Mycorrhiza (1996) 6:69–71

SHORT NOTE

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Mycotrophy in a vascular stem parasite *Cuscuta reflexa*

Accepted: 30 June 1995

Abstract AM-inoculated plants of the love vine *Cuscuta reflexa* showed significant increases in biomass and longevity compared to noninoculated seedlings. The AM-inoculated plants formed arbuscules, vesicles and spores within 5 days of sowing. Seed coats left in the soil by the growing seedlings showed heavy vesicular infections and spore formation. Mycotrophy in stem-parasitizing vascular plants is discussed.

Key words Vesicular-arbuscular mycorrhiza (VAM) · Vascular stem parasite

Introduction

Many stem-parasitic and root-parasitic vascular plants with no true root system depend entirely upon the host for nutrients and water (Hafiz 1986). Arbuscular mycorrhizae (AM) are known in some parasitic vascular plants. Parasitic plants of Orobanchaceae and Orchidaceae initiate formation of AM following root contact of the parasite with a host AM root system (Khalid and Iqbal 1992). Hemi-parasitic plants form AM prior to contact with the root system of an AM host, then lose the mycorrhiza (Lesica and Antibus 1986). The influence of AM on parasitic plant survival and fitness is not understood.

Cuscuta reflexa Roxb., a widely distributed stem vascular parasite, is a climbing plant with a weak, pink-yellow, filamentous stem. Seeds germinate to produce a thin, leafless, upright stem which curves and rotates while seeking a host. Failing contact with a host, it collapses to the ground (MacLean and Ivimey-Cook 1964). The distal part of the young, advancing plant becomes disconnected when contact is made with a host plant. Within its host range are many plants of forage and medicinal value (Hafiz 1986). The present study investigated the mycorrhizal nature of these chlorophyll-less stem parasites, especially prior to parasitism of the host.

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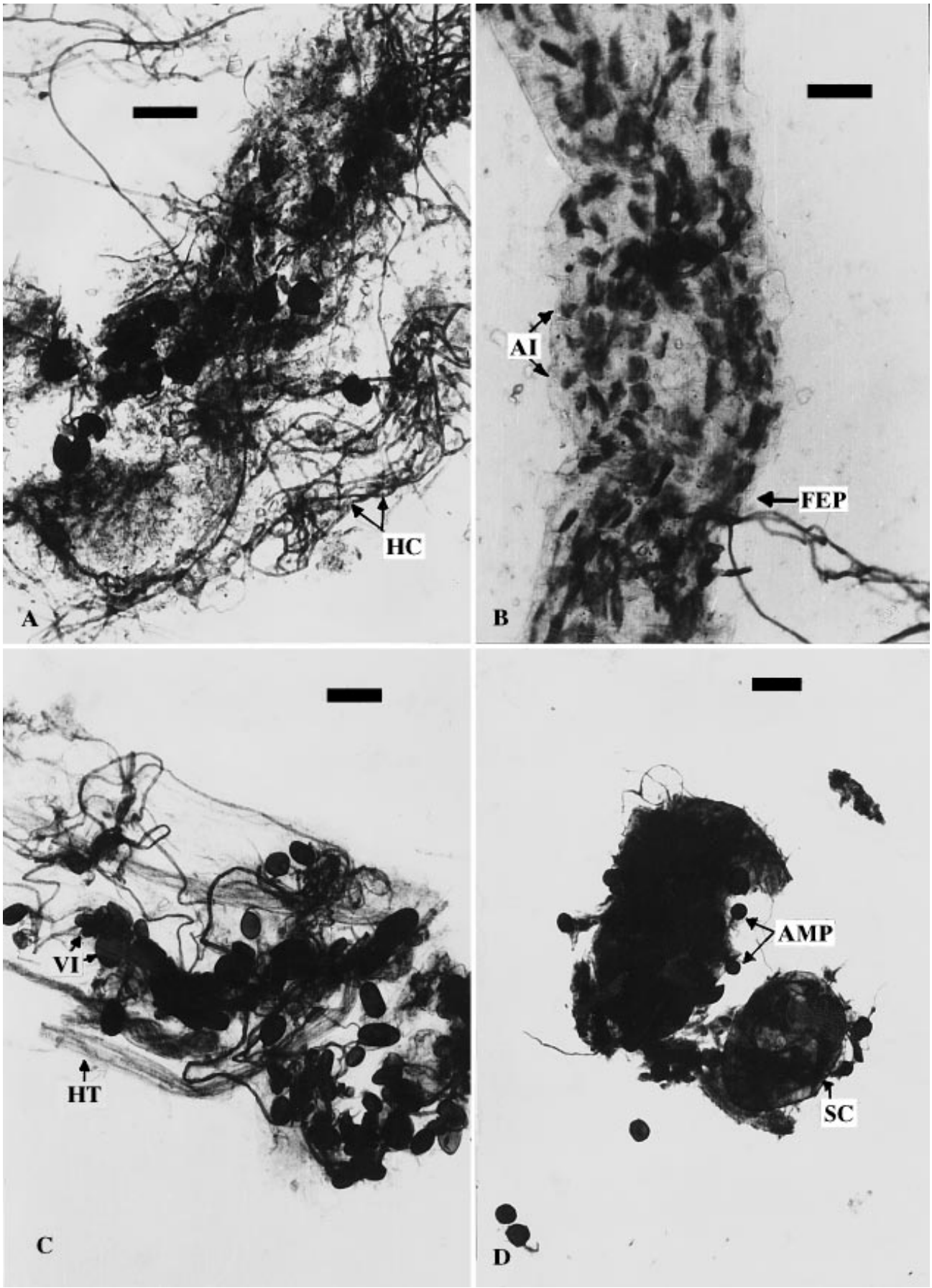
Materials and methods

Seeds of *C. reflexa* parasitizing *Trifolium* spp. and *Citrus* ssp. were collected. After removal of the husks, seeds were immersed in water and floating seeds were discarded. The remainder were surface sterilized in a 1:1 (v/v) mixture of H_2O_2 (20%) and alcohol (70%). Surface-sterilized seeds were sown 1 cm deep in pots with either autoclaved soil or nonsterilized rhizospheric soil from field-grown sunflowers (*Helianthus annuus* L.). Mycorrhizal sunflower root pieces (1 cm long) were mixed with nonsterilized soil 2 cm from the surface. Six pots for each treatment containing three or four seeds per pot were used. Soil moisture was maintained at 20%, and the pots were kept in a growth room at 28 °C and 1600 lx light intensity. Small, dried twigs of plants were pegged in the pots as support for the germinated seedlings. When the seedlings began to wither, they were removed along with rhizosphere soil and washed gently to remove the adhering soil and debris. The entire plants were fixed in formalin:acetic acid, cleared in 5% KOH and stained in 0.05% trypan blue, following the modified methods of Phillips and Hayman (1970). The stained plants were examined under the microscope and the extent of AM infection calculated by measuring the length of mycelium. Duncan's multiple range test was employed to extract the significant differences among the mean values (Snedecor and Cochran 1967). The life spans and biomasses of the control and AM seedlings were also compared statistically.

Table 1 Lifespan and biomass of AM and noninoculated seedlings of *Cuscuta reflexa.* Mean values are significantly different at $P = 0.05$

Lifespan (days)		Biomass (g)	
AM	Noninoculated	AM	Noninoculated
7*	5*	0.6**	0.03**

* L.S.D.$^{(0.05)} = 1.27$
** L.S.D.$^{(0.05)} = 0.1$


A
HC
B
AI
FEP
C
VI
HT
D
AMP
SC

◀ **Fig. 1A–D** AM infections in *Cuscuta reflexa.* **A** Network of hyphae and extramatrical vesicles. **B** Arbuscular infections in underground portion. **C** Vesicles of different sizes and shapes. **D** Seed coats of *C. reflexa* covered with AM propagules and hyphae; *bar* 20 μm (*AI* arbuscular infections, *AMP* arbuscular mycorrhizal propagules, *HC* hyphal cluster, *HT* host tissue, *SC* seed coat, *VI* vesicular infections)

Results

Plants from seeds of *C. reflexa* inoculated with AM roots of sunflower showed significant increases in biomass and life span. The plants formed arbuscules, vesicles and subsequently spores within 5 days of sowing.

Within 48 h of sowing, seedlings of *C. reflexa* emerged. Sprouted seedlings bore the testa on the apex or left it in the soil. The underground parts of the AM-inoculated plants bore a network of hyphae. The pale yellow portion (of control plants) above the soil grew rapidly to 6 cm within 5 days. At this time, the distal part began to wither and the upper part died. If support was provided, the parasite wrapped around the support and survived for 12–15 h. The lower part of the parasite in the soil died. The inoculated seedlings showed a similar pattern of germination and growth but continued to grow for up to 7 days. AM seedlings of *C. reflexa* survived for a significantly longer period than the control. Nonmycorrhizal plants collapsed totally 5 days after germination, whereas AM-inoculated plants survived until day 7 (Table 1).

A network of hyphae was present on the underground parts of AM seedlings (Fig. 1A). These hyphae were entangled, had trapped soil particles and had extramatrical vesicles. Arbuscules, vesicles and subsequently spores were formed within 7 days. These structures were intramatrical and extramatrical in the underground parts. Arbuscules occurred in almost all seedlings (Fig. 1B). Vesicles of different size and shape (oval, oblong to multiangular) (Fig. 1C) with hyphal connections were seen. A mixture of spores of two or three species of *Glomus* were seen associated with the underground parts of the parasite (Fig. 1A, C). The seed coats, when attached to the seedlings and left in the soil, were totally covered with AM propagules and hyphae (Fig. 1D). AM structures were absent in control plants.

AM plants had a greater mean biomass (0.6 g) than control plants (0.3 g) (Table 1).

Discussion

The occurrence of AM associations in parasitic vascular plants extends the range of known functional dynamics in these plants (Khalid and Iqbal 1992). The development of arbuscules, vesicles and spores by the stem parasite mycorrhiza within the short span of 7 days of sowing is of particular interest. Several types of AM propagules form this association and the hastened production of AM structures appears to be related to the host.

It may be safely concluded that mycorrhizae are important to stem-parasitic vascular plants. The mycorrhiza allows these plants to survive longer before parasitizing a host plant. In sites with widely dispersed hosts (in space and time) the additional time may be crucial to their fitness.

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