

The Presence and Importance of VAM Spores in the Soil of Ginseng Gardens

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ABSTRACT

Symbiotic associations between land plants and fungi have been known for more than one hundred years. Vesiculararbuscular mycorrhiza (VAM) are the most common symbiosis in flowering plants and can be recognized in almost all plant families. These fungal associations play a very important role in the growth and survival of plant species. However, with respect to the importance and intensity of the VAM, there is great variation among host species.

Our knowledge of the VAM fungus-plant association in Araliaceae is very limited. After the first reports of the occurrence of VAM in lateral roots of *Panax* species, mycorrhizal structures are now described as special structures representing the so-called *Paris* type. In this type, the development of new spores and vesicles is extremely low. This and the type of colonization of the fungus in *Panax* roots indicates on, one hand, the high intensity of the VAM and, on the other hand, a remarkable dependency for VAM in members of the *Panax* species.

Therefore, it can be easily understood that cultivated *Panax* plants exhibit a significant uptake of nutrients and this leads to an extremely depleted soil at harvest. Further, the soil is nearly free of the spores of VAM fungi as they germinate each year on the newly developing *Panax* roots.

Introduction

Frank (1885) introduced the term “mycorrhiza” as a special type of symbiosis (De Bary 1879; also see Douglas 1994, Sapp 1994). This type of association between fungus and plant represents a very close symbiotic interaction (Burgeff 1932, Schaede 1962) which can be understood as an acquisition and maintenance of one or more organisms by another that results in novel structures and meta-bolism (Zook 1998).

The vesiculararbuscular mycorrhiza (VAM) is the most common symbiosis in flowering plants and takes on a very important role in their survival. In almost all families of cormobiotic land plants (Gerdemann 1968, Kuehn *et al.*, 1991), VAM can be recognized, usually in the roots. VAM is characterized by typical structures. A large volume of the host tissue can be occupied by intercellular running hyphae. From these hyphae, a highly branched arbuscule develops as a fungal haustorium in

the cells of the root cortex (Bonfante-Fasolo 1984). The running hyphae also develops intercellular vesicles as terminal swellings. Later in the fungal development in the root and further colonization of the root cortex, some hyphae develop, from the running hyphae, leave the root and spin around the root surface. Spores will develop at these hyphae that are growing outside of the root in the rhizosphere.

Gallaud (1905) was the first to describe the mycorrhiza of some plant species as modified structures of the VAM fungi, the so-called *Paris* type. After extensive observations on VAM in members of the Gentianales (Knoebel and Weber 1988, Demuth *et al.* 1989, 1991, Tiemann *et al.* 1995, Weber *et al.* 1995, Imhof and Weber 1997), it can be clearly shown, that in many cases, the plant highly influences the structures and growth of the VAM fungus. The different structures and modified type of colonization by the same *Glomus* species in different hosts was explained as a structural incompatibility (Demuth and Weber 1990). The evolution within the Gentianales and the correlated phylogenetic tendency of its mycorrhiza (Imhof and Weber 1997, Weber *et al.* 1997) leads to achlorophyllous and obligate mycotrophic Gentianaceae species (Imhof 1997). VAM structures of the *Paris* type underline the high intensity of this symbiotic association, as well as, the remarkable dependency for the flowering plant.

Recently, Zeuske *et al.* (1997) observed VAM in some Araliaceae. The fungal structures and type of colonization indicate an increasing tendency toward the *Paris* type and *Panax* species (Weber *et al.* 1995a, Whitbread *et al.* 1995) represent a final stage of a progression line within the species studied (Zeuske *et al.* 1997, Zeuske *et al.*, in preparation).

Materials and Methods

Roots of Asian ginseng (*Panax ginseng* C.A. Meyer) were collected:

- a. During the International Conference of Ginseng and Allied Plants, Harbin, China, August 1995, at the Laoshan Plantation Experiment Station and Maoershan Experiment Forestry Farm, Northeast Forestry University.
- b. From plantings in a Pinus forest (W. Dierking Beerenobst, Gilten), close to Hannover, Germany.
- c. From plantings and cultivations in the Botanical Garden, Philipps University, Marburg, Germany.

Roots of North American ginseng (*Panax quinquefolius* L.) were collected:

- a. From plantings and cultivations in the Botanical Garden, Philipps University, Marburg, Germany.
- b. From the Fountain Ridge Ginseng Farms Ltd. (G. P. Smith), Lillooet, British Columbia, Canada, in October 1995, August 1996 and September 1997.

The roots of the 1 through 8 year old ginseng plants were fixed in AFE (50% ethanol, 37% formaldehyde, 100% acetic acid, at 18:1:1). Other roots were dried for later use in the laboratory as

an inoculum. In the most cases, soil for wet sieving in the laboratory was collected together with the ginseng roots.

The fixed material was embedded in paraffin as well as in HistoResine. For the 1 - 15 μ m sections, a rotary microtome and a Supercut 2065 (Reichert-Jung) were used. The paraffin sections were stained with Astra Blue/Auramine O/Safranin O. The sections embedded in plastic were stained with Toluidin Blue. For microscopy, a DMRB microscope (Leitz) was employed.

Results and Discussion

Little is known about the mycorrhiza of the two commercially most important ginseng species. After the first reports of the occurrence of VAM in the lateral roots of *Panax* species, mycorrhizal structures were described as special structures representing the so-called *Paris* type (Weber *et al.* 1995a, Whitbread *et al.* 1995). If we compare the common characteristics of a VAM (see Introduction) with the special structures of the *Paris* type, we cannot describe intercellular running hyphae, rather intracellular colonization only. In the cells of the root cortex, we can not find a highly branched arbuscule (Alexander *et al.* 1988, 1989). Rather, some small reduced arbuscules on coiled hyphae were often found. In some cases, the coiled hyphae swell up, sometimes to the size of vesicles. In all roots with a mycorrhiza of the *Paris* type, the numbers of vesicles and spores are generally poor. Finally, in many cases, there were no vesicles in the roots of the achlorophyllous plants.

Following an evolutionary progression line of the mycorrhiza in the Gentianales, the *Paris* type represents a pre-stage of a mycorrhizal pattern. This is referred to as an "intraradical fungus garden" (Imhof 1997). In this case, in the lacking chlorophyllous plants of the Gentianaceae family (Imhof *et al.* 1994), the microbiont cannot benefit from the association. Therefore, at least for mycorrhiza in achlorophyllous flowering plants, the term "symbiosis" should be changed to the term "parasitism" (if we ignore the strong sense of this term for flowering plants described by Kuijt 1969 or Weber 1993a).

However, VAM structures of the *Paris* type underline the high intensity of this association. The plant partner benefits much more than the fungal microbiont and what results in an extreme dependency. This fact is known from other interactions, too, because dependency is correlated with specialization in general. In addition, specialization is often correlated with the reduction or abbreviation of organs and organelles. Members of the gentian family are examples of lacking chlorophyllous plants. A reduced root system can be noticed in plants like turnips, beet roots or carrots. Ginseng, with its bubble-shaped root, is another example of morphological reduction. Therefore, it is not surprisingly that *P. ginseng* and *P. quinquefolius* show mycorrhizal structures of the *Paris* type as a kind of specialization. But it also indicates that ginseng has a higher dependency on the VAM fungi.

As mentioned previously, the number of vesicles and spores in many plant species is extremely

low (about 10% only), as in plants with the common VAM. In ginseng, they were present in the material which was inoculated in the laboratory with *Glomus* species. But in many samples, collected from ginseng gardens, vesicles are extremely rare, and in some infected roots, no vesicles could be observed. This included the samples which were collected at the end of the vegetative period.

Wet sieving is a method to retrieve spores of VAM fungi from soil and from the surfaces of roots. We used this method for soil samples from Lillooet, as well as soil samples from our cultures of *P. quinquefolius* and *P. ginseng*. In all cases, virtually no spores could be collected. In addition, dried ginseng roots were used as an inoculum for other plants with the result that the development of a VAM in those plants was unsuccessful or very poor.

Therefore, on one hand, *P. quinquefolius* and *P. ginseng* plants show a high dependency for a close association with VAM fungi - they critically need the fungus for growth. On the other hand, because the production of vesicles and spores is extremely limited in this type of VAM in ginseng, successful plant growth in monocultures is not possible. *Panax* plants exhibit a significant uptake of nutrients (Domey und Weber 1997) and this leads to an extremely depleted soil at harvest. Further, after one or two vegetation periods, the soil is nearly free of the spores of VAM fungi, as they germinate each year on the newly developing *Panax* roots. But these produce virtually no new spores and few vesicles.

Of course, in ginseng gardens, successful management of fertilizers can help assure a quality harvest. But, alternatively, the use of VAM spores require consideration from more than one perspective (Weber 1993). The important question is how to enrich the soil of ginseng gardens with vesicles and spores of VAM fungi (Weber in preparation).

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