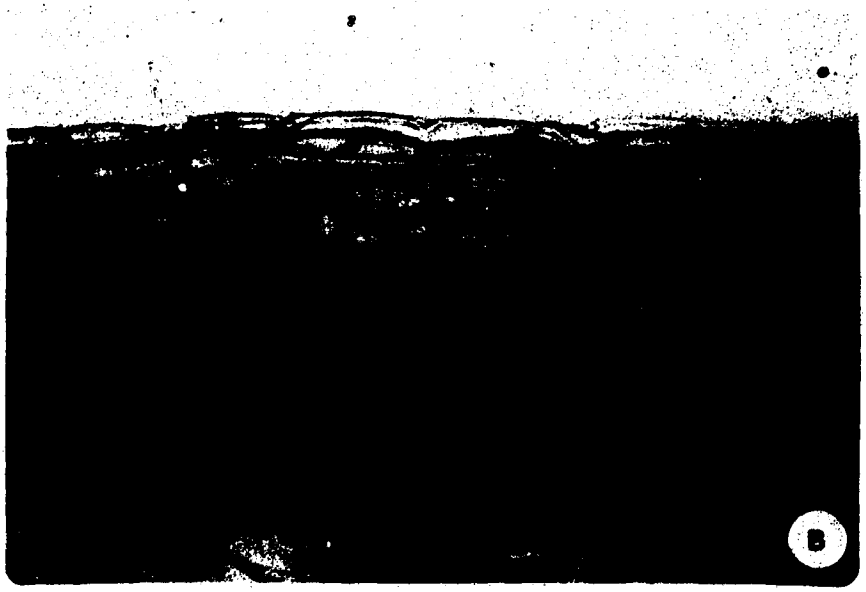
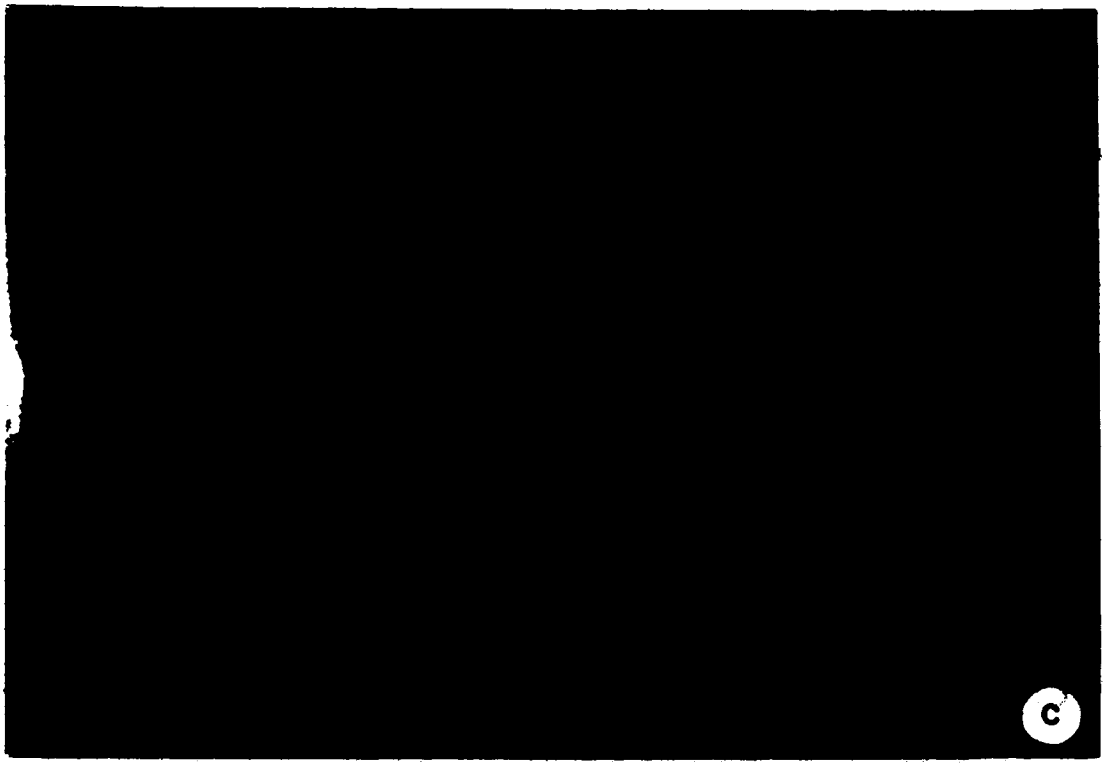

B

Figure 14.

Colonization of alfalfa roots by *Glomus dimorphicum*.

14C. Formation of extraradical spores. x440

14D. Intraradical vesicles filled with lipid droplets. x560


C


D

Figure 15.

Colonization of bean roots by *Glomus dimorphicum*.

15A. Hyphae and intraradical vesicles. x180

15B. Vesicles with arbuscules which appear to be deteriorating (clumping). x680

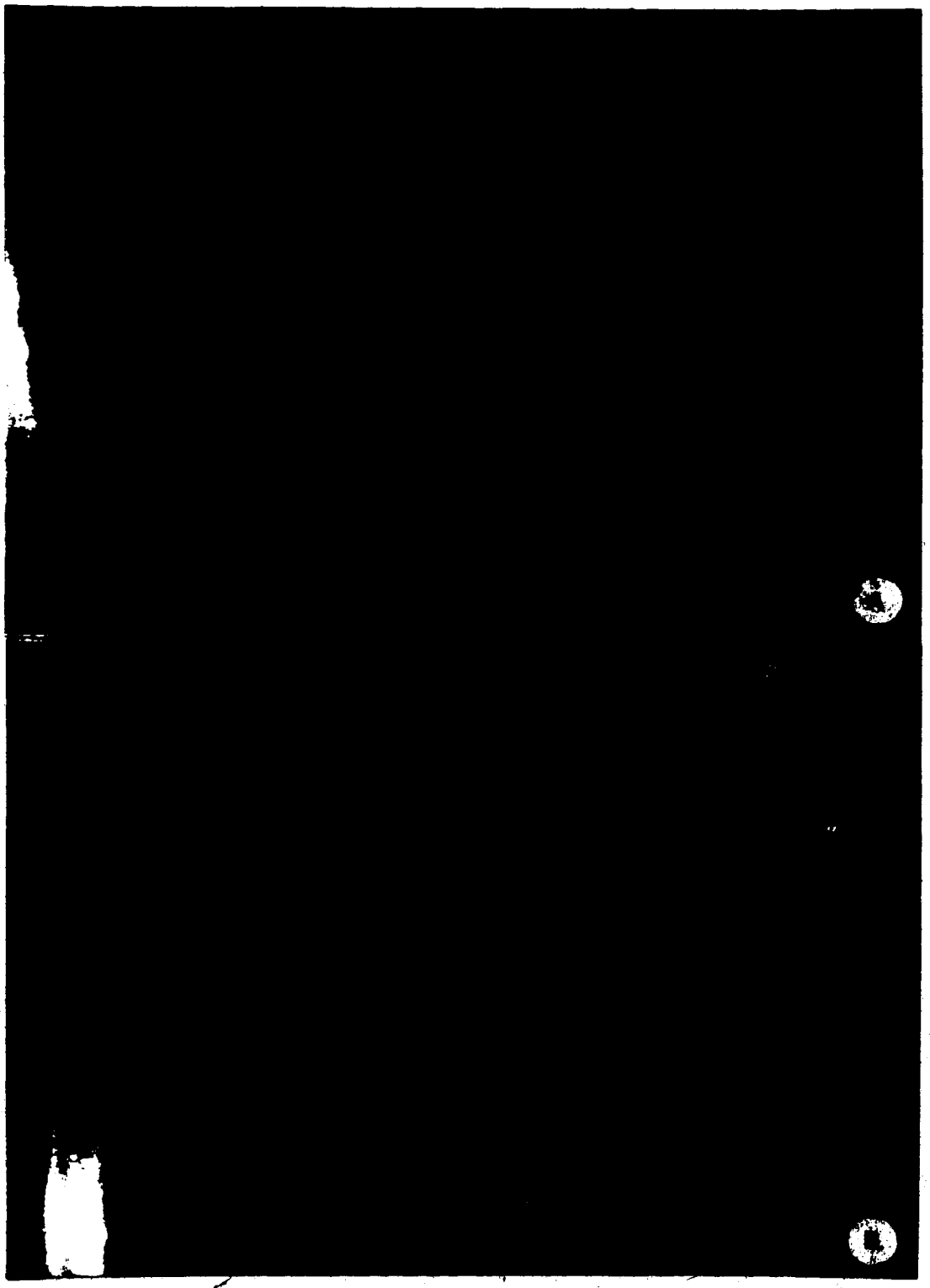


Figure 15.

Colonization of bean roots by *Glomus dimorphicum*.

15C. Intercellular hyphae with arbuscules. Note the arbuscule trunk (arrowhead). x770

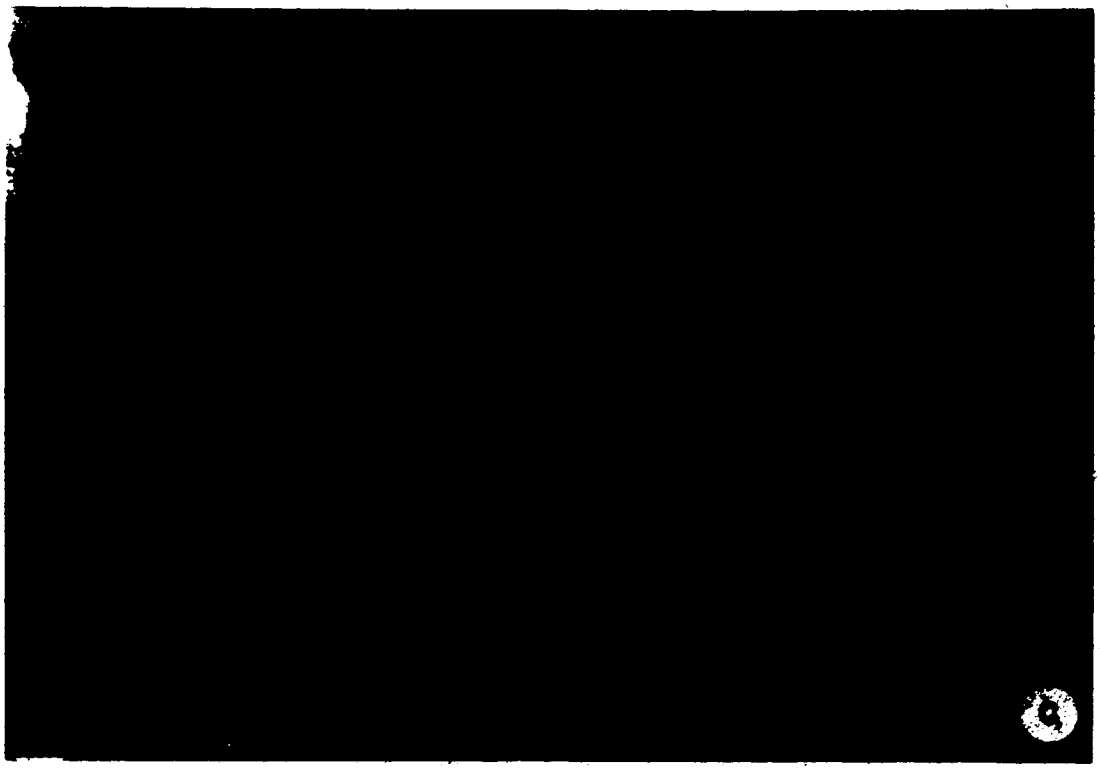
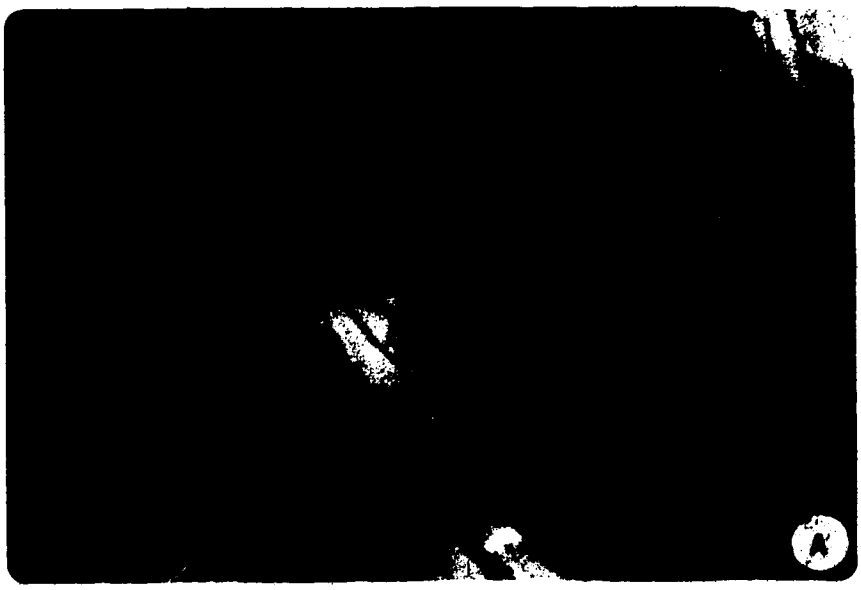


Figure 16.

Colonization of red clover roots by *Glomus dimorphicum*.

16A. Extraradical hyphae and formation of appressoria (arrowhead). x360

16B. Penetration of the epidermal cells. x190



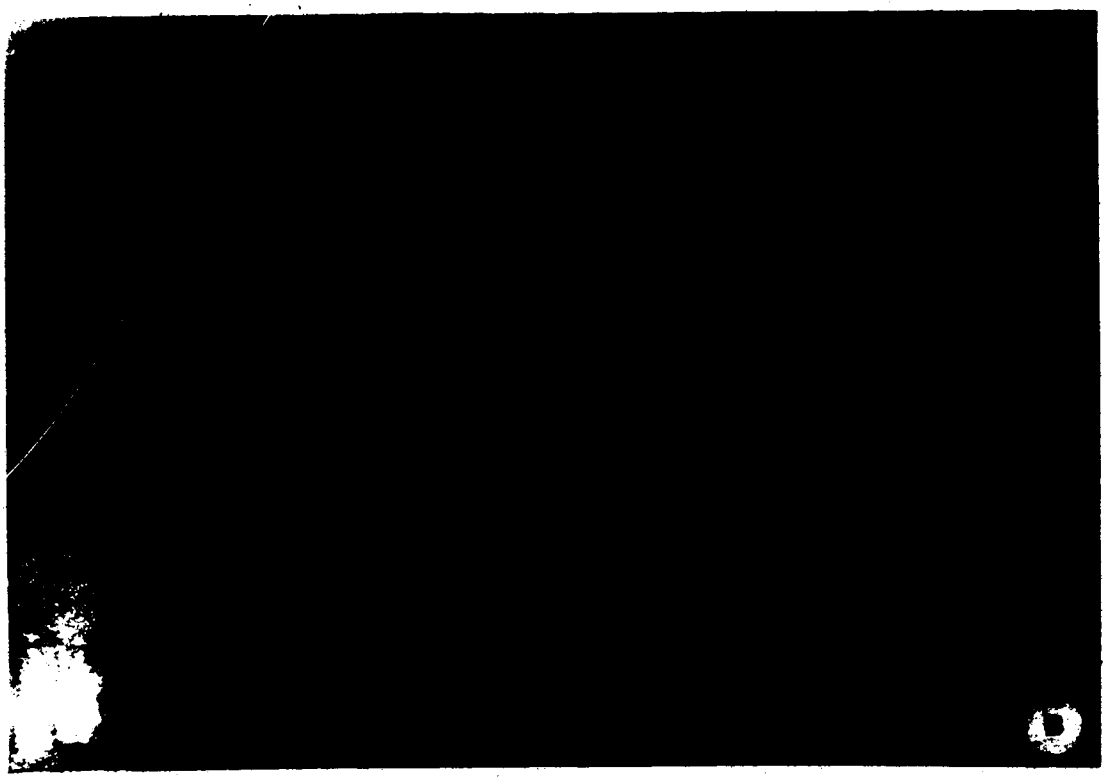


Figure 16.

Colonization of red clover roots by *Glomus dimorphicum*.

16C. Formation of extraradical spores. x70

16D. Intraradical vesicles one of which contains a lipid droplet (arrowhead). x710

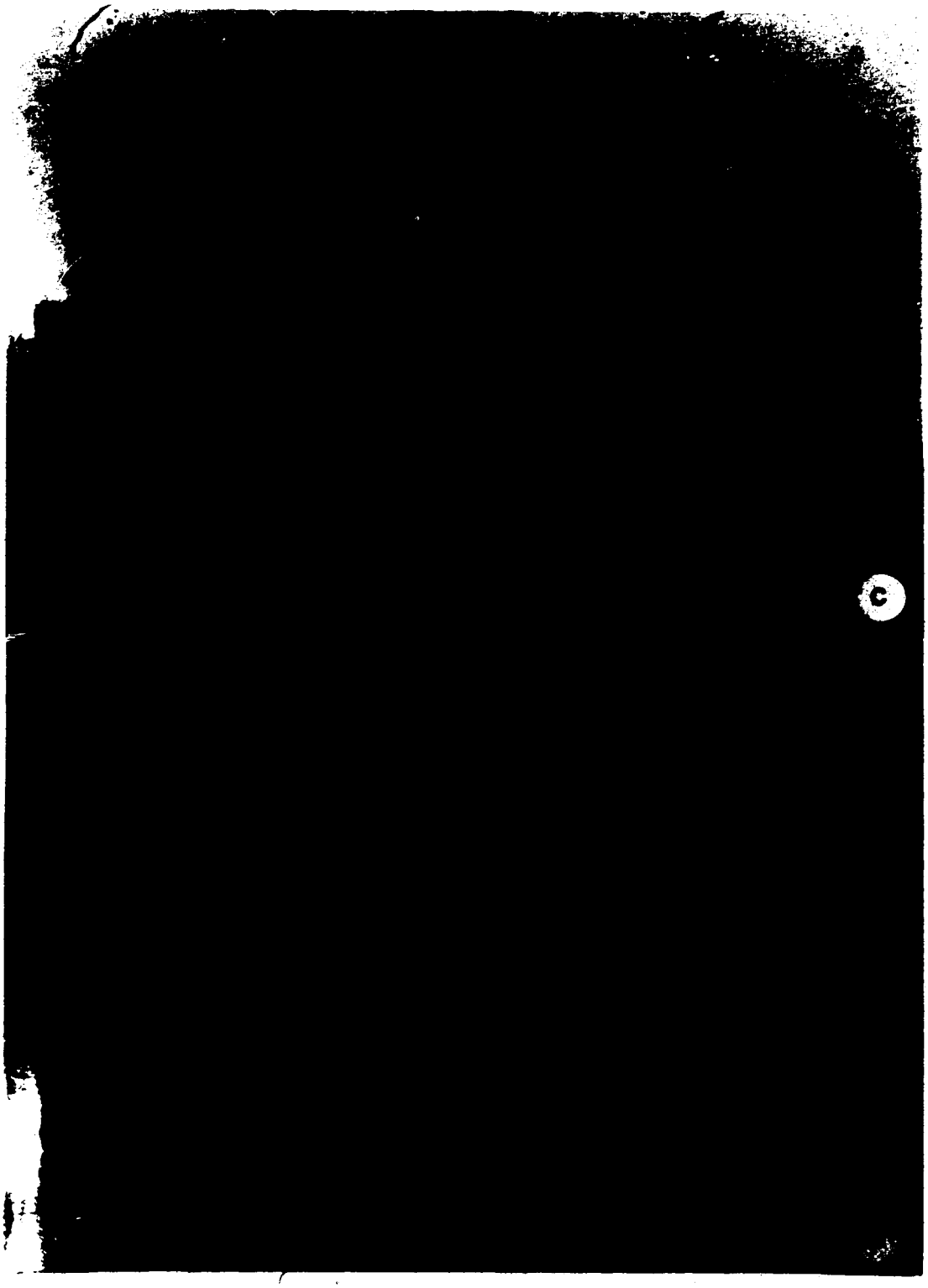


Figure 17.

Corn roots infected by *Glomus dimorphicum*.

17A. Extraradical hyphae and appressoria (arrowheads). x480

17B. Intracellular coiling hyphae (arrowheads). x630


A

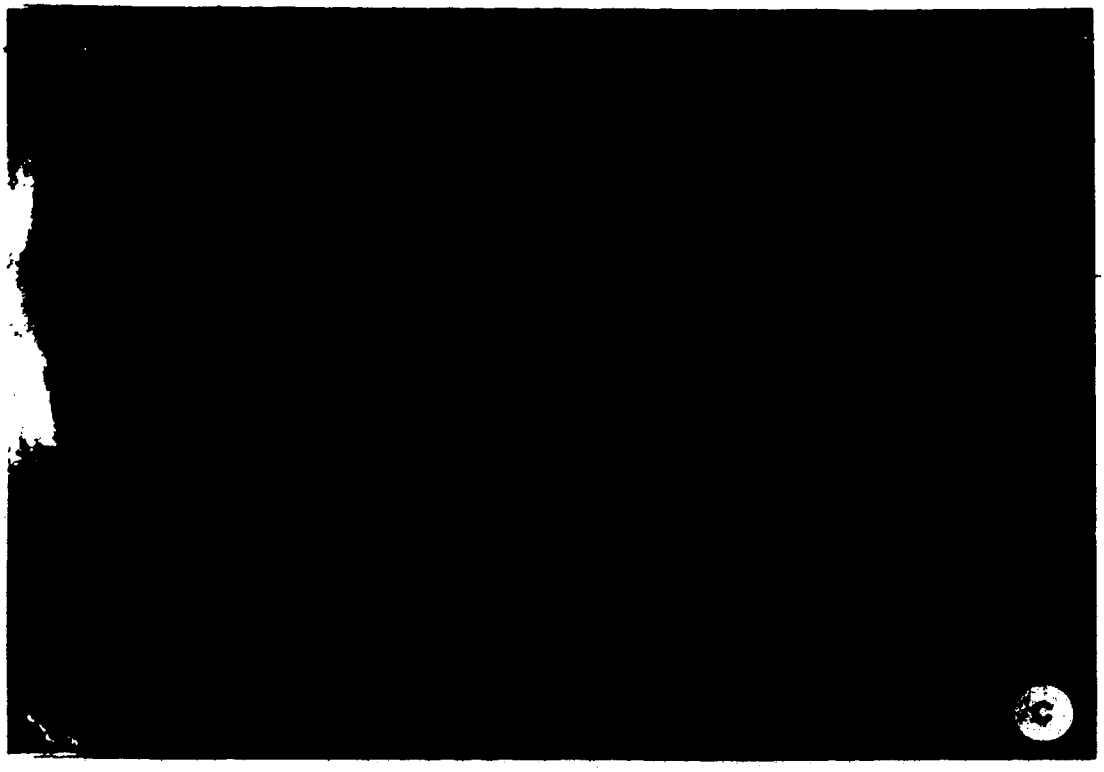

B

Figure 17.

Corn roots infected by *Glomus dimorphicum*.

17C. Intercellular hyphae and arbuscules. x490

17D. Formation of a single chlamydospore outside the root. x400


C

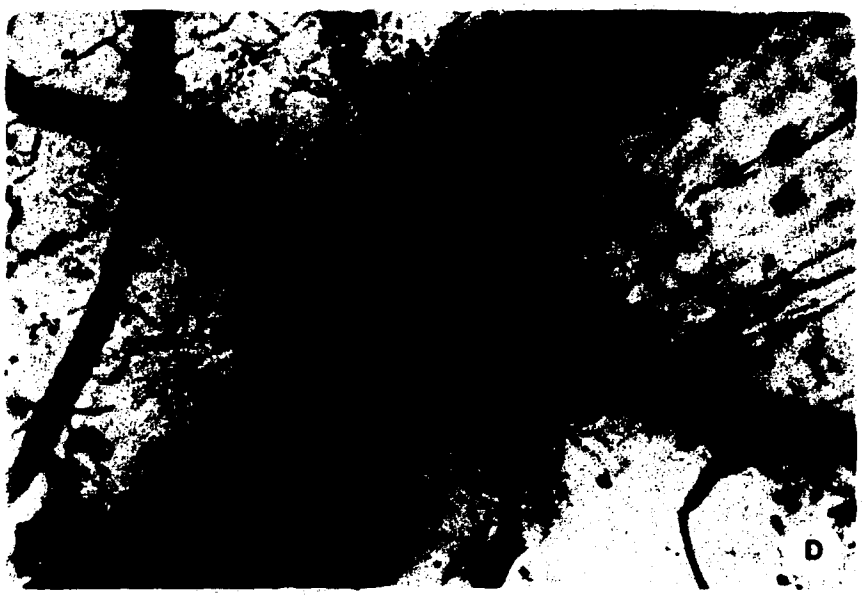

D

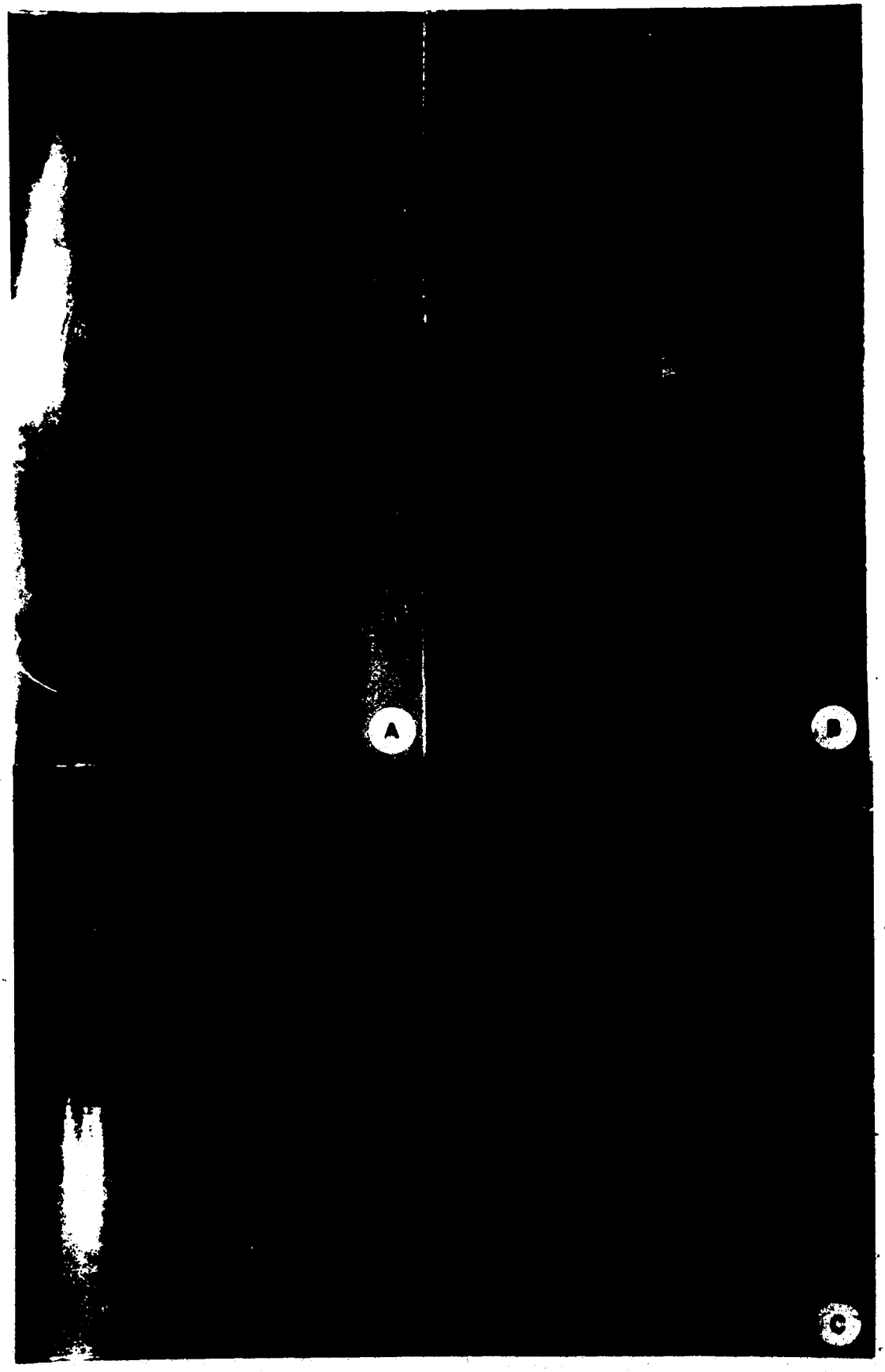
Figure 18.

Roots of wild barley (*Hordeum jubatum*) infected by unidentified indigenous VA mycorrhizal fungi.

18A. Abundant intraradical vesicles. x190

18B. Vesicle containing lipid droplet (arrowhead). x450

18C. Intercellular hyphae and arbuscules. x750


A
D
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Chapter IV

Hyperparasitism of *Glomus dimorphicum*

A. Introduction

Parasitism of fungi by other microbes (hyperparasitism) has been extensively studied and VA mycorrhizal fungi are not immune to this interaction. Studies of hyperparasitized VA mycorrhizal fungi, however, are few. The importance of hyperparasitism of mycorrhizal spores may be underrated. The germination of hyperparasitized mycorrhizal spores may be inhibited (Sylvia and Schenck, 1983) and VA mycorrhizal populations in the field may be reduced (Paulitz and Menge, 1984). The activity of such organisms may also affect experimental results involving supposedly pure VA mycorrhizal cultures and may prevent successful large scale cultures of mycorrhizal fungi (Daniels and Menge, 1980; Ross and Daniels, 1982).

The most frequently observed hyperparasites of VA mycorrhizal spores have been *Phlyctochytrium* Shroet., *Rhizidiomycopsis* Sparrow, and a pythium-like fungus (Ross and Rüttencutter, 1977; Schenck and Nicolson, 1977; Sparrow, 1977; Hetrick, 1984; Paulitz and Menge, 1984). The pythium-like fungus parasitized VA mycorrhizal fungi inside the root. The chytrideaceous fungi were observed to encyst on the fungal spore wall, germinate, and penetrate the spore (Sparrow, 1977). Daniels and Menge (1980) isolated hyphomycetous parasites (*Anguillospora pseudolongissima*

Ranzoni and *Humicola fuscoatra* Traaen) and Sylvia and Schenck (1983) observed species of *Fusarium* Link ex Fr., *Penicillium* Link ex Fr., *Trichoderma* Pers. ex Fr., and *Chaetomium* Kunze ex Fr. contaminating chlamydospores of three *Glomus* Tul. & Tul. species. Spindle cells of *Labyrinthula* Cienk. have also been isolated from the spores of *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. Trappe (Koske, 1981). Perforations of the soil-borne spores of *Cochliobolus sativus* (Ito & Kurib) Drechs. ex Dastur and *Thielaviopsis basicola* (Berk. & Br.) Ferraris by mycophagous amoebae have been documented (Anderson and Patrick, 1978) and recent studies have shown amoebae to be associated with VA mycorrhizal spores as well (Coley *et al.*, 1978).

Varying susceptibility to hyperparasites has been suggested. *Glomus macrocarpum* Gerd. & Trappe was observed to be more susceptible to hyperparasitism than *Gigaspora gigantea* (Ross and Ruttencutter, 1977) and species of VA mycorrhizal fungi producing heavily melanized spores appeared to be more resistant to hyperparasites (Daniels and Menge, 1980; Hetrick, 1984). This suggests that mature spores may be able to survive better in soil than young spores.

The objectives of this study were to document the signs and symptoms of hyperparasitism of the spores of *G. dimorphicum* Boyetchko & Tewari.

B. Materials and Methods

Chlamydospores of *G. dimorphicum* were isolated from field soil from Neerlandia, Alberta by wet-sieving and decanting (Gerdemann and Nicolson, 1963) and collected onto filter paper under suction. Spores were observed directly as follows.

Spores were examined by light microscopy by mounting on slides in lactophenol. Spores were also prepared for scanning electon microscopy (SEM) by vapor fixation with osmium tetroxide overnight, mounting on stubs and coating with gold. Some of the spores were fractured with a dissecting needle before coating with gold in order to observe the interior of the spores. The spores were observed in a Cambridge Stereoscan 150 SEM.

In order to isolate the hyperparasites of *G. dimorphicum*, the spores were surface sterilized in 0.5% sodium hypochlorite for 20 seconds, washed with sterile distilled water and placed onto agar plates containing either 2% bactoagar or hay infusion agar. The presumed hyperparasites isolated were observed by light microscopy.

C. Results and Discussion

1. Symptoms of hyperparasitism

Light microscopy revealed transverse striations in the walls of the mature spores of *G. dimorphicum* (Figures 19A, 19B), similar to those found in the walls of *Gigaspora*

candida Bhatt., Muker., Tewar., & Skoropad (Bhattacharjee *et al.*, 1982). That these structures were not part of the normal wall was ascertained by SEM. The perforations were dispersed singly or in a line on the outer surface of the cell wall and continued through the wall (Figures 20A, 20B). The perforations ranged from 0.25 to 0.50 μm in diameter (Figures 20C, 20D).

The identity of the organisms causing these perforations is uncertain. These organisms are not filamentous as no such structures were seen associated with these spores. It is also unlikely that the soil animals could pass through pores of that size. Anderson and Patrick (1978) however, observed that vampyrellid amoebae could produce perforations in the spore walls of *C. sativus* and *T. basicola* and that they were able to constrict their bodies through such minute holes. Old and Patrick (1976) had earlier suggested that such perforations could be caused by soil bacteria which agglutinate to the spore surfaces of *C. sativus* and *T. basicola* by secreting the bridging polymers and subsequently enzymatically create the perforations. Similar conclusions were reached by Old and Wong (1976). While the evidence is overwhelming that these minute perforations are caused by soil microbiota (Old and Patrick, 1976), conclusive proof of pathogenicity and data on isolation of the responsible microbiota are still lacking.

Both light microscopy and SEM revealed many spores exhibiting reaction zones in the form of papillae (Figures

21A-21F). Papillae are host cell wall thickenings developed in response to invasion by parasites and represent a dynamic host defense response to infection. The formation of papillae as a host response to infection has been documented in vascular plants and in mycoparasitic relationships (Edwards and Allen, 1970; Tsuneda *et al.*, 1976; Akutsu *et al.*, 1980). The present study indicated that the spores of *G. dimorphicum* were hyperparasitized while still being viable.

2. Signs of hyperparasitism

Most spores of *G. dimorphicum* that showed symptoms of hyperparasitism in the form of perforations, papillae, etc., had no signs of the hyperparasites that could be identified definitely. In some cases, irregular aggregates of material were observed by SEM inside the spores (Figure 19B) but it could not be decided if these were masses of spore cytoplasm or perhaps amoebae. In some cases, light microscopy revealed spherical, thick-walled colorless structures containing irregular bodies inside some of the mycorrhizal spores. Hay-infusion agar plates, inoculated with surface sterilized spores of *G. dimorphicum*, produced cultures of amoeba-like organisms that encysted to produce structures similar to those observed above (Figure 22C). It is therefore believed that the spherical bodies inside the spores of *G. dimorphicum* are cysts of amoeba-like organisms. More studies are needed to further characterize this amoeboid

hyperparasite.

Re-inoculation of *G. dimorphicum* spores with the hyperparasites in order to satisfy Koch's postulates was not attempted. VA mycorrhizal fungi are obligate symbionts which, to date, have not been successfully grown in pure culture. The study of hyperparasites of other fungi are usually conducted in dual cultures. However, VA mycorrhizal fungi must be grown in association with a host, normally in pot cultures containing sterilized soil. VA mycorrhizal spores sometimes germinate in agar culture but the germ tubes soon die off after a limited growth. For this reason, it has so far not been possible to use the traditional Koch postulates approach in the case of the VA mycorrhizal fungi.

3. Age of spores vs. hyperparasitism

Much of the literature describing hyperparasitism of VA mycorrhizal fungi does not give evidence whether the spores were viable or dead when attacked (Ross and Ruttencutter, 1977; Schenck and Nicolson, 1977; Sparrow, 1977; Daniels and Menge, 1980). *Spizellomyces punctatum* (Koch) Barr (=*Phlyctochytrium punctatum* Koch), a fungus isolated from many VA mycorrhizal spores and believed to be a hyperparasite (Ross and Ruttencutter, 1077; Daniels and Menge, 1980; Paulitz and Menge, 1984) was shown to parasitize non-viable *Gigaspora margarita* Becker & Hall azygospores (Paulitz and Menge, 1984). The evidence showed that *S. punctatum* is primarily a saprophyte but the

possibility that the fungus may also be a parasite was not ruled out.

Daniels and Menge (1980) and Hetrick (1984) suggested that highly melanized mycorrhizal spores are resistant to hyperparasitism and that light colored spores of mycorrhizal species are more susceptible. From the present study, it appeared that the mature, dark-colored spores are hyperparasitized more than the younger spores. However, exactly when the spores became hyperparasitized is uncertain. Also, young single spores of *G. dimorphicum* containing the outer hyaline wall layer characteristic of this species (see Chapter 2) have not revealed extensive hyperparasitism, thus far. The outer wall layer could very well serve as a means of protecting the spore from hyperparasitism before it eventually sloughs off.

4. Colonization by the same species

Examination of some spores of *G. dimorphicum* revealed colonization by other mycorrhizal spores. Single and grouped spores of a *Glomus* sp. were seen occupying the single spores of *G. dimorphicum* (Figures 23A-23C). It is highly probable that the parasitizing spores were those of *G. dimorphicum*, as this species was the only *Glomus* sp. isolated from the field soil from which the parasitized spores were collected. This phenomenon was also observed in the spores collected from the pure pot cultures of *G. dimorphicum*. Koske (1984) also observed that some dead VA mycorrhizal spores were

occupied by other VA mycorrhizal spores, the dead spores providing a favorable habitat for spore formation. *Gigaspora* spores have also been found in the seeds of *Portulaca* and three other weed species (Taber, 1982). The seeds act as spore receptacles, a niche in the soil favorable for spore development. There is no evidence, however, that viable VA mycorrhizal spores are parasitized by other VA mycorrhizal fungi. Koske (1984) suggested that if mycorrhizal species are able to parasitize each other, problems may arise when introducing VA mycorrhizal fungi into soil containing indigenous populations of VA mycorrhizal spores.

D. Conclusions

Spores of *G. dimorphicum* revealed symptoms and signs of hyperparasitism. The identity of the hyperparasite(s) has remained elusive for two reasons. Firstly, in most cases, only the symptoms and not the signs of hyperparasitism were observed. Secondly, the VA mycorrhizal fungi cannot be grown in pure culture and therefore proof of pathogenesis could not be obtained. SEM and light microscopy studies indicated that the hyperparasites are non-filamentous organisms and at least to a certain extent parasitize viable spores. That Canadian prairie soils are rich in mycophagous amoebae, is indicated through the work of Duczek (1983).

The production of VA mycorrhizal inoculum relies solely on pot cultures in which spores and/or root pieces colonized by mycorrhizal fungi are raised. It is therefore important

to maintain pure cultures of mycorrhizal fungi. Spores of VA mycorrhizal fungi often harbour hyperparasites and the introduction of these contaminants into supposedly pure cultures may severely affect the production of mycorrhizal inoculum and the physiology of the mycorrhizal fungi. As these hyperparasites are often difficult to detect, their presence may yield erratic experimental results (Ross and Ruttencutter, 1977; Daniels and Menge, 1980). It should, therefore, be one of the goals of researchers to improve ways of raising pure cultures of VA mycorrhizal fungi over long periods of time until methods of growing these fungi in the absence of a host can be developed.

E. Figures and Legends

Figure 19.

Glomus dimorphicum spores showing symptoms of hyperparasitism in the form of transverse wall striations and papillae.

19A. Light microphotograph showing transverse striations (arrowhead) and papillae (arrows) in the spore wall. x600

19B. SEM photograph illustrating the perforations (arrowhead) transversing the spore wall. Note the absence of any filamentous structures on the inside of the cell wall. Also note, in this location, the presence of irregular masses of material which could be parts of the cytoplasmic material or amoebae of the presumed hyperparasite. x8000

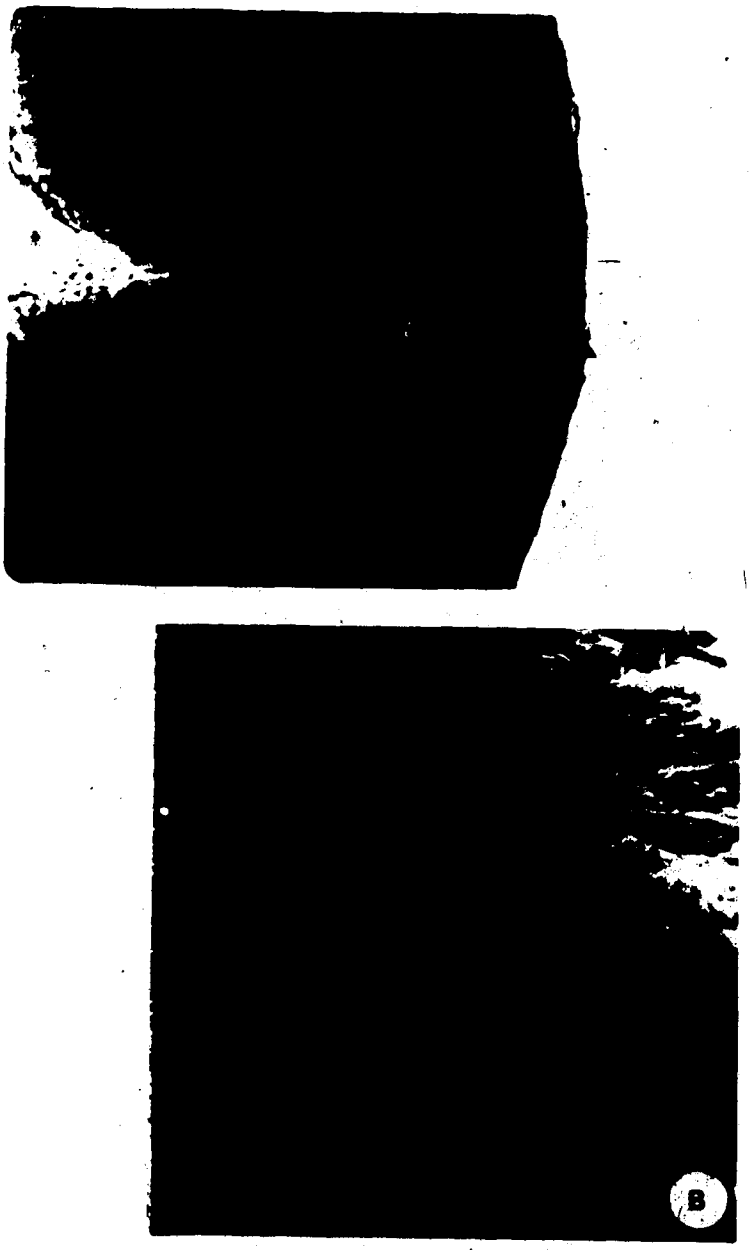
A
B

Figure 20.

Glomus dimorphicum spores showing symptoms of hyperparasitism in the form of perforations.

20A. Perforations on the outer spore wall surface. x6800

20B. The alignment of the perforations in a straight line. x8810

20C. Various sizes of perforations on the outer surface of the spore wall of *Glomus dimorphicum*. x7700

20D. Enlarged view of a perforation observed from the inside of the spore. x19,000

A
C
B
D

Figure 21.

Glomus dimorphicum spores showing symptoms of hyperparasitism in the form of papillae.

21A, 21B. Light microphotographs illustrating the formation of papillae (arrowheads). Figure 21A x620; Figure 21B x580

21C. Light microphotograph showing papillae (arrowheads). x580

21D. SEM photograph showing perforation through spore wall (arrow) and formation of the papilla (arrowhead). x7750


A
C
D

Figure 21.

Glomus dimorphicum spores showing symptoms of hyperparasitism in the form of papillae.

21E, 21F. SEM photographs showing formation of many papillae viewed from the inside of a spore. Note that there are some perforations through the papilla (arrows) (Figure 21F) indicating that the hyperparasite has gained entry into the spore. Figure 21E x470; Figure 21F x2275

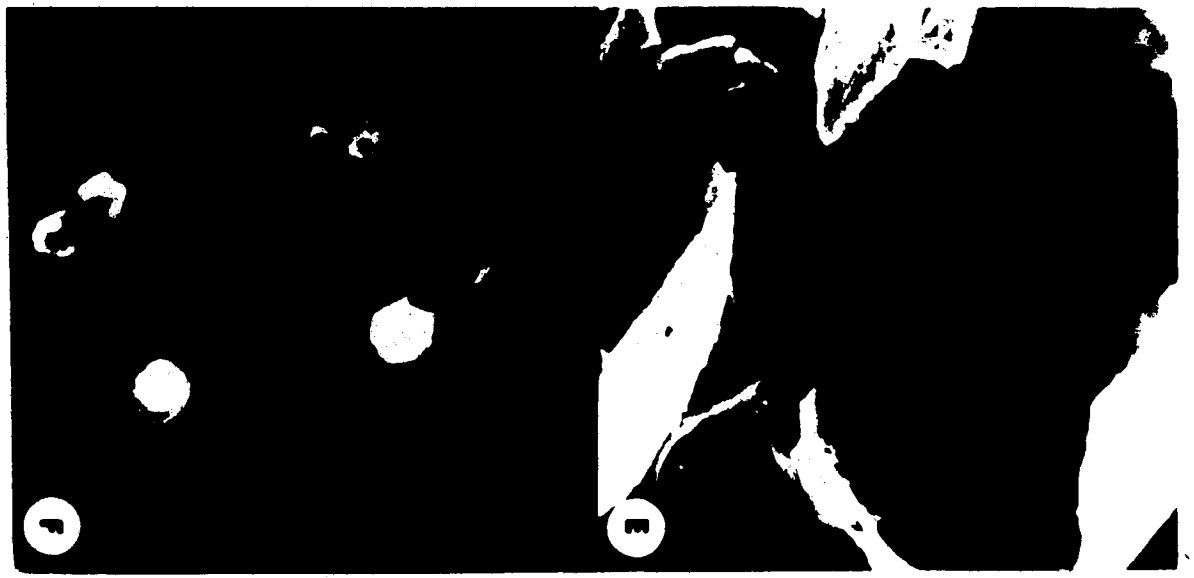
a
b

Figure 22.

Glomus dimorphicum spores showing signs of hyperparasitism.

22A, 22B. Light microphotographs showing thick-walled cysts inside the spore. Also note the transverse wall striations. Figure 22A x110; Figure 22B x600

22C. Cysts of amoeba-like organisms from a culture grown on hay-infusion agar from a spore and stained with lactophenol cotton-blue. Cells of a yeast-like contaminant are dispersed around the cysts. x700

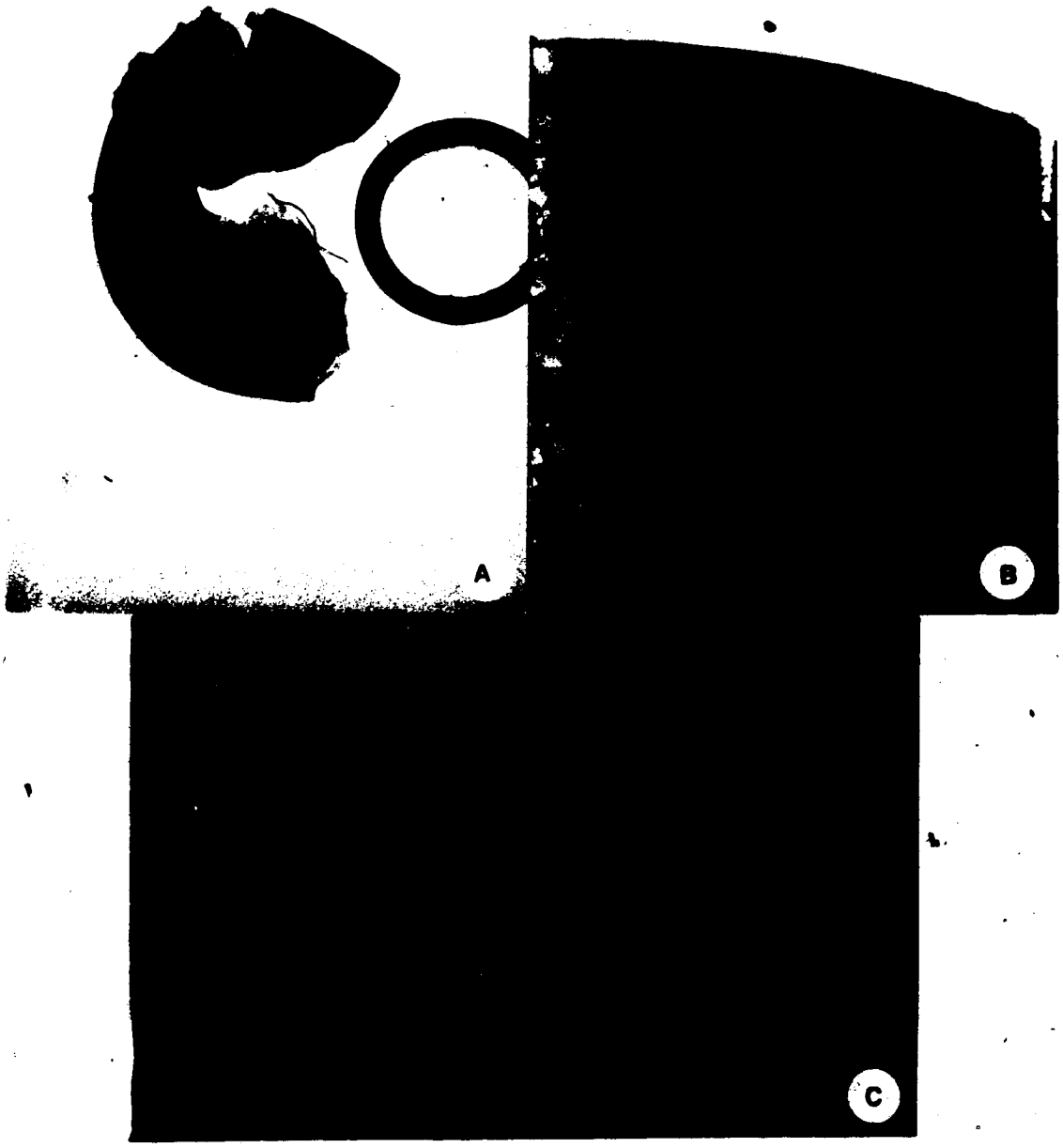
A
B
C

Figure 23.

Self colonization of *Glomus dimorphicum*

23A. A single spore of *G. dimorphicum* present inside a single spore of the same species. The spore formed is still young as evidenced by the presence of wall 1 (arrowhead, see Chapter II for details of this wall). x380.

23B, 23C. Grouped spores of *G. dimorphicum* parasitizing the single spore of the same species. Figure 23B x150; Figure 23C x560


A
B
C

Chapter V

General Discussion and Conclusions

A. Taxonomy

The taxonomy of the VA mycorrhizal fungi is based primarily on the morphological features observed by light microscopy after the spores have been isolated from field soil. The study of the new species, *Glomus dimorphicum* Boyetchko & Tewari, was facilitated through the extensive use of pot cultures. Initially, two spore types were isolated and the preliminary conclusion was that two species had been collected from the field. The pot culture studies, however, proved that the two spore types belonged to one VA mycorrhizal species having a dimorphic nature. Concurrent studies by Smith and Schenck (1985) also recognized two new dimorphic *Glomus* species: *G. ambisporum* Smith & Schenck and *G. heterosporum* Smith & Schenck. These are the first reported cases of spore dimorphism in VA mycorrhizal fungi and have warranted the emendment of the genus *Glomus* to include the characteristic of dimorphism as a taxonomic feature. Also, the extensive pot culture studies in the present investigation and in those conducted by Smith and Schenck (1985) have stressed the need for caution when describing species of VA mycorrhizal fungi directly after isolating the spores from the field soil, a practice commonly followed by many mycorrhizologists. It is also indicated that the previously described species of VA

mycorrhizal fungi, which were described without pure culture multiplication, should be reviewed to determine if they are also dimorphic.

The use of scanning electron microscopy (SEM) was a useful tool for studying the surface morphology of the *G. dimorphicum* spores. This technique provided a three-dimensional view and the high resolving power allowed the investigation of fine detail of the spore surface. Energy dispersive X-ray microanalysis was also a useful technique for studying the chemical nature of the spore wall and future work utilizing this technique may facilitate the study of elemental composition of the spore wall of other VA mycorrhizal species as well.

B. Root Colonization

VA mycorrhizal fungi have a wide host range but the extent and pattern of root colonization differs among hosts. A recent study (Hetrick *et al.*, 1985), along with the present study, showed that infection patterns of *G. epigaeum* and *G. dimorphicum* were influenced by the host. In the past, the patterns were believed to be VA mycorrhizae species specific and it was thought that infection patterns could be used as taxonomic features for fungus species diagnoses (Abbott and Robson, 1978; Abbott, 1982). The current study indicates that such conclusions may be premature as the behavior of a particular VA mycorrhizal species may vary with different hosts.

The field and greenhouse studies showed that there was no significant difference among the four cultivars studied except at the W240 site in 1983. Other barley cultivars should be compared because other research has shown higher levels of VA mycorrhizal infection in barley (Jensen and Jakobsen, 1980; Jensen, 1982). It is possible that Canadian barley cultivars are resistant to VA mycorrhizal colonization. Further studies are also needed to determine whether growth benefits to the barley cultivars are accrued even though the level of root colonization is low.

Although the barley cultivars showed low levels of infection when inoculated with *G. dimorphicum*, the presence of high numbers of spores in the field soil at Neerlandia, Alberta indicated that this species is well adapted to the agricultural practices and to barley which has been grown in this field under monoculture for 15 consecutive years.

Since VA mycorrhizal fungi cannot be grown in axenic culture, the production of intraradical vesicles (chlamydospores) provides a suitable means of raising inoculum for commercial and experimental purposes. *G. dimorphicum* produces appreciable numbers of these structures in certain hosts. It is, therefore, evident that the choice of the host for a particular VA mycorrhizal species is important in the production of root-borne inoculum.

There are many papers reporting the effects of various soil factors such as fertility on the VA mycorrhizal fungi. However, many such reports do not indicate which mycorrhizal

species was used or if a mixture of species was used in the experiment. Since the behavior of VA mycorrhizal fungi is influenced by the host, as is indicated by this study, one cannot be certain, then, that all VA mycorrhizal fungi behave in the same manner under all conditions. Therefore, researchers should identify the VA mycorrhizal fungus utilized in their experiments.

C. Hyperparasitism

The presence of hyperparasites of VA mycorrhizal fungi is often overlooked. As the obligate symbiotic mycorrhizal fungi have not been successfully raised in pure culture, spores and root pieces are used as inoculum sources. Therefore, absence of hyperparasitism is very important, particularly for experimental work and production of inoculum. Unfortunately, little work has been done on the hyperparasites of VA mycorrhizal fungi and proofs of pathogenicity are lacking in all cases. Signs and symptoms of hyperparasitism in *G. dimorphicum* circumstantially indicate the involvement of non-filamentous parasites, perhaps soil amoebae. At least some of the spores are hyperparasitized while still being viable, as papillae formation was observed. Contrary to earlier reports on other species, older melanized spores of *G. dimorphicum* appeared to be more susceptible to infection than the younger hyaline spores.

D. Projections for Future Studies

Very little work on the VA mycorrhizal fungi has been conducted in western Canada. Before meaningful research can be initiated in this area, it is necessary to characterize the indigenous populations of the VA mycorrhizal species and strains which are adapted to the climatic and soil conditions of the area instead of relying on species obtained from elsewhere that may be adapted to other ecological niches. The indigenous species thus collected may then be used to examine their ability to increase nutrient uptake, plant growth and yield.

Most of the work with VA mycorrhizal fungi has dealt with the natural ecosystem, with very little emphasis on agricultural crops and how the mycorrhizae behave in the agricultural system. Agricultural practices such as the application of fertilizer, herbicides and extensive cultivation practices resulting in site disturbance will influence which species of VA mycorrhizal fungus will be prevalent in the soil. By studying the species of mycorrhizae well-adapted to such practices and by examining their ability to improve plant growth and yield, they may be found useful for improving the production of crops such as barley, wheat, clover, alfalfa and some others.

The ability of the VA mycorrhizal fungi to retrieve fixed or immobile nutrients, such as phosphorus, offers a solution to the rising energy and fertilizer costs evident in the agricultural industry. It is certainly possible that

the VA mycorrhizal fungi may be used as "biological fertilizers" and that their usefulness will in future be comparable to that of *Rhizobium*. However, further extensive field trials are necessary, as mostly greenhouse experiments have been conducted on the VA mycorrhizal fungi.

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