THE USE OF ARBUSCULAR MYCORRHIZAL FUNGI IN CONTAINER PRODUCTION OF SELECTED ORNAMENTAL CONIFERS UNDER ORGANIC-MINERAL FERTILIZATION LEVEL

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ABSTRACT

The study investigated the effects of arbuscular mycorrhizal inocula Endorize-Mix, and Endomix, and mode of organic-mineral fertilization, on mycorrhizal colonization and growth of *Chamaecyparis lawsoniana* 'Golden Wonder', *Taxus* × *media* 'Hicksii' and *Thuja occidentalis* 'Smaragd' under a nursery-container production system. The fertilizing combination consisted of compost (0, 10 and 20% v/v) and Osmocote Standard 5-6M (1.5 and 3 g dm^{-3}). The frequency of mycorrhiza in the root system, and growth of plants were evaluated. The use of Endorize-Mix resulted with mycorrhiza formation in all tested plants. The use of Endorize-Mix and Endomix in the container nursery production system allowed for the possibility of achieving well mycorrhized coniferous plants cultivated in pure peat-moss substrate and fertilized at the recommended rate of Osmocote Standard 5-6M. The use of Endorize-Mix ameliorated growth of *C. lawsoniana* and the use of Endomix ameliorated the growth of *T. media* after two years of cultivation.

Key words: *Chamaecyparis*, *Taxus, Thuja*, arbuscular mycorrhiza, compost, slow release fertilizer, nursery production, organic horticulture

INTRODUCTION

Challenges facing the modern nursery business relate to the increasing costs of production, greater regulation, and incorporation of Best Management Practices (BMP). There is a rising demand for the production and marketing of better stress resistant plants to both, environmental and cultivated factors, such as water and/or fertilizer shortage and pest contamination. These demands forced the industry to seek new technological solutions in the cultivation of ornamental trees and shrubs. There are many goals to achieve but the main ones are reducing soluble fertilizer consumption, and reducing irrigation run-off. Also peat moss usage needs to be curtailed because of irreversible environmental devastation as a result of its mining (Josten and Clarke, 2002; Perner et al. 2007). The BMP changes occurring in the nursery industry are highly conductive for mycorrhizal usage. Slow Release Fertilizers (SRF) are much more frequently used than soluble fertilizers and green waste composts are used as organic fertilizer and supplement to potting medium. These treatments seem to be very beneficial for mycorrhizal associations (Linderman and Davies, 2004).

Arbuscular mycorrhizal fungi (AMF) are the most common type of mycorrhiza found in agriculture systems, and used in horticulture. In sustainable productions of horticultural crops, the AMF symbiosis is a crucial component for improving the biological equilibrium between microorganisms in the mycorrhizosphere (Tommerup, 1992). Those plants cultivated in sterile conditions or in growing media devoid of microorganisms are especially prone to react positively for a biodiversity increase of rizosphere. However, the role of the microorganisms is also recognized as critical for the plant response to AMF inoculation. Concomitant use of mycorrhizal fungi with green waste compost (as a source of beneficial microorganisms) can contribute to an increase in positive effects, such as high effectiveness of inoculation, reduction of the use of mineral fertilizers or chemical plant protection products, and increase of nutrient uptake. Use of mycorrhizal fungi with green waste compost can also help overcome transient nutrient and/or water deficiencies (Henderson-Cole and Davies, 1993; Głuszek et al., 2008) In addition, green waste compost added to peat moss-based medium ameliorates physical properties of the medium by preventing its settling. Several studies have dealt with the effect of soil fertilization on AMF activity. However, little is known about the cumulative effect of AMF, green waste compost and SRF, on mycorrhizal colonization and growth, of ornamental conifers cultivated in containers.

The critical factors involved in the sustainable production of mycorrhizal ornamental plants are: the choices of growth substrate and fertilizing method. Therefore, this study investigated the effects of commercially available arbuscular mycorrhizal inocula, and mode of organic-mineral fertilization on mycorrhizal colonization and growth, of three species of ornamental conifers under a nursery-container production system. The selection of commercially available mycorrhizal inocula for our experiment was dictated by how practical it was to apply the products which consist of a broad range of AMF species, already existing in the market.

MATERIAL AND METHODS

The experiment was carried out in the Research Institute of Pomology and Floriculture in Skierniewice, Poland in 2007 and 2008. Rooted cuttings of *Chamaecyparis lawsoniana* (A. Murray) Parl. 'Golden Wonder', *Taxus × media* Rehder 'Hicksii' and *Thuja occidentalis* L. 'Smaragd' were planted in 0.5 dm^3 pots, in the spring of 2007. The next year, plants were transplanted to 3 dm^3 pots. As a base for growing substrate, we used sphagnum peat (Klasmann, pH 5.5-6.0) amended with compost (0, 10 and 20% v/v) and Slow Release Fertilizer (SRF, 1.5 and 3 g dm^{-3}). Thus we had 6 combinations of organic-mineral fertilizer. Commercially available compost (Eko-Kompost, Ekokonsorcjum 'Efekt', Poland, organic matter 46.43%, N 2.44%, P_2O_5 1.39%, K_2O 3.16%, CaO 7.06%, MgO 1.24%, salt concentration 2.2 g dm^{-3}, pH (H_2O) 8.7) is obtained from yard waste and shredded trees and bushes. As a SRF we used Osmocote Standard 5-6 M (16N-11P-11K-3Mg + microelements). Mycorrhization of the plants was carried out by sprinkling the mycorrhizal vaccines directly on roots during planting, according to the manufacturer's recommended rates. We used 2 commercial arbuscular mycorrhizal inocula; Endorize-mix (2.5 g/plant, Agrauxine, France) and Endomix (1 g/plant, Mykoflor, Poland). The control plants were not inoculated. Endorize-Mix contains *Glomus mosseae, G. intraradices* and *G.* spp. Endomix is a mixture of *Glomus etunicatium, G mosseae, G. intraradices, G. fastigatium, G. agregatium.* Plants were cultivated outdoors. Irrigation was applied as needed during rain-free weather via sprinklers located over the plant canopy.

The experiment was a two factorial with six levels of fertilizer (combinations of SRF and compost) and three levels of arbuscular mycorrhizal fungi (AMF) treatments, arranged in a completely randomized design, with five replications.

After the first and second year of cultivation (September) plants were evaluated for height.

For the investigation of mycorrhizal colonization, 3 g fresh root subsamples were randomly taken from 3 plants from each replication. After washing to clear away the soil, and cutting into 1cm fragments, subsamples were cleared by heating with 10% KOH which meant heating in a 90°C water bath for 10 min. Then, subsamples were acidified with 10% HCl. Roots were stained for 2 hours using 0.1% trypan blue in lactogliceroI at 80 °C, then de-stained for 24 hours. Mycorrhizal fungus colonization in the root system (%) was determined according to the method of Trouvelot et al. (1986). Using this method, 30 root fragments from each subsample were taken and mounted on one slide. The fragments were observed under the microscope. Mycorrhizal colonization of each fragment was rated from 0 to 5 according to the range of classes. Obtained values were put into the computer program 'Mycocalc' to calculate frequency of mycorrhiza in the root system. Data were subjected to analysis of variance. Statistical significance was determined at a 5% probability level. Mean separation was carried out with Duncan's Multiple Test.

RESULTS

Mycorrhizal colonization

The use of Endorize-Mix inoculum resulted in high mycorrhizal colonization of roots of all tested plants after the first growing season (Tab. 1). The mean frequency of mycorrhiza in the root system of *C. lawsoniana* was 83%, for *T. media* it was 96%, and for *T. occidentalis* it was 97%. The high level of colonization was maintained in the subsequent year of the plants cultivation in pots. The plants inoculated with Endomix showed weaker mycorrhizal colonization of the roots of *C. lawsoniana* and *T. occidentalis* after the first growing season: 20% and 4% respectively. However, *T. media* plants inoculated with Endomix were highly colonized by AMF at the same level as plants inoculated with Endorize-Mix. After the second growing season, the frequency of mycorrhiza in the root system of *C. lawsoniana* and *T. occidentalis* inoculated with Endomix, increased to 37% and 80% respectively, however the frequency of mycorrhiza in the root system was significantly lower than after application of Endorize-Mix. It is also worth noting that non-inoculated plants showed spontaneous mycorrhizal colonization. After the first growing season, *T. media* plants showed the highest colonization (36%). But after the second year of cultivation, all non-inoculated plants of *C. lawsoniana, T. media* and *T. occidentalis* showed average mycorrhization: 13, 76 and 73%, respectively.

A common nursery practice is to grow ornamental trees and shrubs in peat-moss substrate with the use of slow release fertilizers (SRF). Under these conditions the recommended rate of Osmocote Standard 5-6 M (3 g dm^{-3}) did not significantly affect a formation and presence of mycorrhizal structures in roots of examined coniferous trees inoculated with both of the used AMF inocula, with the exception of a weaker colonization of the roots of *C. lawsoniana* inoculated with Endomix, in respect to less intensive mineral fertilization. But this lack of significance was only true in the first year of the experiment.

A compost addition to peat-moss substrate at the standard mineral fertilization rate (3 g dm^{-3}), generally did not affect mycorrhizal colonization of roots of coniferous plants independent of the use of commercial AMF inocula. However, plants of *T. media* inoculated with Endorize-Mix showed a slightly lower frequency of mycorrhiza in their root systems when they were cultivated in substrate containing 10% compost, compared to plants cultivated with no compost amendment, – but only in first growing season. Plants of *C. lawsoniana* inoculated with Endomix and grown in substrate amended with 20% of compost reacted differently. The plants showed greater mycorrhizal colonization after the second growing

Table 1. Significance levels and interactions between fertilizer and arbuscular mycorrhizal inoculum and effects of these factors on mycorrhizal colonization of *Chamaecyparis lawsoniana* 'Golden Wonder', *Taxus x media* 'Hicksii' and *Thuja occidentalis* 'Smaragd' grown in containers

Factor		Frequency of mycorrhiza in the root system [%]					
		Chamaecyparis lawsoniana 'Golden Wonder'		*Taxus × media* 'Hicksii'		*Thuja occidentalis* 'Smaragd'	
		2007	2008	2007	2008	2007	2008
Significance level							
Mycorrhizal inoculum		**	**	**	**	**	**
Fertilizer		**	**	**	NS	*	**
Mycorrhizal inoculum x Fertilizer		**	**	**	NS	**	NS
Fertilizer							
1.5 g/ 0%		39.7 c[2]	30.1 a	66.6 a	83.3 a	35.4 bc	78.3 ab
1.5 g/10%		32.8 bc	53.3 bc	73.9 ab	92.7 ab	21.4 a	96.0 c
1.5 g/20%		15.6 a	66.8 c	72.9 ab	94.7 b	30.5 a-c	96.7 c
3.0 g/ 0%		27.9 bc	22.3 a	85.5 bc	89.0 ab	42.0 c	64.6 a
3.0 g/10%		25.7 ab	38.0 ab	87.0 bc	89.9 ab	26.3 ab	86.4 bc
3.0 g/20%		35.0 bc	38.6 ab	95.0 c	92.8 ab	32.3 a-c	84.3 a-c
Mycorrhizal inoculum							
Non-inoculated		1.0 a	13.2 a	35.8 a	76.4 a	2.8 a	73.2 a
Endorize-Mix		82.8 c	76.8 c	95.6 b	97.9 b	97.4 b	98.1 b
Endomix		20.1 b	37.0 b	96.0 b	93.0 b	3.8 a	80.0 a
Mycorrhizal inoculum x	Fertilizer[1]						
Non-inoculated	1.5 g/0%	3.6 ab[2]	34.0 cd	14.6 a	47.7 a	0.0 a	67.9 ab
	1.5 g/10%	0.8 a	41.1 c-e	19.3 a	90.0 c-e	1.1 ab	91.3 b-e
	1.5 g/20%	0.3 a	35.3 cd	19.4 a	90.6 c-e	9.8 bc	80.7 a-d
	3.0 g/0%	0.0 a	0.4 ab	15.0 a	64.5 ab	15.2 c	38.8 a
	3.0 g/10%	0.7 a	4.5 ab	78.1 b	73.1 a-c	0.1 a	77.6 a-d
	3.0 g/20%	3.7 ab	0.0 a	76.3 b	83.5 b-d	2.3 ab	75.6 a-d
Endorize-Mix	1.5 g/ 0%	85.9 e	67.5 d-g	88.5 b	95.9 de	100 e	99.6 de
	1.5 g/10%	94.3 e	82.2 fg	96.7 bc	97.2 de	94.7 de	100 e
	1.5 g/20%	46.5 d	90.5 g	97.5 bc	96.4 de	89.9 d	100 e
	3.0 g/ 0%	90.4 e	70.5 d-g	100 c	99.0 de	97.5 de	80.4 a-d
	3.0 /10%	84.9 e	70.1 d-g	84.2 b	95.9 de	95.5 de	98.6 c-e
	3.0 g/20%	84.3 e	76.7 e-g	97.2 bc	100 e	99.9 e	96.7 b-e
Endomix	1.5 g/ 0%	38.3 d	2.4 ab	89.9 bc	93.9 c-e	11.3 bc	49.7 a
	1.5 g/10%	16.0 bc	34.3 cd	90.7 bc	89.5 c-e	0.0 a	90.9 b-e
	1.5 g/20%	16.2 bc	68.7 d-g	86.9 b	96.4 de	3.6 a-c	99.1 de
	3.0 g/ 0%	16.2 bc	16.3 bc	100 c	92.7 c-e	8.9 bc	72.1 a-c
	3.0 g/10%	11.1 bc	50.1 c-f	95.6 bc	95.1 c-e	4.8 a-c	75.4 a-d
	3.0 g/20%	26.6 cd	65.5 d-g	100 c	89.2 c-e	1.6 ab	75.1 a-d

[1]First number means a dose of Osmocote Standard 5-6 M (1.5 or 3.0 g dm^{-3}), second number means a content of compost in a growing medium (0, 10 or 20% v/v)
[2]Means followed by the same letter in columns do not differ significantly at p = 0.05 according to the Duncan Multiple Range Test

season, in respect to plants cultivated in pure peat-moss substrate. It is worth pointing out, that the standard rate of SRF (3 g dm^{-3}) limited spontaneous mycorrhizal colonization of the control plants of *C. lawsoniana*.

A different opposite reaction was observed on plants of *T. media* after the first year of the trial. In that case, we observed a significant increase of spontaneous colonization of non-inoculated plants when we used a higher rate of SRF combined with both a 10 and 20% addition of compost.

It is a general believe that the use of mineral fertilizers in horticultural production limits development of mycorrhiza. On the other hand, the growth and quality of plants cultivated with an insufficient level of nutrients is usually unsatisfactory. For these reasons, an efficient system for fertilizing mycorrhizal plants is desired. The system should be able to deliver well mycorrhized plants with very good growth and quality, which is why mineral fertilizers are partially substituted by organic fertilizers. Our study did not unequivocally confirm that reduced rate of Osmocote Standard 5-6 M (1.5 g dm^{-3}) used simultaneously with 10 and 20% of compost amendment is beneficial for AMF colonization of conifers. What is more, in the case of *C. lawsoniana* and *T. occidentalis* cultivated in peat-moss substrate amended with compost after the first year of the experiment, the plants were generally less colonized by AMF than plants cultivated in substrate with no compost addition, independent of used inoculum. An improvement of root colonization by mycorrhizal fungi was obtained by plants of *C. lawsoniana* and *T. occidentalis* inoculated with Endomix, and fertilized with a reduced rate of SRF combined with compost addition to growing substrate. Although this was true, we observed a significantly higher level of root colonization (91-99%) only in plants of *T. occidentalis*. *T. occidentalis* plants fertilized with the standard rate of SRF were less colonized (75%).

Growth response

The growth of mycorrhizal non-inoculated coniferous plants was not significantly affected either by SRF rate or by using a compost in the peat-moss substrate, even though there were large difference in plants size between species (Tab. 2). Thus, after two years of cultivation in pots, plants of *C. lawsoniana* measured 62 cm, *T. media* 21 cm and *T. occidentalis* 50 cm. Conifer response to AMF inocula were dependent on both, species of tested plants and system of fertilizing. When plants were fertilized in the traditional way (recommended rate of Osmocote 5-6M, no compost addition), the positive effect of Endorize-Mix was observed only on growth of *C. lawsoniana* after two years of cultivation (the plants were higher by 16% than non-inoculated plants). The positive effect of Endomix was observed only on growth of *T. media* also in the second year (the plants were higher by 31% than non-inoculated). However, none of the used vaccines stimulated growth of *T. occidentalis*. The plants of *T. occidentalis* inoculated with Endomix were even shorter after the first growing season than the control, non-inoculated plants. By the second year of cultivation

Table 2. Significance levels and interactions between fertilizer and arbuscular mycorrhizal inoculum and effects of these factors on growth of *Chamaecyparis lawsoniana* 'Golden Wonder', *Taxus x media* 'Hicksii' and *Thuja occidentalis* 'Smaragd' grown in containers

Factor		Height of plants [cm]					
		Chamaecyparis lawsoniana 'Golden Wonder'		*Taxus × media* 'Hicksii'		*Thuja occidentalis* 'Smaragd'	
		2007	2008	2007	2008	2007	2008
Significance level							
Mycorrhizal inoculum		*	**	**	**	**	**
Fertilizer		*	**	*	**	**	**
Mycorrhizal inoculum x Fertilizer		°	°	NS	*	**	**
Fertilizer							
1.5 g/ 0%		22.9 ab[2]	59.9 ab	14.4 a	19.7 a	20.1 d	49.9 bc
1.5 g/10%		25.6 c	63.9 c	14.9 ab	23.3 bc	18.9 cd	48.9 bc
1.5 g/20%		24.3 a-c	56.9 a	14.4 a	21.7 ab	17.2 b	43.8 a
3.0 g/ 0%		23.9 a-c	61.9 bc	16.0 b	24.0 b-d	16.4 ab	48.2 b
3.0 g/10%		25.2 bc	64.2 c	15.6 ab	26.4 d	17.8 bc	55.8 d
3.0 g/20%		22.2 a	59.9 ab	14.7 ab	25.1 cd	15.5 a	52.0 c
Mycorrhizal inoculum							
Non-inoculated		24.6 b	61.5 b	14.1 a	20.8 a	19.1 c	50.3 b
Endorize-Mix		23.0 a	63.4 b	15.6 b	25.1 b	17.7 b	52.2 b
Endomix		24.6 b	58.5 a	15.2 b	24.2 b	16.3 a	46.8 a
Mycorrhizal inoculum x	Fertilizer[1]						
Non-inoculated	1.5 g/0%	24.4 b-d[2]	63.4 c-g	13.7 ab	18.6 a	20.4 g	49.0 b-d
	1.5 g/10%	26.9 d	63.0 c-g	13.9 a-c	18.8 a	18.8 e-g	50.2 b-e
	1.5 g/20%	25.3 b-d	60.4 a-f	13.4 a	19.8 ab	20.1 g	49.6 b-d
	3.0 g/0%	22.0 a-c	58.2 a-e	16.0 b-d	20.8 a-c	19.0 e-g	47.6 bc
	3.0 g/10%	26.1 cd	62.6 c-g	14.0 a-c	23.2 a-e	19.9 fg	56.0 e
	3.0 g/20%	22.8 a-d	61.4 b-f	13.9 a-c	23.6 b-e	16.2 c-e	49.4 b-d
Endorize-Mix	1.5 g/ 0%	21.7 ab	61.4 b-f	14.0 a-c	18.8 a	20.3 g	51.6 c-e
	1.5 g/10%	23.2 a-d	64.0 d-g	16.0 b-d	26.2 d-f	20.0 g	49.4 b-d
	1.5 g/20%	22.8 a-d	56.8 a-d	15.4 a-d	23.8 b-e	18.8 e-g	49.2 b-d
	3.0 g/0%	23.9 a-d	67.6 fg	15.5 a-d	24.0 b-e	17.0 c-f	53.0 c-e
	3.0 g/10%	26.3 c-d	68.8 g	16.7 d	28.8 f	15.4 a-d	56.4 e
	3.0 g/20%	20.0 a	61.6 b-f	16.1 b-d	28.8 f	14.5 a-c	53.6 c-e
Endomix	1.5 g/ 0%	22.7 a-d	54.8 ab	15.6 a-d	21.6 a-d	20.1 g	49.0 b-d
	1.5 g/10%	26.8 d	64.8 e-g	14.8 a-d	24.8 c-f	17.9 d-g	47.2 bc
	1.5 g/20%	24.9 b-d	53.4 a	14.3 a-d	21.4 a-c	12.8 a	32.6 a
	3.0 g/ 0%	26.1 cd	60.0 a-e	16.3 cd	27.2 ef	13.1 ab	44.0 b
	3.0 g/10%	23.1 a-d	61.2 b-f	16.2 cd	27.2 ef	18.0 d-g	55.0 de
	3.0 g/20%	23.9 a-d	56.6 a-c	14.2 a-c	23.0 a-e	15.8 b-d	53.0 c-e

[1]First number means a dose of Osmocote Standard 5-6M (1.5 or 3.0 g dm^{-3}), second number means a content of compost in a growing medium (0, 10 or 20% v/v)
[2]Means followed by the same letter in columns do not differ significantly at p = 0.05 according to the Duncan Multiple Range Test

the differences in the growth of *T. occidentalis* inoculated with Endomix and the non-inoculated control plants were not significant. Using reduced rate of SRF and no compost addition to the peat-moss substrate, none of the tested mycorrhizal inocula stimulated conifer growth during the whole cultivation period. Plants of *C. lawsoniana,* inoculated with Endomix, were even significantly shorter by 14%, after two years of the experiment.

Coniferous plant response to mycorrhizal inocula was also dependent on compost amendment in the peat-moss substrate. Compost added to the substrate, at a rate of 10-20%, caused plants of *T. media* to react more strongly to the use of Endorize-Mix than plants grown in pure peat-moss substrate, independent of the level of mineral fertilization. After two years of the experiment, these plants were higher than mycorrhized plants cultivated in pure peat-moss substrate. In turn, plants of *C. lawsoniana* inoculated with Endomix were significantly higher after two years, when we did not use compost combined with a lower rate of SRF.

DISCUSSION

Approximately 150 species of AMF are known to form symbiotic associations with up to 80% of land plants. The fungi generally show little or no specificity to the plant species they colonize (Ahulu et al., 2007), however there is evidence of preferential selection of the fungi by the host plant species (Gollotte et al., 2004; Landis et al., 2004; Lovelock and Ewell, 2005). The specificity of AMF to the plant species may explain the different level of mycorrhizal colonization of the tested conifers in our study. A possible reason for the high mycorrhizal colonization of all tested plants inoculated with Endorize-Mix, and plants of *T. media* inoculated with Endomix, might be the plants' preferential response to specific fungi contained in the inoculum. Thus, the fungal response to the plants resulted in strong mycorrhizal associations. The lower colonization of *C. lawsoniana* and *T. occidentalis* plants inoculated with Endomix after the first growing season could have resulted from symbiotic incompatibility. These explanations are supported by findings in the reports of Bâ et al. (2000), Scagel (2001), Wang and Qiu (2006). It has also been shown that more than one fungal species can colonize roots of an individual plant in a natural ecosystem (Ahulu et al. 2007). In our experiment, plants of *T. media* inoculated with both mixed fungal cultures (Endorixe-Mix and Endomix) were highly colonized by AMF. These findings suggest that AMF's interaction with *T. media* is nonspecific. It is also of interest to note that control plants of *T. media* were highly colonized by some nonspecific AMF. The spontaneous colonization may have occurred at a very early stage of cultivation, perhaps even during the rooting process of the cuttings. During this period, young nursery stock is exposed to various rooting substrates (usually consisting of sand), which can be contaminated by hyphae and spores of AMF. Bryophytes are used as a base in production of horticultural growing media. It is still a debatable issue if bryophytes have fungal associations (Read et al., 2000). Wand and Qiu (2006) surveyed that 71% of the bryophytes species and families have fungal associations. Zhang and Guo (2007) reported that mosses are colonized by mycorrhizal fungi. Cross-inoculation experiments of Read et al. (2000) have convincingly demonstrated that the same fungi can infect both liverworts and angiosperm and produce similar mycorrhizal structures. Another reason of spontaneous root colonization, especially in the subsequent years of the experiment, can be attributed to the mode of cultivation in the nurseries. Such a cultivation manner allows AMF to be easily moved from container to container by mycorrhizal roots growing outside the pots.

The AMF benefits for plant growth appear to be highest when colonization occurs during the early stages of plant growth (Scagel et al., 2003). However, the utilization of AMF is often limited because of the mycorrhizal inocula's incompatibility with such large chemical inputs as, fertilizer and pesticides. The critical factors in the commercial production of ornamental plants are the choices of growth substrate and fertilizing method. In a conventional production system, plants are cultivated in peat-based substrates supplemented with soluble fertilizers. This is done so that plants achieve an optimal supply of nutrients such as nitrogen and phosphorus (Perner et al., 2007). High levels of soluble fertilizers depress mycorrhizal symbiosis mostly due to the high salinity (Barea, 1991). The main advantage of SRF is that minerals are released gradually and there is no risk of high substrate salinity. Compost containing substrates and SRF may be appropriate for mycorrhizal plants (Linderman and Davis, 2001). Our studies support these findings. We showed that recommended rates of Osmocote Standard 5-6 M used in cultivation of coniferous plants did not generally produce a negative effect on mycorrhiza formation in roots of tested plants. Thus, there is a possibility for using commercial AMF inocula in the standard container nursery production system, when the recommended rate of SRF is used. We observed some contrast reaction of mycorrhizal plants on compost addition to peat-moss substrate. This reaction was expressed in a higher or lower frequency of mycorrhiza in the root system of the plants. In the literature, there are reports revealing similar results. For example, Perner et al. (2007) showed that compost added to the peat, in quantities of 20 and 40%, support AMF colonization of *Pelargonium* plants. However, plants of *Salvia farinacea* cultivated in substrate amended with compost had a lower percentage of root colonization in comparison to plants growing in non-amended substrates (Nowak and Kunka, 2009). Generally all fertilizing combinations used in our experiment were appropriate for plants inoculated with Endorize-Mix and resulted in achieving very good plant mycorrhization. There was a higher mycorrhizal colonization of *C. lawsoniana* and *T. occidentalis,* inoculated with Endomix and cultivated with a reduced rate of SRF combined with a compost addition to peat-moss substrate. This higher colonization may be due to higher sensitivity of AMF derived from Endomix, to minerals released from Osmocote Standard 5-6 M. Our experiments also revealed that recommended rates of SRF limited spontaneous mycorrhizal colonization of control plants of *C. lawsoniana*. The AMF naturally colonized plants of *C. lawsoniana* may genetically differ from commercial AMF in hypersensitive reaction on nutrient addition. AMF showed the opposite reaction to the recommended rate of SRF and compost amendment, as AMF colonized non-inoculated plants of *T. media* in the first year of the experiment. The high level of organic-mineral fertilizer in the growing substrate was beneficial for naturally occurring mycorrhizal symbiosis.

There are numerous reports on the genetic variation in plant responses to inoculation with mycorrhizal fungi (Parke and Kaeppler, 2000; Linderman and Davis, 2004). The gymnosperm species used in our experiment according to Wand and Qiu (2006) are obligately mycorrhizal. The mycorrhizal status of gymnosperms highlights the essential role that mycorrhizas play in the life of these plants, which generally grow in nutrient-poor environments. However, conifers cultivated in nurseries are usually optimally fertilized and watered. With such optimal care, we did not expect any spectacular effects of AMF inoculum on the growth of plants. Our aim was to achieve well mycorrhized plants under commercial nursery production systems that are believed to be better able to withstand the stress of transplanting, and which are able to increase in growth during later stages of plant development (Scagel et al., 2003). The results we obtained in our experiment varied depending on plant species. In the second year of cultivation we observed better growth of *C. lawsoniana* inoculated with Endorize-Mix and *T. media* inoculated with Endomix and cultivated under the traditional fertilizing system (recommended rate of SRF and no compost addition). These second year results may have been due to better nutrient acquisition from growing substrate, when the volume of the substrate is very limited. This hypothesis seems to be convincing, when we take into account the better growth of *T. media* inoculated with Endorize-Mix cultivated in substrates containing organic fertilizer-compost. Amaya-Carpio et al. (2009) reported that addition of rhizosphere microorganisms like AMF may help plants to mobilize and acquire nutrients from the substrate, and ameliorate plants growth. These findings also correspond with those of Frąc et al. (2009) in a study of strawberry inoculated with AMF and mulched with compost. AMF are capable of assisting the plant with uptake of potassium, nitrogen, zinc, copper. As a result of that capability, AMF can increase plant dry weight (George, 2000). In our experiment we also observed a growth reduction of *T. occidentalis* as a result of the use of Endomix in the first year of pot cultivation. It is well known that mycorrhization may depress the plant growth primarily by sink competition for photosynthates (Douds et al., 1988). Some studies indicate that depression due to mycorrhizal colonization is attributed to greater carbon expenditure of the colonized root system (Peng et al., 1993). Indeed, in the case of plants of *C. lawsoniana* fertilized with a reduced rate of SRF with no compost addition and inoculated with Endomix, we observed growth depression even after the second year of the experiment. The growth reduction of plants subjected to AMF inoculum was also reported by Linderman and Davis (2004) in some *Tagests* cultivars. Nowak and Kunka (2009) reported, that mycorrhization had not improved growth parameters and aesthetic value of mealycup sage. Instead, compost amendment of growing substrate proved to be beneficial for its growth and flowering.

CONCLUSIONS

Significant findings of this study for practical horticulture are:

1. The use of AMF inoculum Endorize-Mix in container cultivation of *Chamaecyparis lawsoniana* 'Golden Wonder', *Taxus* × *media* 'Hicksii', and *Thuja occidentalis* 'Smaragd' results in mycorrhiza formation and high frequency of mycorrhiza in the root systems of these plants in just one year of cultivation. For plants of *T. media,* the same results can be obtained using inoculum Endomix.
2. The use of the recommended rate of Osmocote Standard 5-6 M in a container nursery production system with no compost addition to peat-moss substrate allows for well mycorrhized coniferous plants when using commercial AMF inocula Endorize-Mix and Endomix, but after 2 years of cultivation. Under these conditions the use of Endorize-Mix ameliorates the growth of *C. lawsoniana* and the use of Endomix ameliorates the growth of *T. media* after two years of cultivation.
3. Plants of *T. media* are colonized spontaneously in the first year of cultivation without using the AMF inocula. The use of a compost addition at 10 and 20% combined with standard rate of SRF 3 g dm^{-3} is significantly preferential for spontaneous mycorrhizal symbiosis for *T. media.*
4. A compost addition to peat-moss substrate at a rate of 10-20%, ameliorates the growth of plants of *T. media* inoculated with Endorize-Mix independent of the level of mineral fertilization.

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WYKORZYSTANIE SZCZEPIONEK MIKORYZOWYCH W KONTENEROWEJ UPRAWIE WYBRANYCH OZDOBNYCH ROŚLIN IGLASTYCH W ZALEŻNOŚCI OD POZIOMU NAWOŻENIA ORGANICZNO-MINERALNEGO

Grzegorz Falkowski i Bożena Matysiak

STRESZCZENIE

Badano wpływ szczepionek mikoryzowych zawierających grzyby arbuskularne oraz nawożenia mineralno-organicznego na stopień zmikoryzowania oraz wzrost *Chamaecyparis lawsoniana* 'Golden Wonder', *Taxus* × *media* 'Hicksii' i *Thuja occidentalis* 'Smaragd' uprawianych w systemie szkółki pojemnikowej. W doświadczeniu zastosowano 6 kombinacji nawozowych składających się z trzech dawek kompostu

(0, 10 i 20% dodawanego objętościowo) oraz dwóch dawek nawozu o spowolnionym działaniu (SRF, 1,5 i 3 g · dm^{-3}). Oceniano poziom zmikoryzowania roślin oraz mierzono ich wzrost. Wszystkie rośliny, u których zastosowano szczepionkę Endorize-Mix charakteryzowały się wysokim stopniem zasiedlenia systemu korzeniowego przez grzyby mikoryzowe. Stosowanie obu szczepionek pozwoliło uzyskać wysoko zmikoryzowane rośliny iglaste uprawiane w warunkach szkółki pojemnikowej w podłożu torfowym oraz przy standardowym nawożeniu nawozami o spowolnionym działaniu. Zastosowanie szczepionki Endorize-Mix poprawiło wzrost roślin *C. lawsoniana*, a zastosowanie szczepionki Endomix poprawiło wzrost *T. media*.

Słowa kluczowe: *Chamaecyparis*, *Taxus*, *Thuja*, mikoryza arubskularna, kompost, nawóz o spowolnionym działaniu, produkcja szkółkarska, ogrodnictwo ekologiczne