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Mycotrophy of *Annona cherimola* and the morphology of its mycorrhizae

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Abstract. The mycotrophic character of *Annona cherimola* (Magnoliales), a tropical/subtropical plantation crop of interest, is described for the first time. This crop seems to depend on mycorrhizae (arbuscular) for optimal growth, with *Glomus deserticola* being the most effective endophyte tested. Study of the morphology of the arbuscular mycorrhizae in *Annona* roots showed exclusively intracellular hyphal development, with cell-to-cell fungal passage and an abundance of arbuscules and coiled hyphae within cells. Intercellular distributive hyphae were not observed. The morphology and the pattern of spread of the mycorrhizal colonization were similar for the different endophytes involved and appeared to be dependent on the host root. Such features of mycorrhizal colonization are characteristic of host species lacking intercellular air channels and have been described for some species of ecological interest, but they are not commonly noted in the mycorrhizal literature, especially that dealing with crop species. Some ecophysiological consequences of this pattern of colonization are discussed.

Key words: *Annona cherimola* – Mycotrophy – Arbuscular mycorrhizae – *Glomus* species – Morphology

Introduction

Annona cherimola Mill., cherimoya or custard apple, is a tropical plantation crop of interest in fruit culture which has been adapted to subtropical areas and is successfully cropped in the subtropical belt of Europe in Southern Spain (Morton 1987). Research programmes to improve its productivity include clonal selection using micropropagation techniques. Since mycorrhizal inoculation is recognized as a key factor in the survival and development of the plantlets produced in vitro (Gianinazzi et al. 1990; Vidal et al. 1992), the determination of the mycotrophic characteristics of this species is of interest. As far as we know, there is no published information on the mycorrhizal status of *Annona*. The relatively unbranched, thick and generally coarse roots of this species [typical of the Magnoliales, including the Annonaceae (Gaussen et al. 1982)] suggest a mycotrophic habit, probably involving arbuscular mycorrhizae (AM), which is the most common form in plantation crops (Barea et al. 1993).

A preliminary mycorrhizal observation of the rhizosphere of field-grown plants demonstrated the ability of this species to form AM. Study of the morphology of AM fungal development within the root cortex shows the presence of abundant intracellular structures (coils and arbuscules) and a lack of intercellular hyphae. This AM morphology has been noted for other species (Brundrett anbd Kendrick 1990b; Bonfante-Fasolo and Perotto 1992) and Gallaud (1905) corresponds to the *Paris* series, whereas the "intercellular" pattern of spread is followed by the *Arum* series. *Paris* series AM associations have been described for hosts of ecological interest but not for crop plants (Brundrett 1991); however, recent observations suggest this intracellular pattern of AM colonization in potato (McArthur and Knowles 1992; V. Gianinazzi-Pearson, personal communication).

To corroborate the mycotrophic habit of *Annona* plants under controlled conditions, and to determine the degree of dependence of the crop on mycorrhizae for optimal growth, information essential for the management of AM in this crop, a greenhouse experiment was developed with the following objectives: (i) to establish controlled AM associations in *A. cherimola* with different AM endophytes; (ii) to examine in detail the morphology of AM colonization caused by these endophytes in the roots of this crop species; (iii) to select the mycosymbiont most effective in improving plant growth and development for further studies.

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Material and methods

The mycotrophic habit of *A. cherimola* was estimated in a greenhouse experiment. Seeds of *A. cherimola* Mill (cv Fino de Jete) were germinated in the greenhouse on a peat-based substrate. After 11 weeks, uniform seedlings about 3 cm in height were used as the starting plant material. The soil used was collected in the orchards where the crop is cultivated in the region of Southern Spain. This is a sandy soil with a neutral pH and low in assimilable P. The soil was sieved (4 mm), steam sterilized at 100° C for 1 h on three consecutive days and then reinoculated with a soil filtrate containing its own microbiota but without AM propagules.

Seedlings were transplanted (one per pot) into 200-ml pots containing a soil: sand mixture (5:2 by volume). There were three inoculation treatments (and one uninoculated control), each of them replicated nine times, giving a total of 36 pots. The pots were arranged completely randomly. The AM fungi tested were *Glomus fasciculatum, G. mosseae* and *G. deserticola.* Inoculation was carried out by placing 10 g of the corresponding mycorrhizal inoculum in each pot. These inocula, obtained from a stock culture collection, consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and mycorrhizal root fragments. Control seedlings received the same amount of sterilized inoculum.

Plants were grown under greenhouse conditions with temperatures ranging from 19 to 25° C, 16/8 h light/dark photoperiod and a relative humidity of 70–90%. A photosynthetic photon flux density of 400–700 μmol/m²/s was applied as supplementary light.

After 5 weeks growth, seedlings grown in the small pots were individually transferred to 5-l plastic bags filled with the same, but nonsterilized, test soil. The seedlings had soil adhering to their roots at transplanting, and therefore an inoculum of each AM fungus was transferred with the corresponding seedling. Plants were grown for a further period of 23 weeks in the conditions described above. The nutrient solution of Hoagland (Hoagland and Arnon 1938) was used (at 25% full strength) to feed and irrigate the plants at the rate needed to maintain a suitable soil water content monitored by a potentiometric method. A record of the number of leaves formed and of the height of the plants with time was made. Upon harvest, the biomass production (leaves, stem and root weight) and the leaf area were measured. Data were subjected to analysis of variance, and treatment means were further separated by Tukey and Least Significant Difference tests.

Representative root samples of each plant were stained for AM colonization (Phillips and Hayman 1970), and the extent and morphology of the symbiosis were evaluated and studied in detail under a light microscope.

Results

Annona plants showed no apparent growth response to mycorrhiza inoculation after the 5-week growth period in sterilized soil (Fig. 1). The first symptoms of an AM effect were only detected after five further weeks of growth, once transplanted to the nonsterilized soil (Fig. 1). Further development of *Annona* plants, all of them mycorrhizal with either introduced or indigenous fungi, showed significant growth responses to inoculated endophytes when compared to the native (control plants). This is evident from the records of plant height and the number of leaves formed during the assay (Fig. 1), and from most of the growth parameters measured upon harvest (Fig. 2).

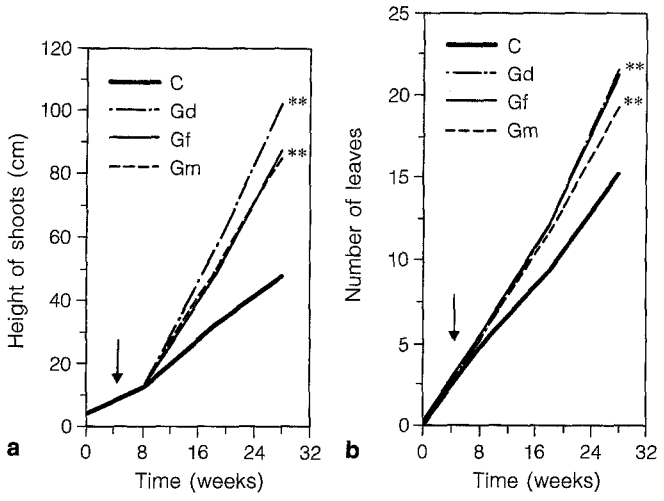

Fig. 1. Change in shoot height (**a**) and number of leaves (**b**) of mycorrhizal (*Gd, Glomus deserticola*; *Gf, Glomus fasciculatum*; *Gm, Glomus mosseae*) and control (*C*) plants of *Annona cherimola* with time. The time of transplantation from a sterilized substrate to an unsterilized soil containing indigenous AM fungi is indicated by →. ** Results significantly different ($P<0.01$) according to Tukey's test

In general, *G. deserticola* was the most effective fungus among those tested. However, as the indigenous endophytes were only able to form AM 5 weeks later than the inoculated ones, only relative comparisons between introduced endophytes can be made. All in all, the observation most pertinent to the aims of this study is that *A. cherimola* is a mycotrophic species. This is the first description for this crop.

Detailed studies on the morphology of AM development in *Annona* roots grown in controlled conditions confirmed the previous observations of field-grown plants.

Figures 3–6 show the main features observed in the morphological study of AM colonization in *Annona* roots inoculated with different endophytes. In summary, the general pattern of AM colonization in the roots of *A. cherimola* was similar for the native endophytes and the three AM fungi inoculated. Hyphae from the appressoria usually branched and become convoluted in outer cortical layers (Fig. 6). Both coarse and fine hyphal coils were observed. Colony extension in *Annona* roots takes place by the growth of convoluted or coiled intracellular hyphae (Fig. 5). Intercellular hyphae were not observed and colonization occurred only be cell-to-cell passage (Fig. 5). This is characteristic of host species lacking air channels. Hyphal constrictions were also seen where the fungus passed through cell walls (Fig. 5). As a consequence of this spreading pattern, fungal colonization of the root occurred slowly and discrete mycorrhizal colonies, without overlapping, were frequently observed (Fig. 5). Cells in the root cortex were profusely colonized with arbuscules (Fig. 4), and these arbuscules sometimes appeared to originate from coarse intramatrical hyphal coils. Arbuscules were not restricted to the inner corti-

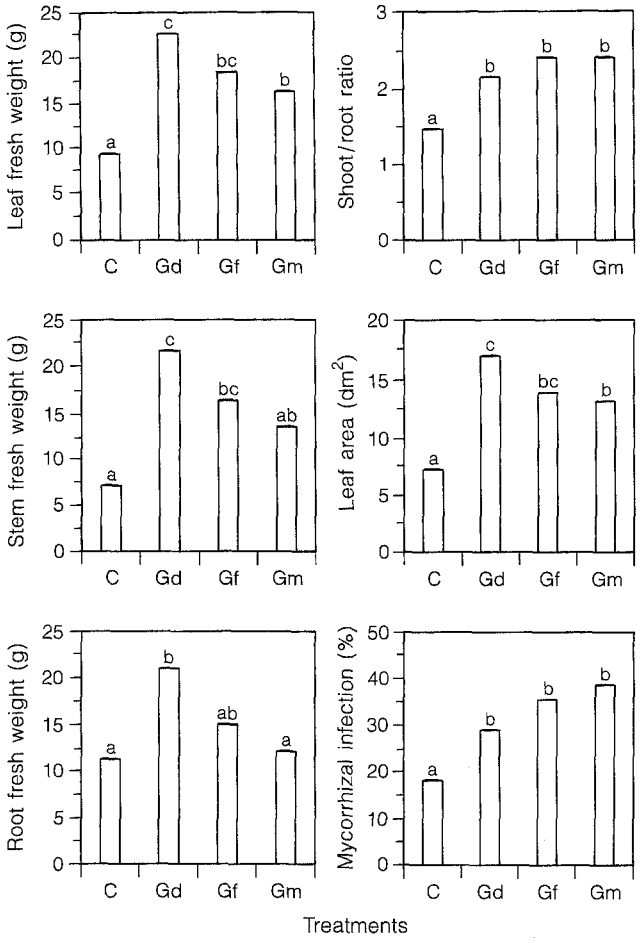

Fig. 2. Effect of different mycorrhizal fungi on biomass production and distribution in *Annona cherimola.* Values marked with different letters differ significantly at the 5% level according to LSD test. For abbreviations, see Fig. 1

cal cells and occasionally seemed to be formed in pairs (Fig. 3). Hyphal masses, probably resulting from arbuscule degeneration, were observed in outer cortical cells (Fig. 4). Fine and dense coiling was seen not only in the outer cell layers but also in the central and inner cortex (Fig. 3). Vesicles formed terminally on intracellular hyphae (Figs. 4, 6) but were rarely found. In general, colonization was preferentially arbuscular, with the presence of fine and dense coils and a few vesicles.

Discussion

Plants grown in controlled conditions clearly developed AM with the introduced endophytes tested and with the native fungi present in the nonsterile soil. Therefore, *A. cherimola* is a mycotrophic crop and seems to depend on mycorrhizae for optimal growth. In fact, inoculation with particular mycorrhizal fungi significantly improved plant growth, with *G. deserticola* being the most effective endophyte among those tested.

The intracellular hyphal development, with cell-to-cell passage, an abundance of arbuscules and coiled hyphae within cells, and a lack of intercellular distributive hyphae found in *Annona* roots is typical of the *Paris* series; in *Arum*-type hosts, intraradical hyphae proliferate between cells by growing through the intercellular air channels present in these species (Gallaud 1905; Brundrett 1991).

Although some of the features of mycorrhizal morphology associated with particular AM fungi, the general pattern of mycorrhizal colonization (cell-to-cell passage versus distributive, intercellular hyphae) and the morphology of the resulting symbiosis appear to depend on the anatomy of the root (Brundrett and Kendrick 1990b; Bonfante-Fasolo and Perotto 1992). This has been corroborated in the present study. In fact, regardless of the endophyte species involved, the AM in *Annona* roots showed features and patterns of spread characteristic of the *Paris* series, although all the endophytes tested form mycorrhizae corresponding to the *Arum* type in hosts such as alfalfa, onion and clover.

Paris-series AM have been described in several hosts, including *Ginkgo biloba* (Bonfante-Fasolo and Fontana 1985), *Acer saccharum* (Brundrett and Kendrick 1988; Yawney and Shultz 1990; Cooke et al. 1992), members of the Gentianaceae (Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983; McGee 1985), *Taxus* (Strullu 1978), and some woodlands species, such as *Erythronium, Asarum* and *Trillium* (Brundrett and Kendrick 1990a, b). However, most of the plant species already studied have an *Arum* pattern of intraradical colonization (Bonfante-Fasolo 1984).

All the endophytes tested in the present study produced many arbuscules. This observation contrasts with descriptions of some woodland plants belonging to the *Paris* series (Brundrett and Kendrick 1990a, b), but in general agrees with descriptions of *Acer saccharum* (Yawney and Shultz 1990). Some vesicles were also present in the roots, mainly in those mycorrhizal with *G. fasciculatum,* but their production was notably low. Mycorrhizal roots of *Acer,* also belonging to the *Paris* series, were reported to lack vesicles (Yawney and Shultz 1990). In this context, Cook et al. (1992) found extensive arbuscular development and few vesicles in the roots of healthy *Acer* trees and the converse when tree health declined. This suggests that the *Annona* plants in this study were healthy and growing in nonstressing conditions. Hyphal masses, probably resulting from arbuscule degeneration, sometimes appeared in the outermost layer of the cortex in the roots of *Annona.*

The abundance of coils and especially arbuscules in *Annona* roots and the scarcity of vesicles, considered as fungal storage organs, suggest efficient AM development. This is supported by the fact that relatively low colonization levels (29.2% in *G. deserticola* inoculated plants) induced significant growth stimulation (a nearly threefold increase in shoot weight).

The AM colonization in *Annona*, as a *Paris*-series host, usually showed discrete fungal colonies in the

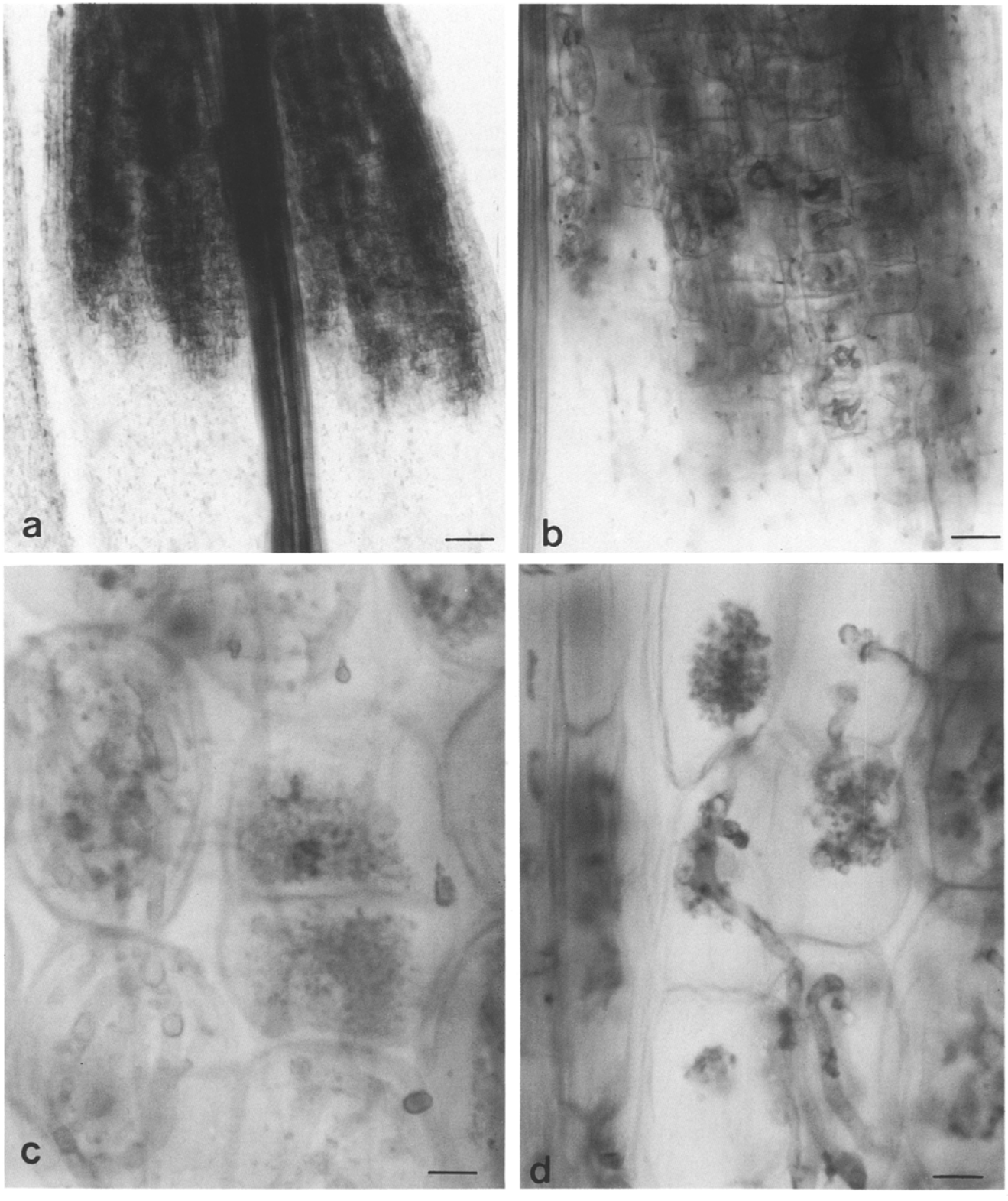

Fig. 3a–d. Morphology of the arbuscular mycorrhizae in *Annona cherimola* produced by native endophytes present in unsterilized soil. **a** General aspect of a partially colonized root lacking intercellular spread; *bar* 100 μm. **b** Coiled and convoluted hyphae colonizing the root from cell to cell. Note the absence of intercellular hyphae; *bar* 40 μm. **c** Coiled and convoluted hyphae in some cortical cells, and arbuscules in an adjacent layer; *bar* 10 μm. **d** Convoluted hyphae responsible for colony spread and arbuscule formation in *Annona.* Note the cell-to-cell passage; *bar* 10 μm

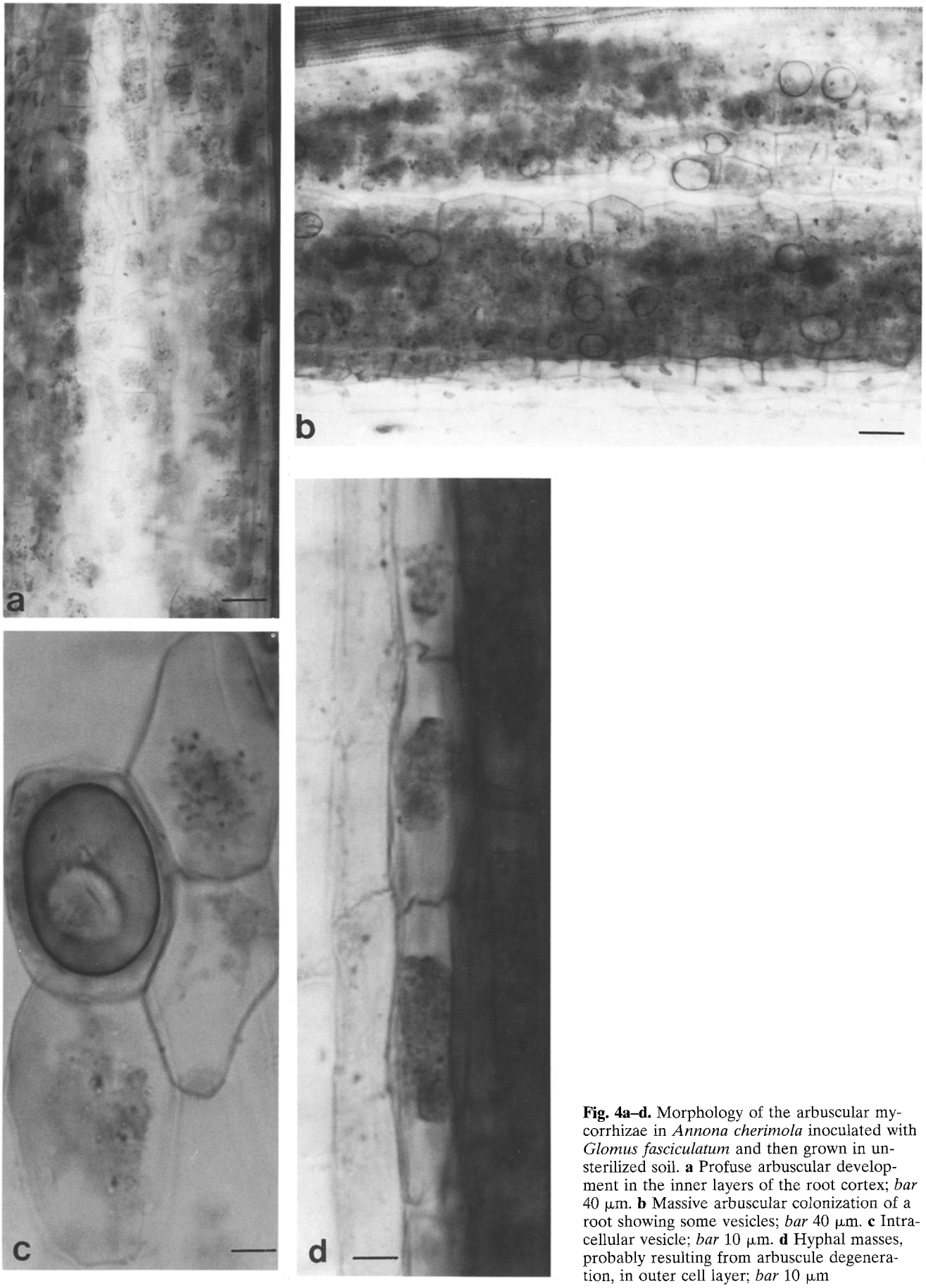

Fig. 4a–d. Morphology of the arbuscular mycorrhizae in *Annona cherimola* inoculated with *Glomus fasciculatum* and then grown in unsterilized soil. **a** Profuse arbuscular development in the inner layers of the root cortex; *bar* 40 μm. **b** Massive arbuscular colonization of a root showing some vesicles; *bar* 40 μm. **c** Intracellular vesicle; *bar* 10 μm. **d** Hyphal masses, probably resulting from arbuscule degeneration, in outer cell layer; *bar* 10 μm

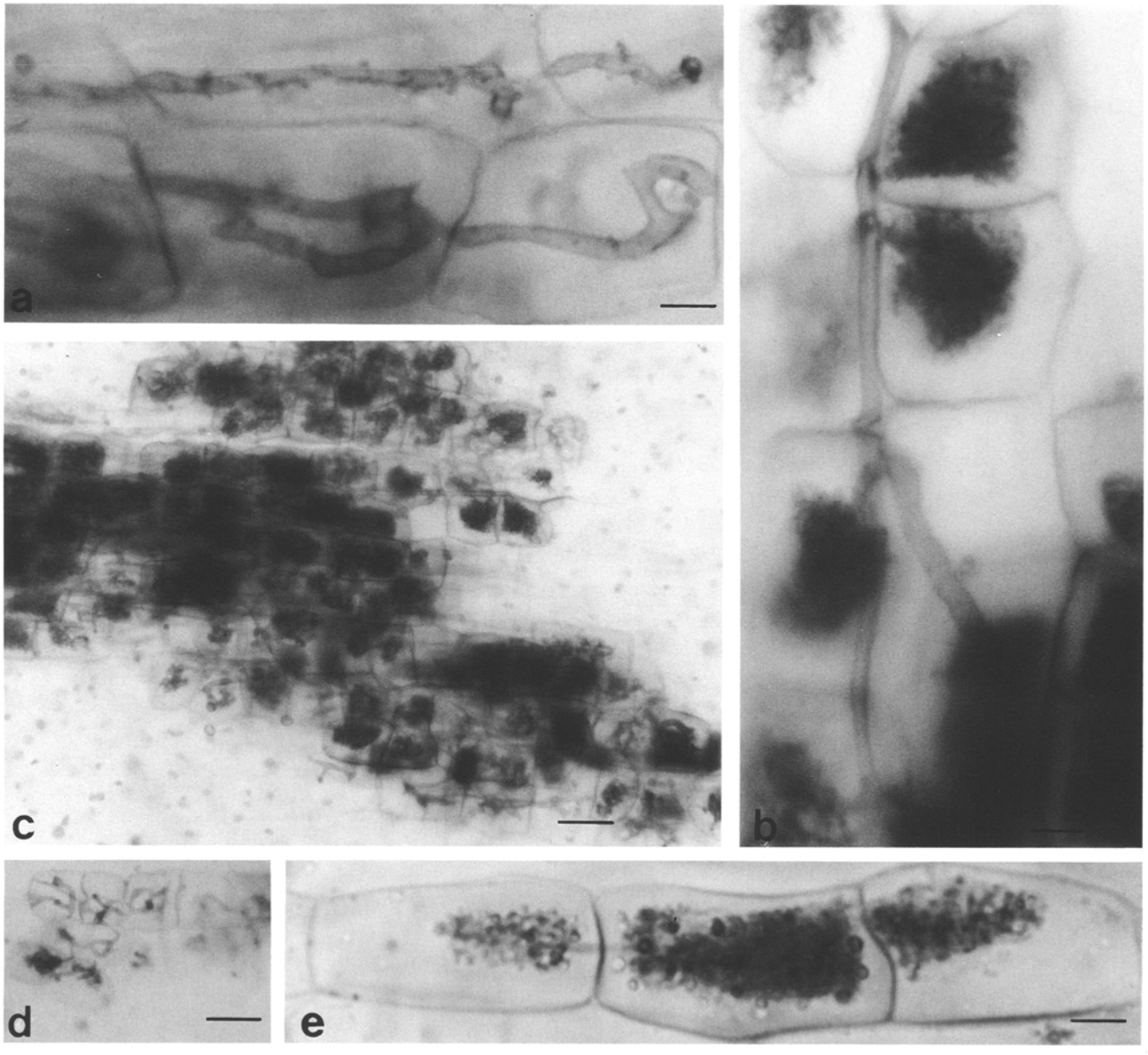

Fig. 5a–e. Morphology of the arbuscular mycorrhizae in *Annona cherimola* inoculated with *Glomus deserticola* and then grown in unsterilized soil. **a** Convoluted hypha colonizing the root from cell to cell. Hyphal constrictions are seen as the fungus passes through cell walls; *bar* 10 μm. **b** Arbuscules arising from intracellular distributive hypha; *bar* 10 μm. **c** Discrete mycorrhizal colony due to the lack of intercellular spread; *bar* 40 μm. **d** Initial colonization by convoluted hyphae responsible for colony spread; *bar* 40 μm. **e** Arbuscules with granular-like branches. Cell to cell passage is observed; *bar* 10 μm

root cortex. In these species, colony growth is usually slow since the hyphae must follow a convoluted path to cross host cell walls (Brundrett and Kendrick 1990a). This fact may account for the rather low levels of root colonization by the mycorrhizal endophytes found in the present study, and emphasizes the need for a well-distributed, high-propagule-density mycorrhizal inoculum to facilitate mycorrhizae formation and root colonization of these plants.

It has been argued that *Paris*-series associations involving woodland species are less efficient than those of the *Arum* series because of the low number of cells colonized with arbuscules compared to those containing coils (Brundrett and Kendrick 1990b). However, the absence of distributive hyphae and the consequently slower rate of root colonization in this type of mycorrhizae might help the plant to control the fungal spread along the root system and thus keep energy losses at low levels; this is important for perennial plants subjected to periods of low activity (Chapin 1980). In the case of *Annona*, both the pattern of AM colonization spread and abundance of arbuscules suggest an efficient symbiotic relationship. If, as described for other hosts with *Paris* associations (Brundrett and Kendrick 1990a), arbuscules have a longer lifespan than in the *Arum* series, this would result in lower in-

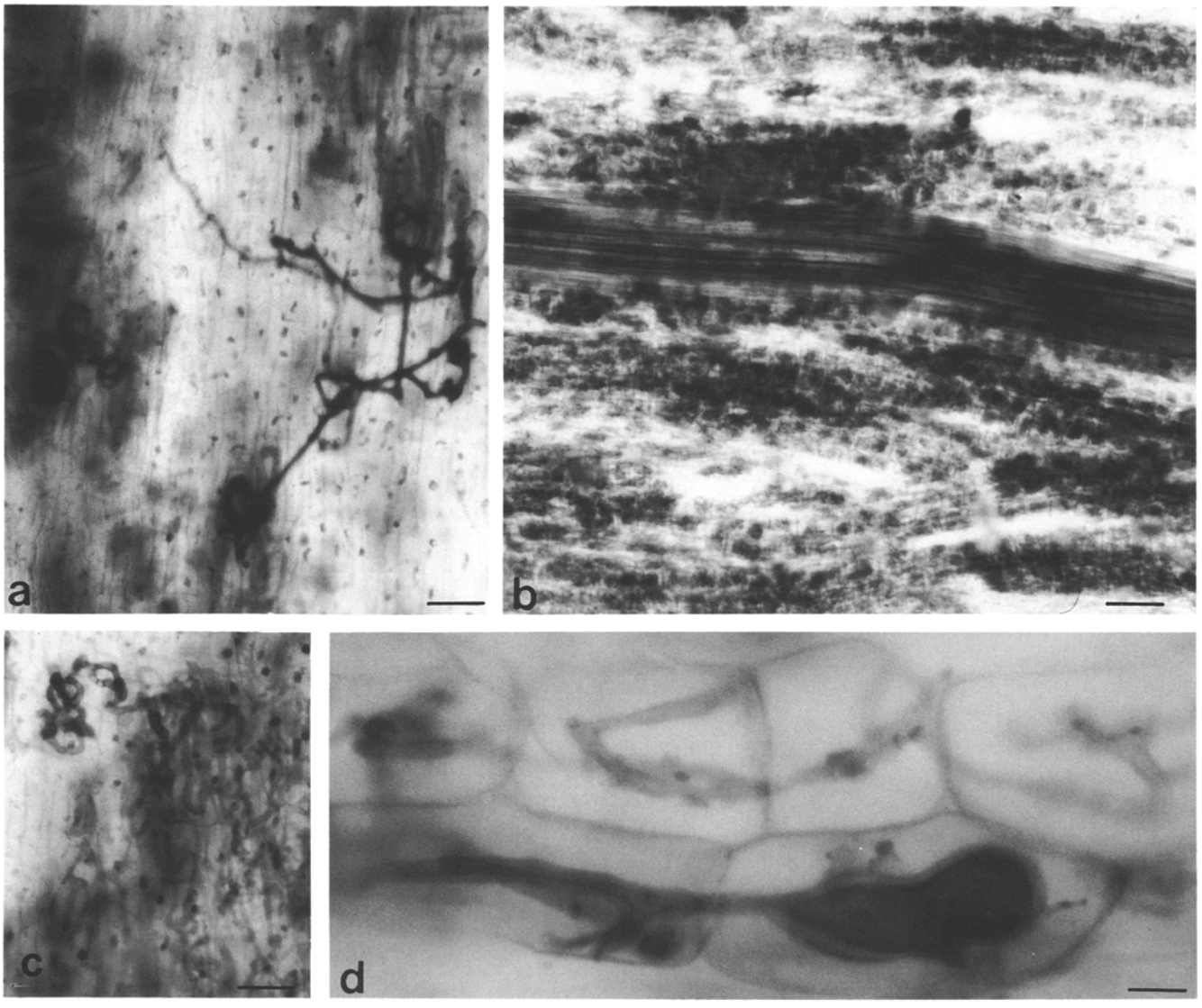

Fig. 6a–d. Arbuscular mycorrhizal morphology of *Annona cherimola* inoculated with *Glomus mosseae* and then grown in unsterilized soil. **a** Appressoria on the root surface leading to coarse hyphal coils; *bar* 40 μm. **b** Massive intracellular development of the AM fungi; *bar* 100 μm. **c** Hyphae forming dense coils; *bar* 40 μm. **d** Intracellular vesicle formation; *bar* 10 μm

vestment in the turnover of fungal biomass and also contribute indirectly to higher effectiveness.

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