# State of Mycorrhizae in Some Ornamental Gymnospermous Tree Species of Pakistan

**S.H. Iqbal, Shahbaz and Ghazala Nasim,** *Department of Botany, University of Punjab, Quaid-e-Azam Campus, Lahore - 20*

**Abstract**

Multiple mycorrhizal infections were found in association with root systems scale leaves, decaying micro sporophylls and epidermal tissues of *Abies pindrow, Auracaria cunninghamii, Cycas circinalis, C. revoluta, Cupresses torulosa, Ephedra gerardiana, E. regeliana, Juniperus communis, Pinus helepensis, P. roxburghii, Podocarpus chinensis, Taxodium mucronatum, Thuja orientalis* and *Zamia floridana*. Endomycorrhizae were prevalent in species of most families including Pinaceae which normally forms the ectomycorrhiza. Fungal hyphae colonized the young roots and formed coils, arbuscules and vesicles inside the cortical cells. Coiled and linear hyphae were very common, digestion stages were observed. Extramatrical mycelium was also found. Some endophytes other than VA, with septate, hyaline or coloured mycelium were found penetrating into the root. A mixture of endogonaceous spores formed the rhizosphere mycoflora.

**Introduction**

The occurrence of mycorrhiza is best known in Gymnosperms (Mohan, 1939; Chaudhri & Akhtar, 1931 ; Anvery & Khan, 1959). Recent studies indicate that presence of vesicular arbuscular (VA) mycorrhiza is a common condition in all Gymnosperms except Pinaceae which form ectomycorrhiza (Anvery & Khan, 1959; Khan 1970; Harley & Smith, 1983. The importance of endomycorrhizae to growth of plants has received considerable attention. Endomycorrhizae are more cogently understood as three major groups: vesicular arbuscular (VA) by far the most widely distributed form of mycorrhizae; ericacious and orchidacious. Other variants occur but are not well knwon (Harley, 1969; Lewis, 1973). Vesicular arbuscular fungi increased the efficiency of nutrient capture particularly phosphorus and this inturn can lead to enhanced growth of plants (Nicolson, 1967: Gerdemann, 1968: Harely, 1969; Mosse, 1973). Moreover, Read (1977) reported that ericoid mycorrhiza enhanced the efficiency of nutrient absorption particularly nitrogen, though an increase in phosphate absorption also occurred (Stribley & Read, 1975). Several types of mycorrhiza are known to occur within the same plant species (Cooper, 1976: Harley, 1969: Iqbal et al., 1980). Largely this may be due to the favourable conditions prevailing in the rhizosphere for the development of more than one type of mycorrhiza.

The present paper presents some additional information on the mycotrophy by VA endophytes in some Gymnosperms of Pakistan grown in plains as ornamentals, with an emphasis on the ecological conditions conducive for the development of mycorrhiza.

**Materials and Methods**

Roots and scale leaves of Gymnosperms were collected from plants grown in Botanical Garden of Quaid-e-Azam Campus of Punjab University and Jinnah Gardens, Lahore, with the rhizosphere soil. Samples of *Ephedra gerardiana* were collected from Saiful Malook and *E. regeliana* from Quetta. The samples were thoroughly washed in water till the soil particles were removed. They were fixed in F.A.A. and stained in 0.05% trypan blue in lactophenol following the procedure of Phillips and Hayman (1970). The stained pieces were mounted in lactic acid and examined under the microscope for mycorrhizal infections. Endogonaceous spores were extracted from the rhizosphere by wet sieving and decanting technique of Gerdemann and Nicolson (1963)

**Results**

**I. Vesicular Arbuscular Mycorrhizal Infections:**

Vesicular arbuscular (VA) mycorrhizal infections were studied in roots, scale leaves and epidermal tissues of *Auracaria cunninghamii, Cycus revoluta, C. circinalis, Cupresses torulosa, Juniperus communis, Ephedra gerardiana, E. regeliana, Taxodium mucronatum, Pinus helepensis, P. roxburghii, Podocarpus chinensis, Abies pindrow and Zamia floridiana.* These plants showed fungal hyphae within the cortical tissues. The hyphae ramified irregularly, at time appeared as filaments and sometime formed aggregated strands. Many entry points were also found on the root. The mycelium was dimorphic. The thickness ranged 2 to 20 um. Vesicles were abundant in all samples. They differ in size and shape. Small rounded (plate Ia), large globose, ovalish (plate Ib) and oblong vesicles were seen (Plate Ic). The size ranged from 28 to 30 um in roots and 70-85 um in scales. Vesicles were both intercallary and terminal (Plate I). Intramatrical vesicles occurred in scale leaves and coralloid roots of *Z. floridana, C. revoluta* and *C. circinalis* (Plate IIa,b,c) Septate and beaded mycelium was also present. Arbuscules occured in very low frequency. However, the *P. roxburghii* arbuscules occupied the entire cavity of the cortical cells. They stained lightly (Plate IId). Decaying microsporophylls and sheathing leaves of *Z. floridana, C. revoluta* and *C. circinalis* were also infected with VA mycorrhizal fungi (Plate IIIa, b). The extramatrical hyphae colonized dead and senescing roots of *A. cunninghamii, T. mucronatum* and *P. chinensis* (Plate IVa). Roots of *P. chinensis* have been found to bear nodules. Most of these nodules were branched and pear shaped (Plate IIIc,d). The nodules were also infected with VA endophytes. Vesicular aggregates were observed (Plate IVb). Meagre VA infections occurred in *E. regeliana* (Plate IVc). Almost uniform vesicular infections were present in *E. gerardiana* (Plate IVd). Septate, coloured mycelium other than that of VA endophytes occurred in the epidermal/cortical cells of various organs (Plate Va).

**II. Ericoid Mycorrhizal Infections:**

The young roots of *A. cunninghamii, Z. floridana* and *T. mucornatum* possessed ericoid mycorrhiza in addition to VA mycorrhiza. The young feeding roots were typically heavily infected. Epidermal and cortical cells were penetrated by septate mycelium which formed hyphal coils within the cells (Plate Vb, c). Degenerating hyphal coils were also seen.

**III. Endogonaceous Spores:**

A variety of spores were recovered from the rhizosphere of each plant. They belonged to *Glomus, Sclerocystis, Gigaspora* and *Acaulospora*. The population of spores in the rhizosphere varied with the plant species.

Five species of Glomus viz. *G. fasiculatum, G. mosseae, G. geosporium, G. microcarpon* and *G. albidus* were found. *Sclerocystis clavispora, Gigaspora decipicus* and *Acaulospora bireticulata* were also recovered from the rhizosphere.

**Discussion**

Invasion of roots of vascular plants by mycelium of VA fungi is a regular phenomenon (Mosse, 1981). Several reports suggest that VA mycorrhiza increases nutrient uptake benefitting the plants (Nicolson, 1968; Gerdemann, 1969; Harley & Smith, 1983 Mosse, 1973). Some observations have been made on mycorrhizal status of Gymnosperms in Pakistan (Khan, 1970). Bakhshi and his co-workers (1974) contributed valuable data on mycorrhizal status of Gymnosperms of India. Previous reports indicate that all Gymnosperms except Pinaceae possessd VA mycorrhiza (Khan, 1970). Two exceptions to this generality are reported. Occurrence of VA mycorrhiza in Pinaceae, and an Ericoid mycorrhiza in *A. cunninghamii, T. macronatum* and *Z. floridana.*

The present study reveals that normally ectomycorrhiza was almost present in all gymnospermous flora of temperate forests, where the relative humidity is not a limiting factor. Ectomycorrhizal fungi flourish and fruit well. The fungal hyphae extending into the soil serve as an extension of root systems that are more effective for nutrient absorption. Water stress influences the ectomycorrhizal association in conifers (Mexal, 1973), whereas Gymnosperms grown in plains possess VA mycorrhiza which is independent of the moisture content of the soil. In plains occurrence of VA mycorrhizal infections are a result of nutrient stress caused by intense competition between individual plant communities (Read, 1976). Though sporadic ectomycorrhizae occurred but of very low intensities or they were only frequently encountered in range land habitats (Trappe, 1977). Incidence of mycorrhizal colonization and availability of spores varies with season and soil moisture availability (Stafteldt & Vogot, 1975), habitat (Miller, 1979) and composition of plant community (Hirrel & Gerdemann, 1980). Consequently VA mycorrrhiza performs the same function in such conditions (i.e. enhances the growth by helping in uptake of water and nutrients) thus it can be concluded that VA mycorrhizal fungi maintain themselves both in time and space by infecting almost any plant that grows sufficiently close to the propagule. The occurrence of mycorrhiza was significantly influenced by topographic variations, is indicated by heavier ectomycorrhizal infections in plants growing at higher altitudes, whereas higher intensities of VA infections occurred at lower altitude. This is in line with the findings of Read *et al.* (1976) Read, (1980). Therefore, the type of mycorrhiza and its abundance depends on certian ecological factors. These results are in line with Cooper's (1976) idea of complete and partial displacement of endomycorrhiza. Therefore, this seasonal occurrence of ectomycorrhizal and endomycorrhizal associations is governed by wet and dry spell.

Moreover, the occurence of multiple mycorrhizal infections showed that different mycorrhizal fungi under different conditions produce histologically different mycorrhizae on the same host. The presence of ericaceous, coiled and septate mycelium showed that ericoid mycorrhizae do occur in some species of Gymnosperms but that they are rare.

Nodular infection in *P. chinensis* by VA endophytes opens up a new Vistas of synergistic interactions in gymnospermous plants. *Podocarpus* show a dual mutualistic relationships with VA fungi and bacteria like organells (Azcon & Barea, 1975; Daft & El-Giahmi, 1974, 1975, 1976; Abbot & Robson, 1977; Manjunath et al., 1984;. Endophytic infections by VA fungi caused greater growth of the nodules (Plate III c,d).

Scarcity of arbuscule does not attribute the plant as non-mycorrhizal one (Koske *et al.,* 1985). But the presence of high level of vesicular infection is a clue to higher degree of mycotrophy.

TABLE: VESICULAR ARBUSCULAR MYCORRHIZAL AND OTHER INFECTIONS FOUND IN THE ROOTS OF SOME GYMNOSPERMS OF PAKISTAN

| | Type (Morphological features) | Figure | Infected parts | Host species |
|---|---|---|---|---|
| 1 | Aseptate hyphae producing small rounded thin walled vesicles. | Ia | Coral roots, scale leaves, epidermal tissue micro-sporophylls. | *Z. floridiana* |
| 2 | Aseptate hyphae with globose, ovalish vesicles. | Ib | Coral roots | *C. circinalis*<br>*C. revoluta* |
| 3 | Aseptate hyphae with elongated vesicles. | Ic, IVc, IVd | Roots | *T. mucronotum*<br>*E. regeliana*<br>*E. gerardiana* |
| 4 | Aseptate hyphae bearing intercallary and terminal, spherical vesicles. | Id | Roots, Scales | *P. chinensis*<br>*Z. floridiana*<br>*T. mucronotum* |
| 5 | Rounded thin walled, intramatrical vesicles. | IIa, IIb, IIc | Scales, Coral roots. | *C. circinalis*<br>*C. revoluta*<br>*Z. floridiana* |
| 6 | Arbuscules. | IId | Roots | *P. helepensis* |
| 7 | Aseptate, dark coloured, external hyphae. | IIIa, IIIb | Sheathing leaves & Microsporophylls | *Z. floridiana*<br>*C. circinalis* |
| 8 | Characteristic, branched and pear shaped nodules. | IIIc, IIId | Roots | *P. chinensis* |
| 9 | Aseptate hyphae producing thin walled spherical vesicles. | IVa | Decaying roots & sheathing leaves | *A. cunninghamii* |
| 10 | Vesicular aggregates. | IVb | Roots, Nodules | *P. chinensis* |
| *11* | Endophytes other than VA with septate hyphae | Va | Roots | *J. communis* |
| *12* | An ericoid septate hyphae forming coils inside cortical cells. | Vb, Vc | Roots | *Z. floridiana*<br>*A. cunninghamii.* |

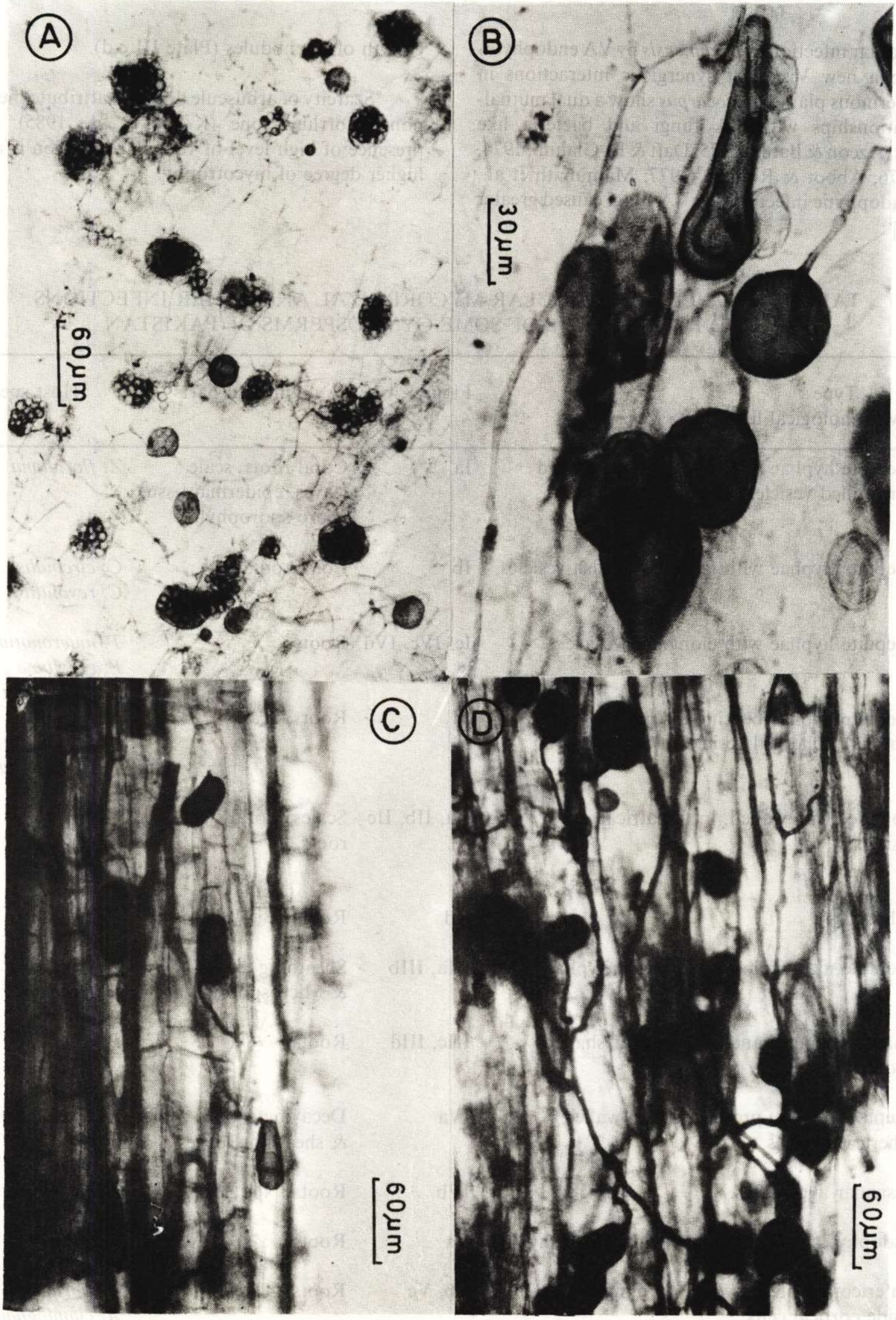
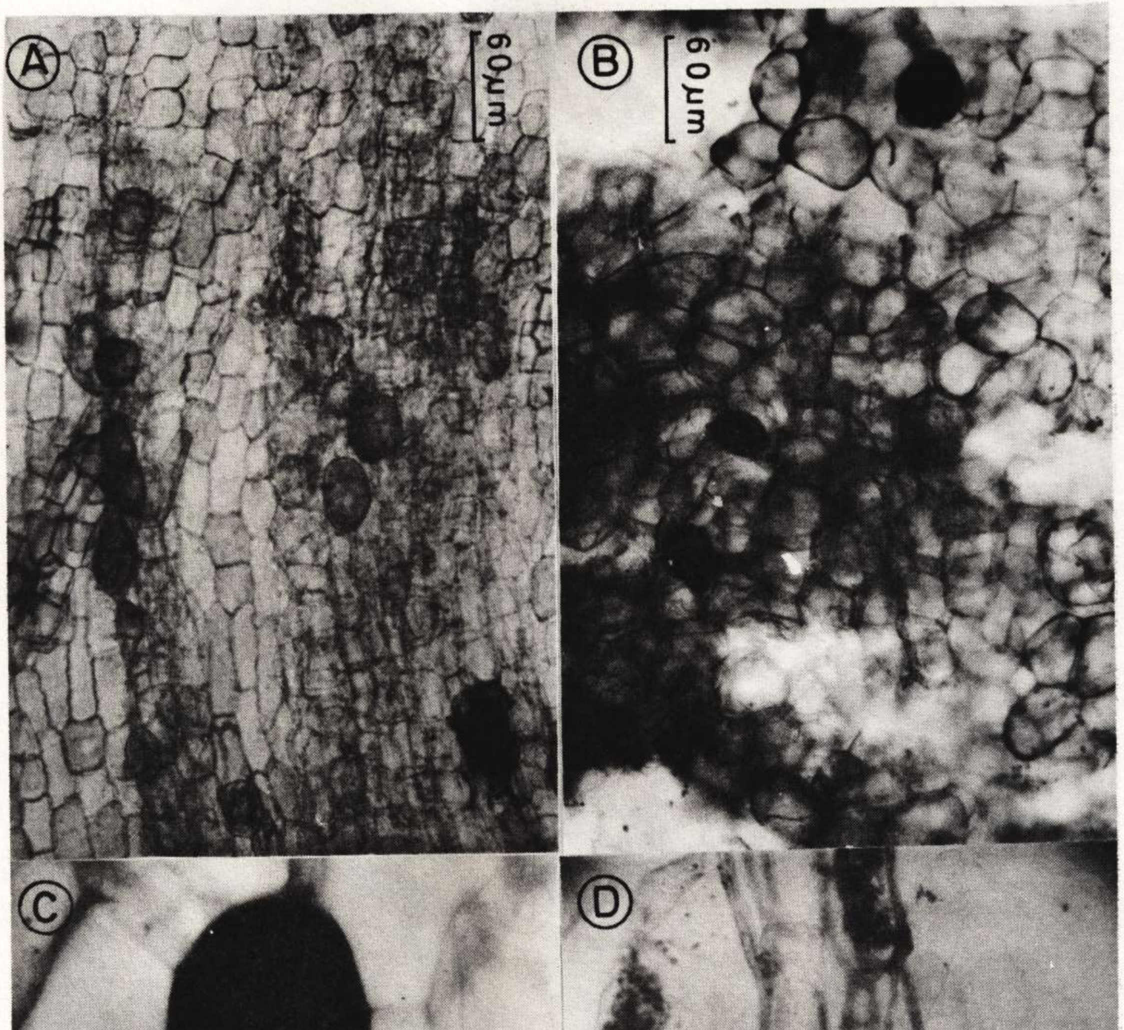



Plate I:

a: Small rounded vesicles in roots of *Z. floridiana*.
b: Vesicular infections in roots of *C. circinalis*.
c: Vesicular infections in roots of *T. mucoranatum*.
d: Heavier vesicular infections in *P. chinensis*.

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|---|---|---|
| Plate II: | a: | Arbuscular infections in *P. roxburghii* |
| | | Intramatrical vesicles in: |
| | b: | Scales of *Z. floridiana*. |
| | c: | Roots of *C. circinalis*. |
| | d: | Vesicles enlarged. |

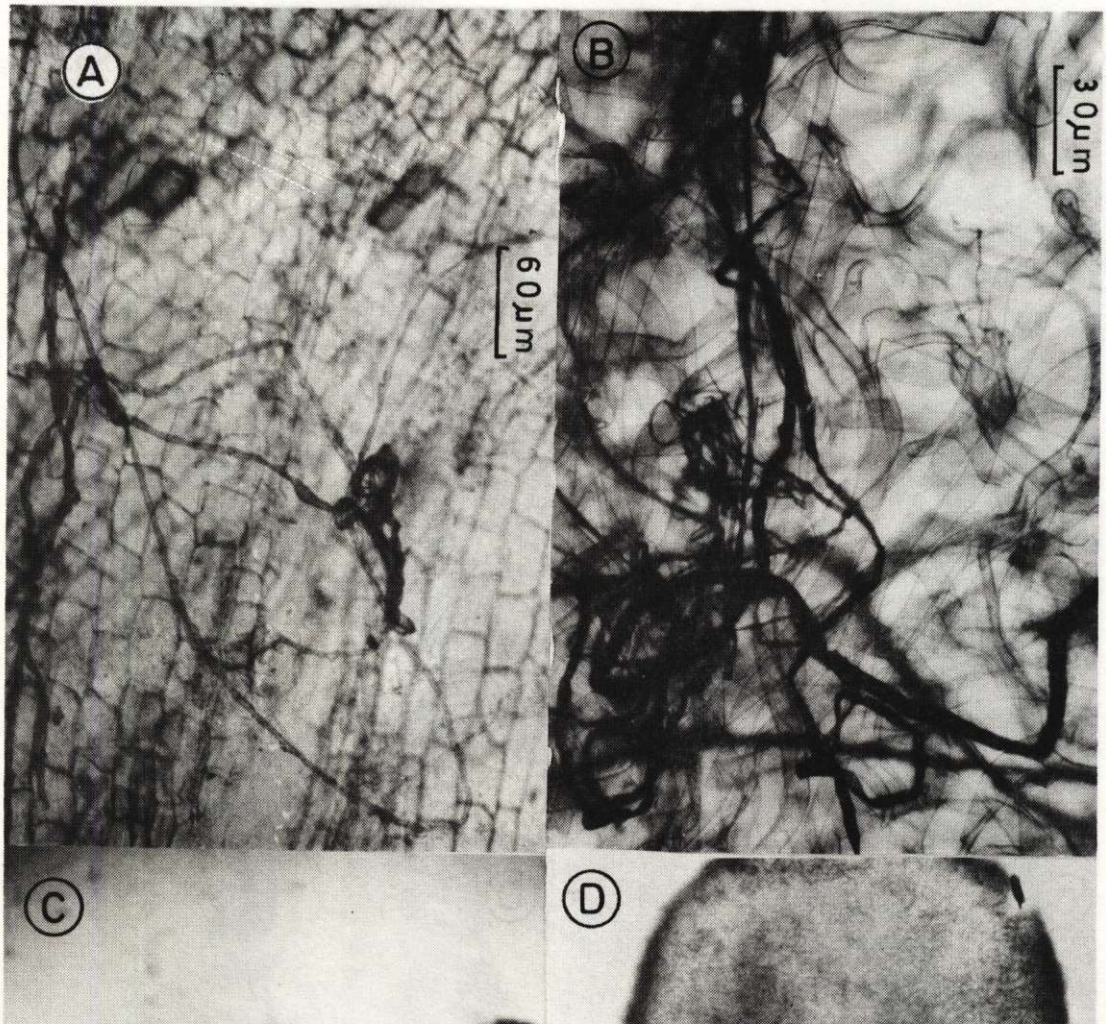
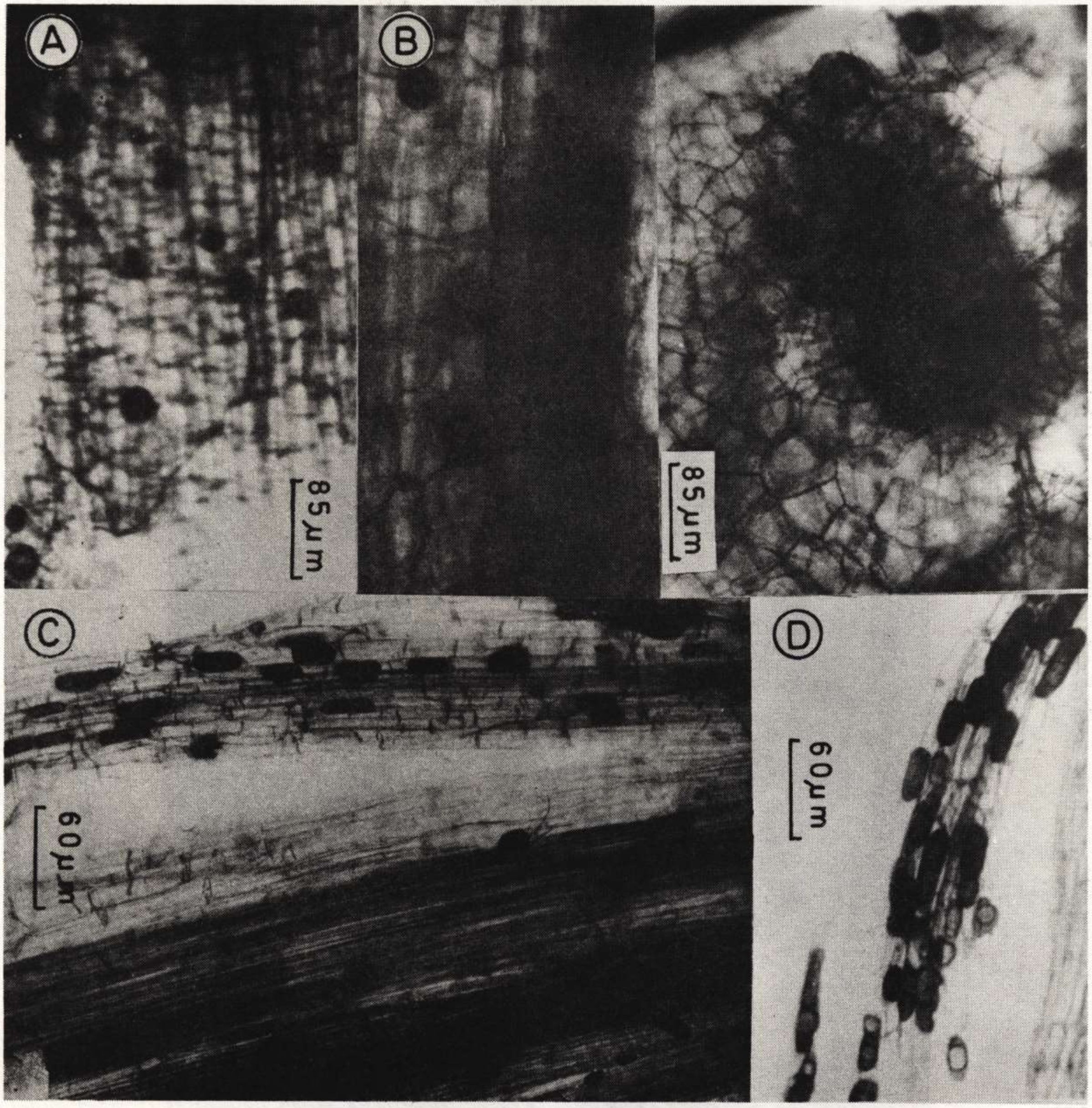



Plate III:

- a: Extramatrical hyphae colonized sheathing leaves of *Z. floridiana*.
- b: Extramatrical hyphae colonized decaying microsporophylls of *C. circinalis*.
- c: Branched, pear shaped nodules roots of *P. chinensis*.

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| Plate IV: | a: | Vesicular infections in dead roots of *A. cunninghamii.* |
| | b: | Vesicular aggregate in nodules of *P. chinensis.* |
| | c: | *Vesicular infections in E. regeliana.* |
| | d: | Vesicular infections in *E. gerardiana.* |

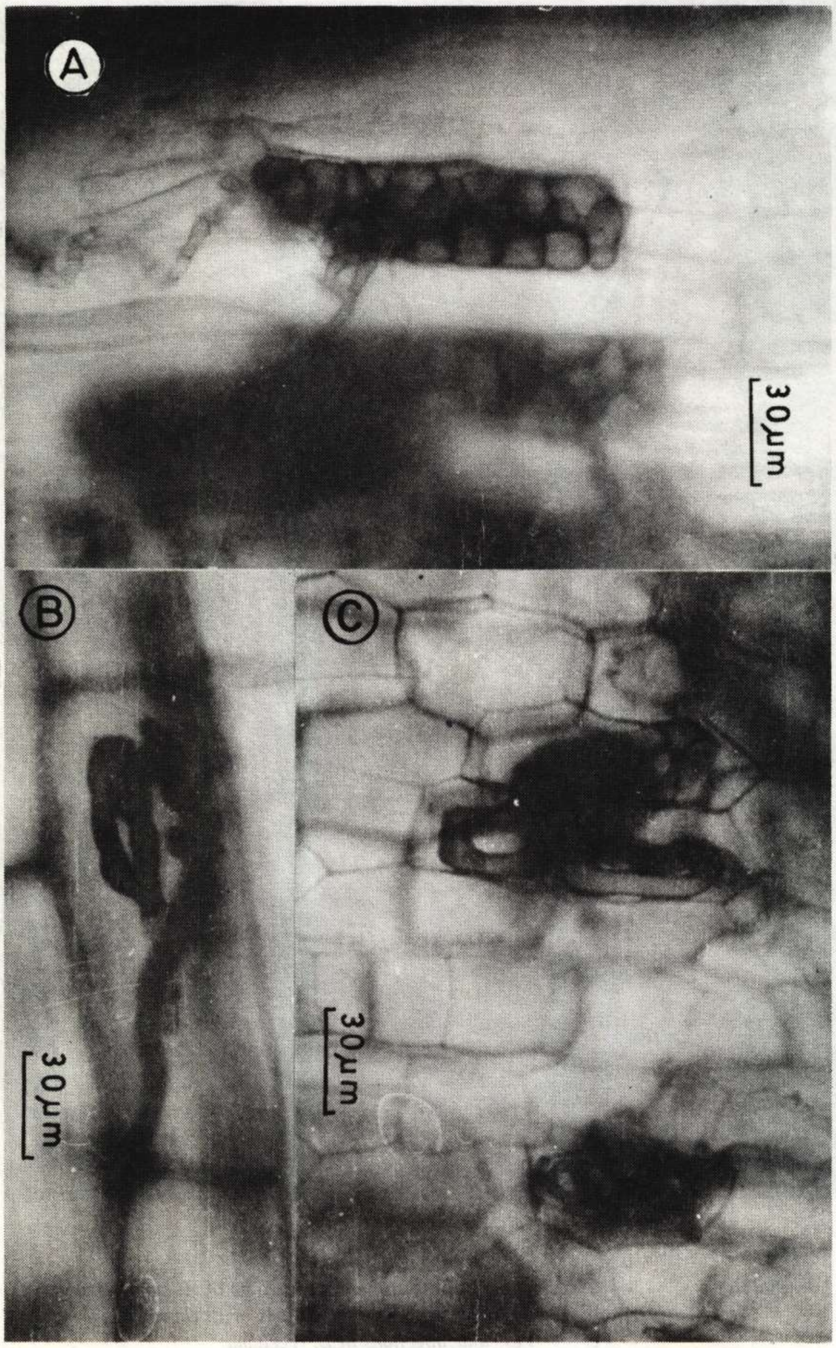


Plate V: a: Infections other than VAM in *J. communis*.
b, c: Coils of septate mycelium in cortical cells.

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