The Strain-Inherent Variability of Arbuscular Mycorrhizal Effectiveness: I. Development of a Test System Using *Petroselinum crispum* Hoffm. as Host

F. FELDMANN[1,3]* W. KRUSE[1], C. BOYLE[2], and R. LIEBEREI[1]
[1]*Institute for Applied Botany, University of Hamburg, Marseiller Str. 7, 20309 Hamburg, Germany;* [2]*Institute for Microbiology, Technical University of Braunschweig, Spielmannstr. 7, 38106 Braunschzweig, Germany;* [3]*Corresponding address: Konstantin-Uhde-Str. 13, 38106 Braunschweig, Germany, E-mail. c.boyle@tu-bs.de*

Abstract

Nearly nothing is known about the variability of arbuscular mycorrhizal strains and the genetic variation within populations of arbuscular mycorrhizal fungi. *Petroselinum crispum* Hoffm. was found to be a very suitable host plant for a standardized experimental system for the study of such population biological aspects of these fungi, including long term studies and changes in strain characteristics. It was easy to handle under laboratory conditions, reproducibly grown in large numbers on small space, well defined according to the genetic background and furthermore easy to analyse with respect to morphometric, physiological, biochemical and gene-technological methods. Another aspect of this system was a phase of slow development of the root systems permitting easy analysis of the fine roots for mycorrhizal colonization. *P. crispum* is available as maternally defined seed material of several varieties and, above all, has been studied in various aspects of plant-pathogen interaction. Our data show how to standardize the

*The author to whom correspondence should be sent.

testing system using *Glomus etunicatum* Becker & Gerdemann as microsymbiont and how to apply it to strain-comparative and long-term studies.

1. Introduction

Knowledge about the variability of effectiveness of the symbiosis between arbuscular mycorrhizal fungi (AMF) and higher plants is of central importance for the consequent use of the arbuscular mycorrhizal technology in practice. The genetic background of mycorrhizal effectiveness as well as data about the contribution of population biological parameters of AMF are still lacking (Giovannetti and Gianinazzi-Pearson, 1994).

In order to be able to study the genotypic variation within populations regarding the effectiveness of *Glomus etunicatum* Becker & Gerdemann, we had to develop an adequate test system for the recognition of effectiveness resulting from interactions between AMF strains. The test system had to be suitable for long-term studies on population biological aspects of changes in strain characteristics, must be standardized and highly reproducible, and must also fulfil the requirement to reveal effectiveness of single spores on host plants (compare Feldmann, 1998).

The macrosymbiont for such tests must be easy to handle under laboratory conditions, reproducibly grown in large numbers on small space and well defined according to the genetic background. It should be a facultatively mycotrophic plant, in order to permit observation of the influence of environmental factors and resistance reactions to root colonization. Furthermore, that plant should be easy to analyse with respect to morphometric, physiological, biochemical and gene-technological methods. Another aspect of this system must be a slow-developing root system consisting of fine roots to facilitate analysis of mycorrhizal colonization behaviour.

Out of a range of monocotyledones and dicotyledones tested, *Petroselinum crispum* Hoffm. (parsley) was the most suitable species for the cited experiments. It fulfilled the above requirements, is available as maternally defined seed material of different varieties and, above all, has been studied in various aspects of plant-pathogen interaction (Jahnen and Hahlbrock, 1988; Schmelzer et al., 1989; Franken and Gnaedinger, 1994). For standardized tests and for quantification of plant reactions (e.g. growth responses or physiological /molecular/biochemical responses) a detailed description of the plants' status of development is needed. On such a basis, the reaction to inoculated fungal material (strain, monospore culture) can easily be used for comparative studies.

2. Materials and Methods

Fungal material and mycorrhizal analysis

The AMF strain of *Glomus etunicatum* Becker & Gerdemann and *G. intraradices* Schenck & Smith were cultivated for six years on several hosts under greenhouse conditions following the method of Feldmann and Idczak (1992).

Mycorrhizal analysis followed the standard techniques as described by Schenck (1984). DAPI staining was carried out according to Panwar et al. (1987).

Plant cultivation

Maternally defined seed material of *Petroselinum crispum* cv. "Mooskrause" was used for all experiments. The plants were grown in climate chambers under the following conditions: light of SON-T AGRO 400 Philipps lamps (360 µE m^{-2} s^{-1}), 14h/d; 60–80% relative humidity; 18–20°C at night, 22–24°C during the day; irrigation below field capacity; fertilization once per week with 10% pot volume of a nutrient solution, pH 5.5, containing 15% N (10% nitrate, 5% ammonium), 7% P_2O_5, 22% K_2O, 6% MgO, 0.03% B, 0.05% Mn, 0.01% Zn.

During permanent cultivation over more than 170 days the inflorescence was cut to prevent fruiting and death of the plant. In this case the pot size was increased from 0.3 to 4 litres volume in four steps.

Inoculation

The seeds of *P. crispum* were grown in sand for 35 days, a subpopulation of equally sized seedlings was selected (phase III plants, see results), which had already developed the primary leaf and the second ramification of the root system. Two potting treatments were tested: in the first the seedlings were planted into pots of 2.5 ml volume and inoculated there with a single spore or spore population using a micropipette to locate them directly onto the root surface. After 14 days, substrate, spore and plant were transferred to a pot of 25 ml and then after further 14 days to a pot of 50 ml volume (called the "small pot treatment" in Fig. 3). In the second treatment the seedlings were directly planted and inoculated in a pot of 50 ml volume and transferred twice to pots of the same size (called the "large pot treatment" in Fig. 3). The plants were harvested and evaluated 60 days after inoculation.

3. Results

Homogeneity within populations of parsley seedlings

A prerequisite for comparative studies of microsymbiont influence on its host plant is the homogenous and exactly described development of the macrosymbiont.

Maternally defined seeds of the parsley cultivar "Mooskrause" developed within 170 days into fully differentiated vegetative plants with a strongly ramified root system. The plants were not yet in their final stage of physiological maturity, as they did not respond to external stimulus with flower induction.

Table 1. Growth characteristics of *Petroselinum crispum* seedlings and description of developmental phases

Phase	Plant age at the end of phase	Shoot characteristic	Root characteristic	Increase of total biomass
I	14–42 d	Epicotyl, cotyledones and primary leaf completely developed	Primary root developed, first ramification of the root system	Slow
II	21–64 d	Second leaf developed, formation of rosette	Initiation of the second ramification	Slow
III	28–104 d	Moderate formation of leaves, increase of petiole length	Moderate increase of root system ramifications	Moderate
IV	56–140 d	Intensive formation of leaves, spread of leaf area	Drastic increase of root system ramifications	Accelerated
V	77–170 d	Formation of leaves, spread of leaf area, growth of petioles moderate	No further degree of root ramification	Moderate

The plants were grown in climate chambers under the following conditions: light of 360 µE $m^{-2} s^{-1}$, 14h/d; 60–80% relative humidity; 18–20°C at night, 22–24°C during the day; irrigation below field capacity; fertilization once per week with 10% pot volume of a nutrient solution, pH 5.5, containing 15% N (10% nitrate, 5% ammonium), 7% P_2O_5, 22% K_2O, 6% MgO, 0.03% B, 0.05% Mn, 0.01% Zn.
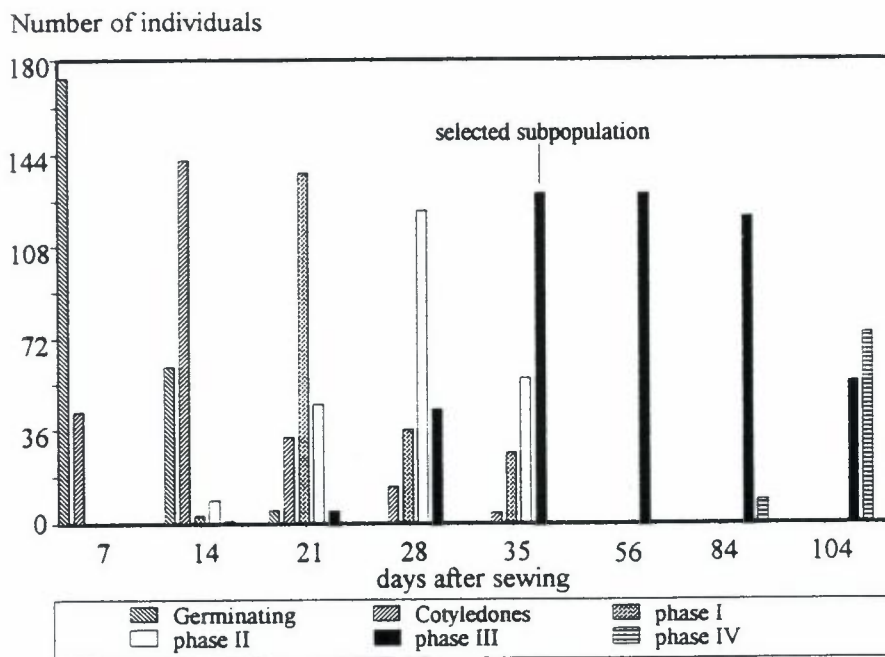
Number of individuals

Figure 1. Developmental phases as basis for selection of *Petroselinum crispum* seedlings for use in the standardized testing system. For description of developmental phases see Table 1.

Five developmental stages were defined (Table 1) using morphological parameters. The time span of the phases varied from 28 days for plants in phase I up to 93 days for plants in phase V. Phase III plants were characterized by moderate morphological differentiation of leaves and petioles and a moderate ramification of the roots. Thirtyfive days after sowing at least 71% of all plants had reached phase III (Fig. 1); 94% of this subpopulation remained in phase III for 49 days. The onset of phase IV was characterized by the beginning of accelerated biomass production correlated with an intensive formation of root and of shoot (Fig. 2). Plants in phase III were found to be infected by AM fungi (Fig. 3) within less than 14 days after inoculation.

A standard test system developed according to these data consists of parsley plants of phase III, selected 35 days after sowing and inoculated immediately afterwards. Potential changes in morphological and physiological parameters can be followed and compared against the background of a homogenously developing control population over a period of at least 49 days following inoculation.

Fresh weight [g]

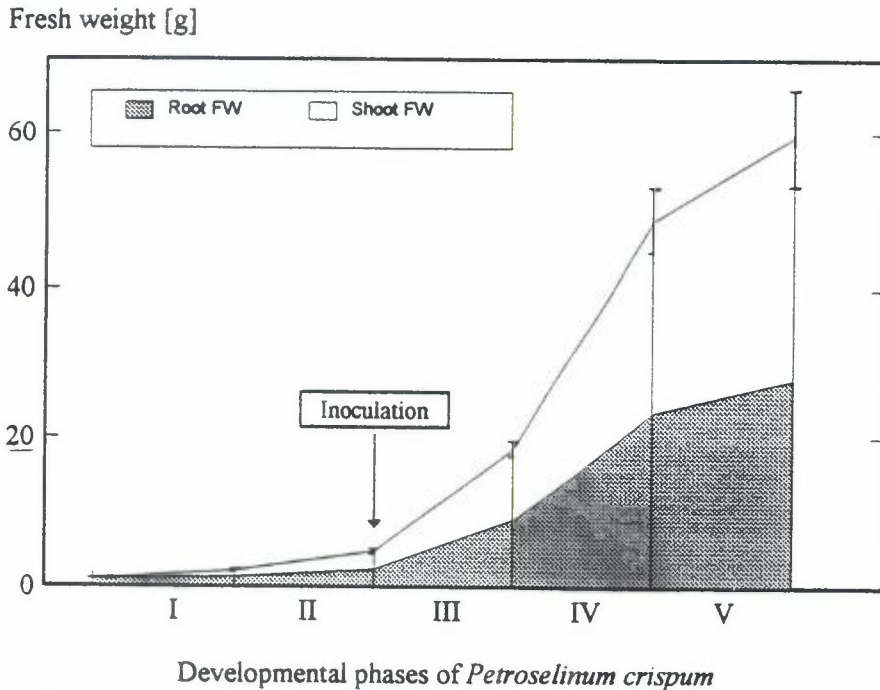Developmental phases of *Petroselinum crispum*

Figure 2.   Biomass production of *Petroselinum crispum* seedlings without mycorrhization. During the tests the inoculation was set at the beginning of Phase III. For description of the developmental phases see Table 1.

The homogenously developing test plant population can be grown in large quantities, allowing excellent high sample size for statistical analysis in morphometric studies. The reproducibility of the growth parameters of *P. crispum* make the test system useful as well for long-term studies of strain characteristics and host/single spore interactions (Feldmann, 1998).

Adaption of the system to single spore inoculations

The culture conditions of the plant had been defined in preceding studies, especially regarding abiotic parameters and phosphate concentration in culture media, as described above. The success of inoculation, i.e. the onset of root colonization depends on the contact of the growing root with the spores introduced into the substrate and, thus, it depends on the amount of spores per volume of root penetrated substrate. The root to pot volume ratio gives the possibility to reach high degrees of colonization even by inoculation with a single spore.
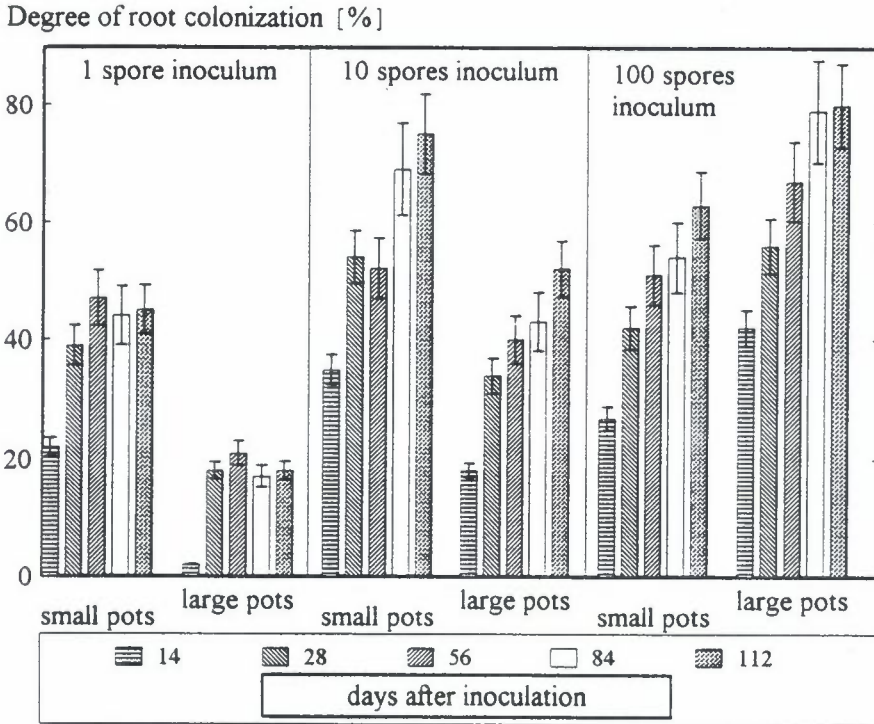
Degree of root colonization [%]

Figure 3. Colonization of *Petroselinum crispum* roots (phase III) by *Glomus etunicatum* HH13 in different potting treatments. Small pots: inoculation into vessels of 2.5 ml volume, careful transfer of plants inclusively substrate to pots of 25 ml after 7 days followed by a second transfer to pots of 50 ml after further 14 days. Large pots: direct planting and inoculation into pots of 50 ml, two transfers into other pots of same size at the same time as described for the small pot treatment. n=5/treatment.

Single spore inoculation resulted in a maximum of 48% colonized roots within 56 days after inoculation, provided the inoculation was carried out in a sequence of small pot volumes as described above. After 28 days the degree of root colonization in single spore assays using small pot volumes is comparable to results of 100 spores in the same pot volumes. Single spore inoculation carried out directly in 50 cm^3 pot volume resulted in 22% root colonization as a maximum (Fig. 3). Inoculation with higher amounts of spores revealed root colonization up to 78% in small substrate volumes, whereas inoculation of plants grown in 50 cm^3 substrate the root colonization remained less than 60%.

In contrast to these results obtained with low spore numbers, inoculation with high spore numbers (100 spores) led to different pattern of colonization.

Inoculating the seedlings with 100 AMF spores per 2.5 cm^3 substrate resulted in a lower maximal colonization as compared to a high volume of substrate. Calculating the spore density demonstrates that the root colonization was higher when spore amounts of two to four spores per cm^3 of soil were inoculated (10 spores/small pots and 100 spores/large pots in Fig. 3) than in the highest inoculum density of 40 spores per cm^3 (100 spores/small pots, Fig. 3).

Obviously there is an optimal amount of spores per substrate volume for effective incubation of plant and AMF. The root system of plants inoculated with the highest amount of spores per volume did not reveal any morphological symptom, indicating pathogen like interactions, thus the lower degree of root colonization might be caused by either an competition of spores on each other or on negative influencing spore/host root interactions.

Spore type

The long-term research on *Glomus etunicatum* allowed to distinguish three spore types in permanent AMF cultures on maize or parsley as hosts (Table 2). Spores isolated from experimental cultures younger than 60 days generally do not contain spore types 2 and 3. Thus it is assumed that spore types 2 and 3 are later aging stages of *G. etunicatum* spores, and spore type 1 is the youngest infective spore type. All three spore types are able to germinate and have successfully been used for infection studies. In single spore inoculations, under time limiting conditions, only the spore type 1 led to root colonizations. Besides its morphological parameters the number of nuclei found after DAPI-staining is strikingly different, spore type 1 reveals about 25 time more nuclei than spore types 2 and 3 (Table 2).

Table 2. Spore types within a population of *Glomus etunicatum* after long term cultivation on various hosts

Spore type	Sample size	Diameter (μm)	Colour	Muronym*	Nuclei (n/spore)
1	29	85.9± 9.6	White	A (EL)	247.5±40.4
2	18	93.2±23.2	Yellow	A (L)	7.1± 4.8
3	11	116.7±29.1	Brown	A (L)	9.5± 3.9

*The classification of the wall composition follows the description of Schenck and Perez (1988): A = wall group A; E = evanescent wall; L = laminated wall; nuclei content after DAPI staining. The spores were collected randomly from a population of strain HH13.

Table 3. Growth and colonization parameters of *Petroselinum crispum* under the influence of AMF strains (*Glomus etunicatum* HH6, HH13; *Glomus intraradices* 267)

Plant and fungal parameter	Mycorrhizal strains	Plant developmental phases		
		III	IV	V
Leaf area per plant (cm^2)	Control	27.0±0.1	160±0.1	211±0.1
	HH 6	34.3±0.1	179±0.2	240±0.2
	HH 13	34.4±0.1	182±0.2	243±0.3
	HH 267	33.6±0.2	177±0.2	235±0.2
Leaf number per plant (n)	Control	12±1.0	21±1.0	24±2.0
	HH 6	14±1.0	23±0.0	27±1.0
	HH 13	14±1.0	23±0.0	27±1.0
	HH 267	13±1.0	22±1.0	25±1.0
Ramification of the root system (n)	Control	5±0.0	10±0.0	10±0.0
	HH 6	2±0.0	5±0.0	5±0.0
	HH 13	2±0.0	5±0.0	5±0.0
	HH 267	4±0.0	7±0.0	7±0.0
Root length (cm)	Control	83±1.2	1,052±60.5	1,969± 44.2
	HH 6	60±8.1	762±39.8	1,446± 61.1
	HH 13	53±5.2	619± 1.1	1,134±124.6
	HH 267	81±6.0	936±11.3	1,714± 23.9
Colonized root length (cm)	Control	0	0	0
	HH 6	33±3.9	450±26.2	1,148±125.4
	HH 13	21±1.8	282±23.2	902± 91.3
	HH 267	24±1.9	281±19.2	514± 48.2
Degree of root colonization (%)	HH 6	55±11.2	60±9.1	79±13.6
	HH 13	40±8.9	46±11.1	80±11.4
	HH 267	30±2.3	30±5.6	30± 4.5
Colonization intensity (%) in root tip segments (1 cm)	HH 6	56±15.4	68±21.3	95±23.3
	HH 13	47±12.3	51±18.7	85±17.7
	HH 267	12± 8.5	15± 5.8	15± 6.6
Arbuscules in root tip segments (1 cm) (n)	HH 6	27±12.6	5±1.7	12±12.4
	HH 13	20± 9.7	9±7.6	15± 7.6
	HH 267	5± 2.3	5±3.3	5± 2.2
Vesicles in root tip segments (1 cm) (n)	HH 6	47±25.2	66±18.6	189±122.4
	HH 13	46±13.7	56±28.3	95± 86.6
	HH 267	8± 3.4	10± 5.4	10± 5.5

The plants were grown in climate chambers under the following conditions: light of 360 µE m^{-2} s^{-1}, 14 h/d; 60–80% relative humidity; 18–20°C at night, 22–24°C during the day; irrigation below field capacity; fertilization once per week with 10% pot volume of a nutrient solution, pH 5.5, containing 15% N (10% nitrate, 5% ammonium), 7% P_2O_5, 22% K_2O, 6% MgO, 0.03% B, 0.05% Mn, 0.01% Zn.

Considering the development of a standardized test system, it must be underlined that successful inoculation depends on various factors. Using *G. etunicatum* the application of two to four young spores, type 1, per cm^3 substrate to phase III plants of *P. crispum* is most effective.

Comparison of strains and spores

Germinating parsley plants (phase I) were inoculated with spore populations (100 spores) of different AMF strains, *G. etunicatum* HH6, HH13 and *G. intraradices* HH267. The root tips of phase III, IV and V plants were analysed qualitatively and quantitatively for the fungal structures (Table 3). Besides the analysis of whole root systems colonized, root tips produced in each phase were used to obtain data on the formation of mycorrhizal structures.

Inoculated plants undergo distinct changes in the development of their root system: Ramification and root length growth were reduced significantly in plants inoculated with *G. etunicatum* HH 6 and HH 13, whereas root systems of plants inoculated with *G. intraradices* developed similarly to the control plants. Degree of colonization and formation of root system structure varied significantly with the strain used (Table 3).

The selective response of the parsley plants to the two strains of *G. etunicatum* and the species *G. intraradices* gives the chance to study the fungal influence on the macrosymbiont regarding the fungal developmental stage. The analysis is facilitated using clearly defined plant cultures and exactly described developmental stages of the plants. Low standard deviations in Table 3 indicate the homogenous constitution of the test plant population. The influence of the microsymbiont on both, the morphogenetic and the physiological responses can easily be detected using such a standardized systems with plants of identical physiological status.

Long term studies

The influence of the plant on its microsymbiont is often discussed, however, experimental systems to analyse the characteristics of spore populations produced after experimental inoculation are rarely developed. Using the parsley system the mycorrhizal effectiveness index (MEI) sensu Plenchette et al. (1983) was estimated in strains, propagated over three years on maize. The derivating spore populations were used each time to reinoculate maize and to inoculate parsley as macrosymbionts. The MEI decreased on maize in all combinations. On parsley, not used for inoculum propagation but inoculated with the fungal material from maize, the MEI rose with *G. etunicatum* HH6 and *G. intraradices* HH267, but decreased with *G. etunicatum* HH13 (Table 4).

These differences in MEI led to the assumption that during cultivation and propagation of the AMF strain on maize the fungi adapted to the macrosymbiont in a way, that a contribution of the microsymbiont to the plant development decreased without significant impairments of the fungal development and spore formation.

When parsley is used for permanent cultivation and propagation of AM fungi, the same decrease of MEI with various populations of *G. etunicatum* could be detected as compared to maize (Table 5). A change from decreased to enhanced MEI was never determined. Strains without any growth promoting effect as HH 476, HH 271, HH 236 remained ineffective throughout an experimental period of 23 to 39 months.

These results indicate changes in the populations of microsymbiont, leading from effective to ineffective strain characteristics, meaning that selective processes take place in short times of AMF cultivation on one single host. The importance of these findings for inoculum production in commercial scale are discussed in detail.

4. Discussion

The estimation and prediction of effectiveness of an arbuscular mycorrhizal symbiosis is the main interest of everyone, who wants to use the symbiosis as a management factor in plant production systems. Because of the recalcitrance of the obligate biotrophic AM fungi to pure, axenic culture the study of host selectivity and changes in strain characteristics is hampered (Giovanetti and Gianinazzi-Pearson, 1994). We know that the host genotype/AMF genotype combination under influence of certain environmental factors modulates the expression of a mycorrhizal effect and determines the effectiveness of a mycorrhizal symbiosis.

The range of mycorrhizal effectiveness varies from mutualistic to parasitic symbiosis: Varma and Schuepp (1994) found positive and negative effects of *Glomus intraradices* depending on the host variety and the environment. Hendrix et al. (1992) found parasitic influence of *Glomus macrocarpum* on tobacco while some other authors report positive growth response of different host to the inoculation with that AMF (Blaszkowski, 1993; Declerck et al., 1995; Srivastava and Mukerji, 1995).

It is only marginally understood how such variability of effectiveness can be evolved. The polynuclear nature of AMF spores at least partially could explain the genetic base (Wood and Cummings, 1992), e.g. if heterocaryosis does exist. There is evidence for preference between the interacting symbiotic partners on the species level (Dhillion, 1992). Probably preferential development of the

Table 4. Reproducibility of mycorrhizal effectiveness (mycorrhizal effectiveness index) on *Petroselinum crispum* seedlings after sequential inoculum production on *Zea mays*

Mycorrhizal strains	Test year	Mycorrhizal effectiveness on host plant (MEI)	
		Zea mays	*Petroselinum crispum*
Glomus etunicatum	1.	20±3.2	9±1.4
HH6	2.	15±1.4	13±2.1
	3.	7±0.9	21±1.9
Glomus etunicatum	1.	37±2.8	11±1.0
HH13	2.	14±1.1	−8±1.7
	3.	14±0.8	−4±0.7
Glomus intraradices	1.	24±2.7	−13±3.8
HH 267	2.	4±1.3	−5±1.2
	3.	5±0.5	21±2.9

The inoculum production for each test on AMF effectiveness on *Petroselinum crispum* cv. "Mooskrause" was carried out in the same year on *Zea mays* cv. "Felix". The given data for *Zea mays* demonstrate the effectiveness of the AMF strains during the inoculum production process. The mycorrhizal effectiveness index (MEI) was calculated sensu Plenchette et al. (1983). n=15, three repetitions of the experiment.

Table 5. Effectiveness (MEI) of AMF strains (*Glomus etunicatum*) after permanent cultivation on *Petroselinum crispum*

Mycorrhizal strains	Cultivation period (months)	Initial MEI	Final MEI after permanent cultivation
HH13, population 1	39	34±2.5	3±0.3
HH13, population 2	28	30±1.9	15±1.3
HH13, population 3	26	24±0.4	5±0.9
HH13, population 4	20	35±0.3	11±1.2
HH6, population 1	39	15±0.6	−2±0.1
HH6, population 2	28	24±0.6	5±0.3
HH476	23	7±1.3	8±0.4
HH271	23	−3±1.1	0±0.2
HH236	23	−10±1.3	0±0.1

Biomass (fresh weight) of non-mycorrhizal control plants: 14.6±1.2 g; mycorrhizal effectiveness index (MEI) calculated sensu Plenchette et al. (1983). Plant cultivation was carried out in greenhouses without additional light from May to October. Further management: standard conditions as given in Material and Methods.

symbiosis between distinct genotypes will be found in future studies, besides the fact that we do not know how this could work.

There are already many controverse data which give advice to analyse population biological aspects of such phenomena. The strain inherent variation of AMF genotypes and the variability of AMF could be a key for the understanding of several complicated interactions between the symbionts. The elements of the strains are its single spores. If a population of AMF is composed by individual spores with distinct characteristics, e.g. distinct potential to create effects in hosts, a lot of contradictory observation could be explained. But actually nothing is known about the strain inherent variation and variability of AMF.

To be able to make a first step to elucidate the strain inherent variability of *Glomus etunicatum* we developed a test system using parsley as host. Parsley was easy to handle under laboratory conditions, reproducibly grown in large numbers on small space, well defined according to the genetic background and furthermore easy to analyse with respect to morphometric, physiological, biochemical and gene-technological methods (Jahnen and Hahlbrock, 1988). Another aspect of this system was a phase of a slow developing root system consisting of easy to analyse fine roots for mycorrhizal colonization. *P. crispum* is available as maternally defined seed material of several varieties and, above all, has been studied on various aspects of plant-pathogen interaction (Franken and Gnaedinger, 1994).

By using the pot size as an important variable it was possible to compare single spore effectiveness with the effectiveness of populations of competing individual spores. This opens the chance to population biological studies. Additionally to that the consequent management of the naturally biannual plant species *Petroselinum crispum* – especially the removal of the inflorescence – allows long term studies over several years.

The preceded fitting of cultivation conditions (data not shown here, Feldmann, 1997) clearly pointed out that parsley var. "Mooskrause" is a facultatively mycotroph host for *G. etunicatum*. Environmental conditions, e.g. the P-concentration in the soil solution, have drastic influence to the degree of colonization and effectiveness. The facultative mycotrophy of the test plant is an important advantage of the developed test system. Only a facultatively mycotroph host can show incompatibility triggered by environmental factors (sensu Janos, 1987) or internal plant and fungal factors. Therefore especially facultatively mycotroph hosts are suitable for detailed studies of host/fungus interrelationships.

In contrast, obligately mycotroph plants like Manihot esculenta as used by Sieverding (1991) can be useful e.g. in the quality control of commercial inoculum (Feldmann and Boyle, 1997): if a tested inoculum is not effective on

obligately mycotroph plants, the probability that it will be effective in facultatively mycotroph hosts is low.

The developed test system using parsley as host will be applied to population biological studies including the determination of single spore effectiveness (Feldmann, 1998) and agro-ecological studies on AMF in progress of a project to recultivate degraded plantations in the humid tropic of Brazil (Feldmann et al., 1995).

Acknowledgements

REFERENCES

Blaszkowski, J. 1993. Effects of five *Glomus* spp. (Zygomycetes) on growth and mineral nutrition of *Triticum aestivum* L. *Acta Mycologica* **28**: 201–210.

Declerck, S., Plenchette, C., and Strullu, D.G. 1995. Mycorrhizal dependency of banana (*Musa acuminata*, AAA group) cultivar. *Plant and Soil* **176**: 183–187.

Dhillion, S.S. 1992. Evidence for host mycorrhizal preference in native grassland species. *Mycological Research* **96**: 359–362.

Feldmann, F. 1997. Stability and reproducibility of AM effectiveness. *Mitteilungen der Biologischen Bundesanstalt* **332**: 54–65.

Feldmann, F. 1998. The strain-inherent variability of arbuscular mycorrhizal effectiveness: II. Effectiveness of single spores. *Symbiosis* **25**: 131–143.

Feldmann, F. and Boyle, C. 1997. Qualitätskontrolle von kommerziellem Inokulum arbuskulärer Mykorrhizapilze. *Mitteilungen der Biologischen Bundesanstalt* **332**: 66–81.

Feldmann, F. and Idczak, E. 1992. Inoculum production of VAM fungi for use in tropical nurseries. In: *Methods in Microbiology: Experiments with Mycorrhiza*, A.K. Varma, J.R. Norris, and D.J. Read, eds. pp. **24**: 339–357.

Feldmann, F., Idczak, E., Martins, G., Nunes, J., Gasparotto, L., Preisinger, H., Moraes, V.H.F., and Lieberei, R. 1995. Recultivation of degraded, fallow lying areas in central Amazonia with equilibrated polycultures: Response of useful plants to inoculation with VA-mycorrhizal fungi. *Angewandte Botanik* **69**: 111–118.

Franken, P. and Gnaedinger, F. 1994. Analysis of parsley arbuscular endomycorrhiza: Infection development of mRNA levels of defense-related genes. *Molecular Plant-Microbe Interactions* **7**: 612–620.

Giovannetti, M. and Gianinazzi-Pearson, V. 1994. Biodiversity in arbuscular mycorrhizal fungi. *Mycological Research* **98**: 705–715.

Hendrix, J.W., Jones, K.J., and Nesmith, W.C. 1992. Control of pathogenic mycorrhial fungi in maintenance of soil productivity by crop rotation. *Journal of Production in Agriculture* **5**: 383–386.

Jahnen, W. and Hahlbrock, K. 1988. Cellular localization of nonhost resistance reactions of parsley (*Petroselinum crispum*) to fungal infection. *Planta* **173**: 197–204.

Janos, D.P. 1987. VA mycorrhizas in humid tropical ecosystems. In: *Ecophysiology of VA Mycorrhizal Plants*, G.R. Safir, ed. CRC Press, FL, USA, pp. 107–134.

Panwar, R., Singh, U.S., and Singh, R.S. 1987. Fluorescent staining of nuclei in filamentous fungi. *Stain Technology* **78**: 205–208.

Plenchette, C., Fortin, and J.A., Furlan, V. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil* **70**: 199–209.

Schenck, N.C. 1984. Methods and principles of mycorrhizal research. *The American Phytopathological Society*, St. Paul, MN, USA, pp. 244.

Schenck, N.C. and Perez, Y. 1988. Manual for the identification of VA mycorrhizal fungi. *INVAM*, 1453 Fifield Hall, University of Florida, Gainesville, FL, USA. pp. 241

Schmelzer, E., Krüger-Lebus, S., and Hahlbrock, K. 1989. Temporal and spatial patterns of gene expression around sites of attempted fungal infection in parsley leaves. *The Plant Cell* **1**: 993–1001.

Sieverding, E. 1991. Vesicular-arbuscular mycorrhiza management in tropical ecosystems. Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn.

Srivastava, D. and Mukerji, K.G. 1995. Field response of mycorrhizal and nonmycorrhizal *Medicago sativa* var. local in the F1 generation. *Mycorrhiza* **5**: 219–221.

Varma, A. and Schuepp, H. 1994. Infectivity and effectiveness of *Glomus intraradices* on micropropagated plants. *Mycorrhiza* **5**: 29–37.

Wood, T. and Cummings, B. 1992. Biotechnology and the future of VAM commercialization. In: *Mycorrhizal Functioning*, M. J. Allen, ed. Chapman & Hall, New York, pp. 468–487.