Effect of inoculation with arbuscular mycorrhizas on rooting, weaning and subsequent growth of micropropagated *Malus* (L.) Moench

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The importance of different rooting methods and the effects of arbuscular mycorrhizal (AMF) inoculation on the rooting rate, weaning survival and subsequent growth of microcuttings of *Malus* rootstock YP and the Finnish crab apple cultivars Hanna and Marjatta were studied in four experiments conducted at the Laukaa Research and Elite Plant Unit, Finland. Sucrose in the *in vitro* rooting medium gave the best support to weaning survival in comparision with glucose and fructose. Directly rooted microcuttings had a higher survival rate than *in vitro* rooted microcuttings. AMF inoculation did not improve the rooting rate in direct rooting. However, the *Glomus hoi* strain V98 caused severe rotting of microcuttings and thus lowered the rooting rate of cv. Hanna. AMF inoculation increased the mean shoot height of established plants, particularly inoculation with *G. hoi* V98, *G. claroideum* V43a or *G. fistulosum* V128. This effect, however, varied considerably in different host-fungus combinations. Some AMF strains, e.g. *G. hoi* V104, caused strong growth retardation. After the rooting and weaning stage, many uninoculated plants lapsed into arrest of growth. This phenomenon was less frequent in AMF inoculated plants. Optimal timing of AMF inoculation and nutritional level of rooting and weaning substrate are discussed.

Key words: Apple rootstock, crab apple, carbon drain, *Glomus claroideum*, *G. fistulosum*, *G. hoi*, *G. mosseae*, rooting of microcuttings

Introduction

The beneficial effect of arbuscular mycorrhizal fungi (AMF) on the growth of plants is well documented (MOSSE 1973, HARLEY and SMITH 1983). Apple seedlings are usually reported to show a strong positive growth response to inoculation with AMF (BENSON and COVEY 1976, HOEPFNER et al. 1983, GNEKOW and MARSCHNER 1989, AN et al. 1993), even at moderate or high phosphorus availability (PLENCHETTE et al. 1981, GNEKOW and MARSCHNER 1989). In micropropagated apple plants AMF may be even more beneficial than in seedlings (GIANINAZZI et al. 1990, BRANZANTI et al. 1992), although there are problems such as high sensitivity of apple microcuttings to rot following inoculation, particularly in direct rooting (AVANZATO et al. 1994).

The basic principles in rooting of apple microcuttings are rooting *in vitro* on agar gelified medium or direct, *post vitro* rooting on artificial or compost substrate (DIRR and HEUSER 1987). Also combining of these two rooting methods was shown to be successful on rooting of apple microcuttings (ZIMMERMAN and FORDHAM 1985). In rooting of apple microcuttings, there may be large

fluctuations due to various accidental external factors (DENISSEN et al. 1992). The timing of harvesting shoots from the subculture medium (DE KLERK and TER BRUGGE 1992, UOSUKAINEN 1992) and the type of carbohydrate used in a gelified rooting medium (MONCOUSIN et al. 1992, UOSUKAINEN 1992) are crucial. A too long auxin treatment may lead to rooting inhibition and disturbances in the apical growth of micropropagated shoots (GEORGE and SHERRINGTON 1988). Also light, photoperiod, temperature and macronutrients of the rooting medium have an effect on root initiation, root growth and the quality of the root system (ZIMMERMAN and FORDHAM 1985, GEORGE and SHERRINGTON 1988).

A good rooting result in the laboratory does not always lead to good weaning and undisturbed continuous growth of the rooted plantlets. There may be great losses after transplanting to soil in greenhouses or in growth chambers (PREECE and SUTTER 1991). Microcuttings, rooted on agar, have difficulty in inducing a fully effective root system when the plants are transferred to soil (DE BERGH and MAENE 1981, GEORGE and SHERRINGTON 1988, PREECE and SUTTER 1991, UOSUKAINEN 1992). During the rooting process, problems may arise also from internal latent bacterial contaminants (BOXUS and TERZI 1985, 1988, CONSTANTINE 1986, YEOMAN 1986, POULSEN 1988, LEIFERT et al. 1991, SAVELA and UOSUKAINEN 1994). Also the quality and texture of the growth substrate and the thermohydrical stress influence the rooting and survival of rooted plantlets, particularly in direct rooting (AVANZATO et al. 1993).

Good rooting techniques are needed to obtain plant material of uniform quality which, together with correct timing of AMF inoculation, are essential factors in AMF utilization. The aim of this study was to optimize these two steps and to minimize the effect of accidental external factors in AMF utilization.

Material and methods

The study consisted of four experiments. Apple rootstock YP, and two Finnish crab apple culti-

vars, Hanna and Marjatta, were used in this study. All these cultivars are offspring of *Malus baccata* (L.) Moench. Both 'Hanna' and 'Marjatta' cultures consisted of one meristem clone, but in the YP experiments, two meristem clones (YP–130 and YP–148) were used. Microcuttings were 26–35 mm long and harvested from a proliferation medium (UOSUKAINEN 1992) six weeks after subculture. In the first three experiments microcuttings were rooted in test tubes, size 22 mm x 150 mm, sealed with aluminium caps allowing gas exchange. An acute auxin treatment (LANE and McDOUGALD 1982) for root induction was given by immersing the microcuttings in 100 mg/l IBA 1:1 alcohol-water solution for 15 min. During the root initiation phase microcuttings were on the hormone-free rooting medium (UOSUKAINEN 1992). Rooted microcuttings were transplanted to soil after 7 weeks. In experiment 4, the direct rooting method, combined with a procedure of acute auxin treatment, was used. Shoots were dipped for 7 sec in a water solution of NaOH (0.5N) dissolved IBA (100 mg/l). After dipping the microcuttings were stuck directly in rooting substrate.

The AMF strains used as inoculants were *Glomus claroideum* Schenck & Smith strain V43a (experiment 2), *G. hoi* Berch & Trappe strains V98 (exps. 1 and 4) and V104 (exps. 1 and 2), *G. mosseae* (Nicol. & Gerd.) Gerdemann & Trappe strain V57 (exp. 4) and *G. fistulosum* Skou & Jakobsen strains V92 (exp. 1), V107 (exp. 1) and V128 (exp. 4). The inoculum was a mixture of infected maize roots, hyphae, spores and growth substrate. As a control, a similar soil and root inoculum without AMF was used. Inocula (1 ml/microcutting) were placed in the planting hole prior to transplantation.

Root colonization by AM fungi was estimated at the end of the experiment 1 and at the end of the first repetition of the experiment 4 using the grid-line intersect method (GIOVANNETTI and MOSSE 1980) for root samples stained in 0.05 acid fucsin according to KORMANIK and McGRAW (1982). Statistical analyses were done using SAS, and means were separated by using the Duncan's Multiple Range Test.

In YP experiment 1, the effects of fructose, glucose and sucrose (30 g/l), as carbohydrate source in the *in vitro* rooting medium, combined with AMF inoculation at transplanting to soil, on the survival and growth of 76 micropropagated YP plants were studied in a pot experiment with seven replicates. There were five mycorrhizal treatments: (1) uninoculated (No AMF), (2) inoculated with V92, (3) inoculated with V107, (4) inoculated with V98 and (5) inoculated with V104. Rooted, 7-week-old microcuttings were planted in 0.25 l plastic Vefi pots containing a substrate mixture of steam sterilized peat and sand (3:1) limed with 4 g/l Dolomite lime (Saxo oy, Finland) to give an approximate pH of 6, and fertilized with 1.8 g/l of the controlled-release fertilizer Osmocote Plus (8–9 months, 18N:11P:10K, Sierra Ltd, UK). The plants were transplanted to soil and inoculated on September 2, 1991. Plants were kept in continuous growth in a greenhouse for 34 weeks in long day. From October to March, plants were given artificial light (Philips HPI 400 W). The daily temperature was 18°C during the light photoperiod and 15°C in the dark. The experiment was completed on April 24, 1992. Shoot height was recorded at 18 weeks, 24 weeks and 34 weeks after inoculation.

In YP experiment 2, the effect of AMF inoculation on the subsequent growth during the first two growing seasons of *in vitro* rooted YP microcuttings was studied in a pot experiment with 14 replicates. Rooted YP microcuttings, altogether 78 plants, originated from a rooting medium containing sucrose 30 g/l. There were four AMF treatmens: (1) uninoculated, (2) inoculated with V43a, (3) inoculated with V104 and (4) inoculated with a mixture of V43a and V104. Rooted microcuttings were planted in 0.25 l plastic Vefi pots containing a substrate mixture of steam sterilized peat, sand and finesand (1:1:1). The mixture was limed with 3 g/l Dolomite lime (pH of the mixture about 6) and fertilized with 0.5 g/l of Osmocote Plus (8–9 months).

The rooted plantlets were transplanted to soil and inoculated on January 9, 1992. Plants were in continuous growth in the greenhouse until May 1992. The plants were kept in long day under artificial light (Philips SON-T Agro 400 W) from January to March 1992. The daily temperature was 18°C during the light photoperiod and 15°C in the dark. In May 1992, the plants were replanted in 0.5 l black plastic pots and kept outdoors from May to September 1992. At the beginning of October 1992, the plants were replanted in 2 l plastic bag containers and stored at 4°C in the dark until the beginning of May 1993. After the cold storage, the plants were kept outdoors until October 11, 1993, whereafter the experiment was finished. During the first growing season, shoot heights were recorded 9 weeks after transplanting, on the second growing season, immediately after winter storage and again at the end of the season, 76 and 91 weeks after transplanting and inoculation, respectively. Also shoot base diameter was measured after 76 and 91 weeks, respectively.

In YP experiment 3, the optimal timing for transplanting microcuttings to soil was studied on apple rootstock YP. Rooting and survival were compared between microcuttings kept on hormone-free *in vitro* rooting medium for 0 (=direct rooting), 2, 3, 4 or 5 weeks before transplanting to soil. The experiment was repeated four times between June 1992 and January 1993. The rooting and weaning survival of 480–500 shoots in each treatment, altogether 2457 shoots, was estimated. The rooting rate was recorded before transplanting. Survival rates were estimated after two months in the greenhouse. Also pH of the rooting medium was measured during the *in vitro* rooting stage.

In crab apple experiment 4, the effect of AMF inoculation on the rooting rate of crab apple cultivars Hanna and Marjatta was studied by using direct rooting with simultaneous AMF inoculation with three different AMF and a control inoculum. Inoculations with control (No AMF) and AMF V128 were repeated four times. Inoculations with AMF V57 and AMF V98 were repeated 3 and 2 times, respectively. Repetitions were started on February 18th, on April 20, on July 5 and on November 4 in 1993. Altogether 334 microcuttings of cv. Marjatta and 241 microcuttings of cv. Hanna were stuck. Steam sterilized rooting

substrate, peat:sand:perlite (4:1:5), consisted of peat (Vapo Peat XL/B0, Finland), sand (local, 0.5–1.5 mm) and perlite (coarse, 3–5 mm, Nordisk Perlite, Denmark). The substrate was fertilized with 1 g/l Osmocote Plus (8–9 months) and Dolomite lime 5 g/l. Microcuttings were rooted in plant propagators (WARD Seed Tray 355 mm x 215 mm with Seed Tray Cover Ward Reg. Des. N:o 938270), one treatment/tray during rooting and weaning. Trays were placed for four weeks into a growth chamber. The light source consisted of a combination of fluorescent lamps (Warm White Super and Floralux), 3500 lux-3700 lux inside the propagators. The temperature during the rooting and weaning periods was 20°C during a 16-hour light period and 16–18°C during the 8-hour dark period. Rooting and weaning were estimated after four weeks.

After the rooting and weaning stage, subsequent growth of the microcuttings, which were transplanted to soil and inoculated with *G. fistulosum* V128 on February 18th, was also estimated. Two months after transplanting and inoculation, plant heights were measured, small plants were replanted in plastic pots (12 cm) and potted plants were placed in a randomized order on a table in the greenhouse. The potting substrate was similar to the rooting substrate. Plant heights were measured on May 6th, 2.7 months after inoculation, again on June 24th, during the most active growth, and finally in the autumn, on September 15th, 7 months after transplanting to soil.

Results

In YP experiment 1, weaning survival of apple rootstock YP was highly dependent on the carbohydrate source of the *in vitro* rooting medium. After 18 weeks in the greenhouse, the weaning survival rate was 0%, 21.1% and 50% for fructose, glucose and sucrose, respectively. Due to the poor survival rates, the interactions between the carbohydrates and the mycorrhizal fungi were not recorded after the weaning stage.

AMF inoculation increased the mean shoot heights, especially at 24 and 34 weeks. This effect
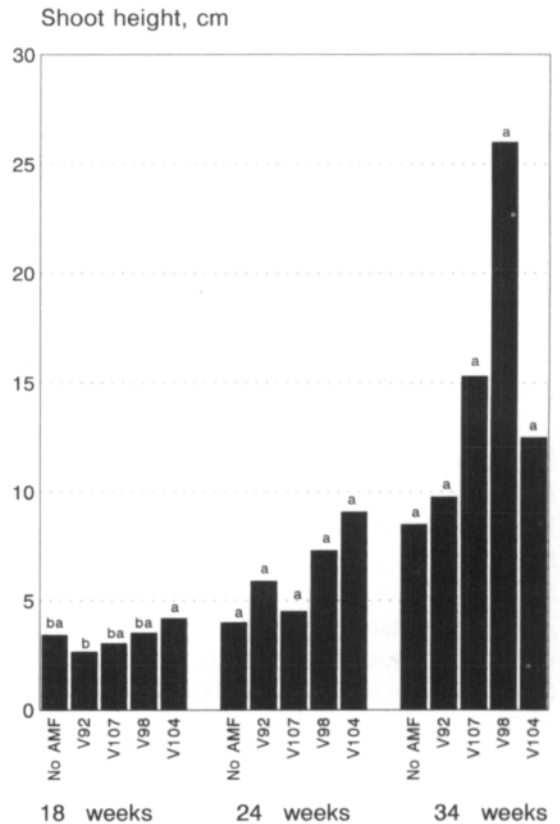
Shoot height, cm

Fig. 1. Effect of arbuscular mycorrhizal (AMF) inoculation (V92, V107 = *G. fistulosum*; V98, V104 = *G.hoi*) on shoot height of apple rootsock YP at three different times. For each time, values sharing the same letter do not differ at p=0.05 according to Duncan's multiple range test. No AMF = non-inoculated control plants.

was, however, not statistically significant (Fig. 1). Plants inoculated with *G. hoi* V98 resulted eventually in 3 times higher plants than uninoculated ones. Also a higher rate of uninoculated plants lapsed into arrest of growth than inoculated plants, eventually (Table 1). At 18 weeks, 22% of uninoculated and 18 % of inoculated plants were growing vigorously, being >5 cm high. At 24 weeks, 12.5% of uninoculated and 22% of inoculated plants were >10 cm high, at 34 weeks 12.5% and 55.5%, respectively. At the end of the experiment, the AMF colonization in inoculated plants varied from 25% (V92) to 37.5% (V98) . Due to spread of AMF from adjacent pots, AMF colonization was 6.3% in the uninoculated plants.

Table 1. Effect of arbuscular mycorrhizal (AMF) inocula-
tion (V92, V107 = G.fistulosum; V104 = G. hoi) on the
proportion (%, as means of 4–9 surviving plants) of ap-
ple rootstock YP plants in vigorous growth (i.e. plants
>100 mm) in height, at 24 weeks and 34 weeks after onset
of pot experiment. No AMF = non-inoculated control
plants.

Mycorrhizal treatment	Plants in vigorous growth, %			Root colonization, %
	18 weeks	24 weeks	34 weeks	34 weeks
No AMF	22.2	12.5	12.5	6.3b
V92	0	25.0	25.0	25.0ba
V107	25.0	0	40.0	30.0a
V98	33.3	20.0	100.0	37.5a
V104	14.3	42.9	57.1	26.4ba
Inoculated, mean	18.2	22.0	55.5	29.7

The results from the YP experiment 2 are il-
lustrated in Figure 2. After nine weeks, there
was a significant growth increase in YP root-
stocks due to inoculation with *G. claroideum*
V43a. The other AMF strains also caused a mean
growth increase, which was, however, not statis-
tically significant. At 76 weeks, after cold stor-
age and one month of new growth, the positive
effects of inoculation had decreased. However,
plants in the treament V43a + V104 were, on
average, still bigger than plants in other treat-
ments, but the differences were not statistically
significant. Prior to cold storage, this treatment
had the shortest plants. Plants inoculated with
V104 alone were poor in growth after cold stor-
age. There were only small differences in the
stem base diameter between inoculated and unin-
oculated plants. After cold storage, V104 showed
also the smallest stem base diameter which cor-
relates with the poor growth in this treatment.
The uninoculated treatment had the highest pro-
portion of surviving plants throughout the exper-
iment, the percentage being 86% at the end of
the experiment. Corresponding percentages for
the AMF treatments V43a, V104 and V43a+V104
were 57%, 29% and 64%, respectively. Root
infection was not evaluated in this experiment.

At 9 weeks, the proportion of plants in active
growth (>10 cm high) was greater in all AMF
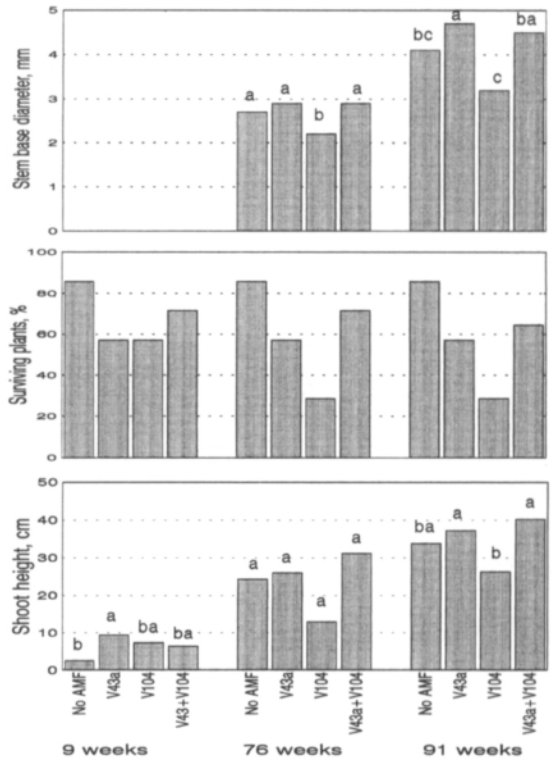treatments (Table 2) than in the control. At 76

Fig. 2. Effect of arbuscular mycorrhizal (AMF) inocula-
tion (V43a = *G. claroideum*, V104 = *G. hoi*) on shoot
height, proportion of surviving plants and stem base di-
ameter of apple rootstock YP at three different times. For
each time, values sharing the same letter do not differ at
p=0.05 according to Duncan's multiple range test. No AMF
= non-inoculated control plants.

and 91 weeks, however, inoculation with *G. hoi*
V104 had caused a strong growth retardation,
while 100% of the plants inoculated with the com-
bination of V43 and V104 were >30 cm high,
eventually.

In YP experiment 3, the roots started to emerge
after 10 days on the *in vitro* rooting medium. The
mean rooting rate of microcuttings at transplant-
ing and the mean weaning survival of the same
plantlets after 2 months in the greenhouse showed
negative correlation (Fig. 3). Microcuttings that
had been on the *in vitro* rooting medium for 5
weeks had the highest mean rooting rate, 41%,
but the mean survival rate was only 27%. Direct-
ly rooted microcuttings had the highest mean sur-
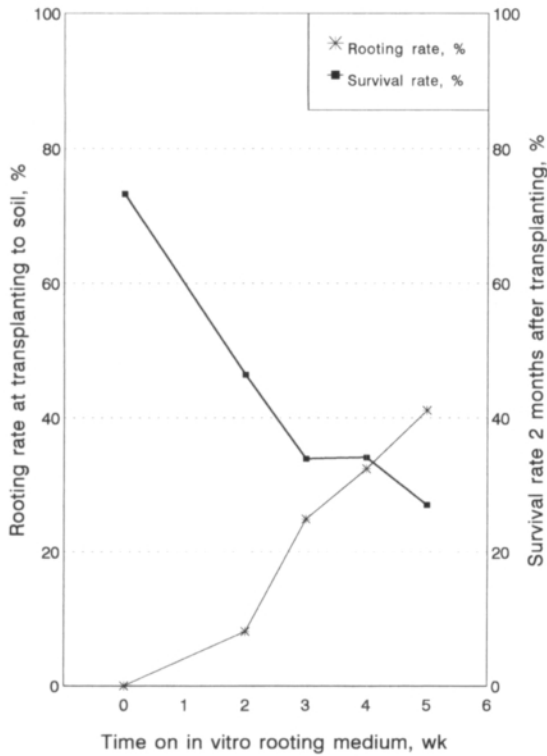vival rate, 73.3%. Although there was seasonal

Fig. 3. Effect of length of *in vitro* rooting phase (wk) on rooting and survival rates (%) of apple rootstock YP microcuttings. Rooting rates were estimated at transplanting to soil and survival rates 2 months after transplanting.
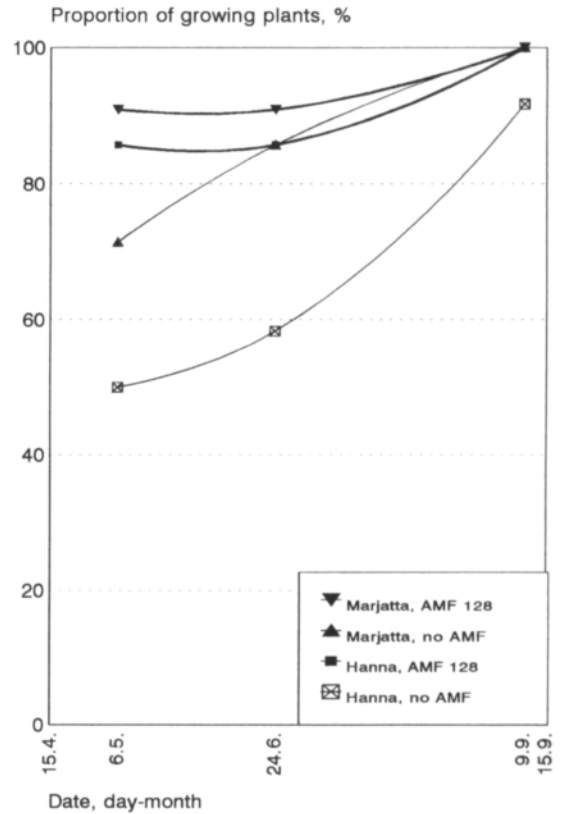

Fig. 4. Effect of arbuscular mycorrhizal (AMF) inoculation (V128 = *G. fistulosum*) on the continuous growth of directly rooted microcuttings of crab apple cultivars Marjatta and Hanna. No AMF = non- inoculated control plants. The proportion of growing plants (%) after the rooting and weaning stage was estimated on May 6, June 24 and September 9 in 1993.

variation in percentual rates between repetitions, the rooting rates correlated with the survival rates. In visual observation, an increasing number of symptoms resembling nutritional deficiency was recorded in the microcuttings during the *in vitro*

Table 2. Effect of arbuscular mycorrhizal (AMF) inoculation (V43a = G. claroideum; V104 = G. hoi) on the proportions (%, as means of 4–12 surviving plants) of apple rootstock YP plants in vigorous growth, i.e. >100 mm high at 9 weeks, >250 mm at 76 weeks and >300 mm at 91 weeks after onset of pot experiment. No AMF = non-inoculated control plants.

AMF treatment	Plants in vigorous growth, %		
	9 weeks	76 weeks	91 weeks
No AMF	0.0	41.7	58.3
V43a	37.5	37.5	75.0
V104	28.6	0.0	25.0
V43+V104	22.2	77.7	100.0

rooting. The pH value of the *in vitro* rooting medium rose during the 5- week period from 4.9 to 6.3.

According to the results obtained from crab apple experiment 4, AMF inoculation did not improve the rooting rate in direct rooting (Table 3). *Malus* cultivars differed in their reaction to different AMF. Particularly AMF V98 seemed to be incompatible with cv. Hanna. In visual observation, at the end of the rooting stage, the uninoculated plantlets seemed to be more vital than the inoculated ones. Most of the inoculated plants had dry or pale green leaves often with a tinge of red. These symptoms were transient in their na-
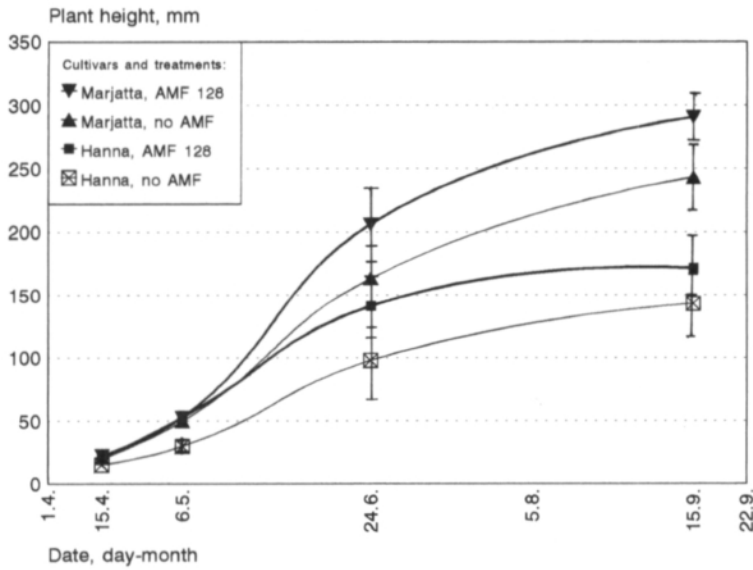
Fig. 5. Effect of arbuscular mycorrhizal (AMF) inoculation (V128 = *G. fistulosum*) on the growth of micropropagated crab apple cultivars Marjatta and Hanna. No AMF = non-inoculated control plants. The plant height (mm) was measured on April 15, May 6, June 24 and September 15.

ture and resembled symptoms caused by nutritional deficiencies.

The plantlets, inoculated with *G. fistulosum* V128, continued their growth and development soon after the weaning stage. The uninoculated plants, instead, had a great tendency to lapse into

Table 3. Effect of arbuscular mycorrhizal (AMF) inoculation on rooting rate (%) of direct rooted microcuttings of crabapple cultivars Marjatta and Hanna, recorded four weeks after inoculation and transplanting to soil. Inoculation treatments (AMF V57 = G. mosseae; AMF V98 = G. hoi; AMF V128 = G. fistulosum and No AMF = no inoculation, given to coincide with transplanting to soil), total number of treated microcuttings and rooting rate (%, as means of 4 repetitions).

Cultivar	Treatment	Number of microcuttings	Rooting rate, %
Marjatta	No AMF	103	77.7
Marjatta	AMF V57	73	80.8
Marjatta	AMF V98	58	69.0
Marjatta	AMF V128	100	65.0
Hanna	No AMF	75	66.7
Hanna	AMF V57	55	63.6
Hanna	AMF V98	40	30.0
Hanna	AMF V128	71	77.5

arrest of growth (Fig. 4). The greatest difference was observed 2.7 months after inoculation and transplanting to soil. At that point, on May 6th, approximately 90% of inoculated plants had started active growth. However, at the same time only about 60% of uninoculated plants were in active growth. Even 4 months after transplanting and inoculation, only 58.3% of uninoculated 'Hanna' plants had started to grow in height. After 7 months, on September 15th, 8.3% of uninoculated 'Hanna' plants had remained at the stage of dormancy.

The growth curves of directly rooted and *G. fistulosum* V128 inoculated 'Marjatta' and 'Hanna' plants (Fig. 5) illustrate that the inoculated plants were, throughout the growing season, at least 19% higher than the uninoculated plants. However, the difference in growth was statistically significant only in cv. Hanna, on May 6th, 2.7 months after transplanting to soil. At that point the inoculated 'Hanna' plants were 76% higher than the uninoculated plants. The major differences in growth decreased towards the end of the growing season. Only sporadic AMF root colonization was observed after 4 or 7 months despite a

moderate to abundant sporulation in cv. Marjatta and a moderate sporulation in cv. Hanna.

Discussion

The experiments conducted with apple rootstock YP and crab apple cultivars Hanna and Marjatta show both a negative and a positive effect from inoculation of AMF fungi at the beginning of the weaning stage of the microcuttings. Of the positive effects, increased growth as a result of inoculation with *Glomus claroideum* V43a, *G. fistulosum* V128 and *G. hoi* V98 can be mentioned. Most of the AMF inoculated plantlets obtained continuous normal growth soon after the weaning stage, but growth lapses of several months were common in uninoculated plantlets. Therefore, the inoculated plants were more uniform at the end of the growing season. From a practical point of view, this means a great benefit for the nursery: due to the balanced growth, the majority of the plantlets can be sized into one class. AMF inoculation can be profitable to nurseries also due to the shorter time needed for raising the plantlets to saleable trees. According to our results, the culture time may be a quarter to one third shorter than with uninoculated plants.

In apple, the benefits of early inoculation of micropropagated plantlets should be re-evaluated. In direct rooting of microcuttings, AMF inoculation did not give such a positive effect on rooting as has been reported on bigger cuttings (JOHNSON et al. 1980, GNEKOW and MARSCHNER 1989). On the contrary, *G. hoi* V98 seemed to be incompatible with crab apple 'Hanna', causing severe rotting of the microcuttings. A similar negative effect of AMF inoculation on *Malus* microcuttings in direct rooting was also observed by AVANZATO et al. (1994) using *G. mosseae* as the inoculant. The poor general condition of the inoculated microcuttings after the rooting and weaning stage, possibly due to nutritional deficiencies, may be caused by too rapid and intense root colonization by the AM fungi.

AMF inoculation of *in vitro* rooted plantlets simultaneously with transplanting to soil did not seem to be beneficial, either. As a rule, in on agar rooted microcuttings, most AMF-host combinations resulted in lower survival rates during plant establishment to soil than no inoculation. Strains belonging to the species *G. hoi*, in particular, seemed to cause severe rotting of the plantlets. In the present study, the rotting of microcuttings and micropropagated plantlets was probably not caused by a pathogen, because (i) the control plants were inoculated with the same soil inoculum but without AMF, (ii) some AMF strains gave better rooting results than others and caused only little or no rotting, (iii), rotting occurred slowly, after three weeks. Thus the rotting did not start until the roots had emerged and root growth had started. Bacterial contaminants that cause rotting of microcuttings (BOXUS and TERZI 1985, 1988, CONSTANTINE 1986, YEOMAN 1986, POULSEN 1988, LEIFERT et al. 1991, SAVELA and UOSUKAINEN 1994) appear virulent earlier and are more acute in their nature. Apple microcuttings need about 4 weeks to develope a good root system and they seem to change to fully autotrophic nutrition slowly. During this period plantlets normally release more CO_2 than they are capable to assimilate through photosynthesis (GEORGE and SHERRINGTON 1988). It is therefore concluded that the rotting of the microcuttings was actually caused by the mycorrhizal fungi. Infection with AMF during the early acclimatization phase probably led to a carbon drain which was not compensated for by the plant during the prevailing stress conditions. Carbon drain is a phenomenon not uncommon in mycorrhizal experiments. GNEKOW and MARSCHNER (1989) suggested that carbon drain takes place in AMF inoculated apple cuttings in a field situation at high P levels.

It is a well-known fact that *Malus* microcuttings are extremely sensitive to environmental stresses (DIRR and HEUSER 1987, AVANZATO et al. 1993). The adaptation to *post vitro* conditions, as such, is an extreme stress to a small plantlet due to a high energy requirement. Therefore, AMF inoculation may become an overwhelming stress for a small apple microcutting at the rooting and weaning stage. If the small plantlet, often in poor nutritional and hormonal balance, is sub-

mitted simultaneously to another extreme stress, such as AMF infection, the result may be a host-parasite relationship instead of a symbiosis.

According to AVANZATO et al. (1994), the rotting might have been due to some pathogen in the inoculum. To overcome the rotting problem, he suggests that the inoculum should be applied as sterilized spores. In France, RAVOLANIRINA et al. (1989) have developed a method of using surface sterilized AMF spores in combination with a *post vitro* rooting procedure. Besides pathogens, there is also a risk of insect pests, and therefore it is equally important to control the sanitary status of AMF inoculum as it is to control the phytosanitary status of plant material (CASSELLS 1991).

Referring to the above, the importance of an optimal rooting method and the correct timing of inoculation must be emphasized. The carbohydrate source of the rooting medium has a great effect on the *in vitro* rooting of apple microcuttings (MONCOUSIN et al. 1992). Rooting experiments conducted with apple cv. Compact Spartan showed that sucrose was an indispensable component in the agar gelified rooting medium (P. Druart, pers. commun.). UOSUKAINEN (1992) showed that both sucrose and glucose in the rooting medium, combined with optimal auxin treatment, resulted in a high rooting rate with apple rootstock YP. The present study showed that the carbohydrate source of the rooting medium has also a great effect on weaning survival of the microcuttings, when *in vitro* rooted plantlets are used. The highest weaning and survival rates of inoculated rooted plantlets were obtained by using sucrose in *in vitro* rooting medium.

Macronutrients are important to root induction, root emergence and root growth, but high salt levels, high nitrogen levels in particular, are frequently inhibitory to root initiation (GEORGE and SHERRINGTON 1988). Rooting experiments with the apple cv. Compact Spartan showed that NH_4NO_3 was an inhibitor of root emergence and further growth. Decreases in average root number/plantlet, smaller roots and a lower rooting rate were typical. On the other hand, the macronutrients KH_2PO_4 and, particularly, $Ca(NO_3)^2$ stimulated root growth (P. Druart, pers. commun.). However, maximum AMF root colonization and sporulation occur in soil of low fertility. Both phosphorus and nitrogen may significantly reduce root colonization if present at high levels and there exists a balance between these two elements (DANIELS HETRICK 1986). Thus, there is a contradiction between the optimal conditions for rooting and AMF root colonization. This contradiction can most likely be reconciled only by using intermediate conditions rather than optimal conditions for one or the other partner of the symbiosis. For example, AMF inoculation after the weaning stage, instead of immediately after the *in vitro* stage, may give a more positive growth response, as was found by AZCON-AGUILAR (1994) in *Annona cherimola*. The level of mineral nutrients in the weaning substrate also needs to be modified to meet the requirements of the developing root systems and undisturbed growth of apple microcuttings. These elements must be further examined and their effects must be controllable before AMF inoculation can be introduced to nursery men.

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SELOSTUS

Arbuskeli-mykorritsasiirrostuksen vaikutus mikrolisättyjen omenapuiden (*Malus*) juurtumiseen, viljelyolosuhteisiin sopeutumiseen ja sopeutumisvaiheen jälkeiseen kasvuun

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Maatalouden tutkimuskeskus

Maatalouden tutkimuskeskuksen Laukaan tutkimus- ja valiotaimiasemalla tutkittiin eri juurrutusmenetelmien sekä arbuskelimykorritsa- eli AMF- siirrostuksen vaikutuksia omenapuun mikropistokkaiden juurtumiseen, kasvihuoneviljelyyn sopeuttamiseen ja sopeuttamisen jälkeiseen kasvuun. Tutkimuksen tavoitteena oli optimoida sekä juurrutus- että siirrostusolosuhteet siten, että satunnaisten tekijöiden häiritsevä vaikutus saataisiin mahdollisimman vähäiseksi ja toisaalta mykorritsasymbioosista saatava hyöty mahdollisimman suureksi.

Tutkimus toteutettiin neljänä kokeena. Koekasveina olivat omenapuun perusrunko YP sekä suomalaiset koriste-omenapuut 'Hanna' ja 'Marjatta'. Tutkittaessa kasvien selviytymistä kasvihuoneviljelyyn sopeuttamisvaiheesta todettiin, että *in vitro*-juurrutusalustan hiilihydraattilähteenä sakkaroosi paransi kasvien selviytymistä verrattaessa glukoosi- ja fruktoosipitoisilla alustoilla juurrutettuihin versoihin. Paras juurrutus- ja sopeuttamistulos saatiin kuitenkin juurruttamalla mikropistokkaat suoraan turvepohjaisella kasvualustalla *in vitro*-juurrutuksen sijasta. AMF-

siirrostus ei parantanut juurtuvuutta suorajuurrutuksessa. *Glomus hoi* kanta V98 aiheutti vaikea-asteista mikropistokkaiden tyvien mätänemistä ja täten jopa heikensi 'Hanna'-lajikkeen juurtuvuutta. AMF-siirrostus kuitenkin lisäsi juurtuneiden kasvien keskimääräistä verson tuorepainoa, erityisesti siirrostus kannoilla *G. hoi* V98, *G. claroideum* V43a tai *G. fistulosum* V128. Tämä vaikutus vaihteli huomattavasti eri isäntä-sieniyhdistelmissä, ja mm. AMF-kanta *G. hoi* V104 jopa heikensi YP-perusrungon pikkutaimien kasvua. Omenapuun mikropistokkaille oli tyypillistä, että taimien kasvu pysähtyi juurrutus- ja sopeuttamisvaiheiden jälkeen. Sekä YP- perusrungon että koriste-omenapuiden AMF-siirrostetuilla taimilla tämä ilmiö jäi selvästi harvinaisemmaksi kuin siirrostamattomilla taimilla.

Tasalaatuinen taimiaines, optimaalinen AMF-siirrostuksen ajankohta sekä juurrutus- ja sopeuttamisvaiheessa käytettyjen kasvualustojen ravinnetaso ovat onnistuneen tuloksen kannalta kriittiset tekijät. Näiden tekijöiden vaikutukset on hallittava ennenkuin AMF-siirrostuksella saadaan aikaan toimiva symbioosi.