NOTES

INOCULATION RATE OF ARBUSCULAR-MYCORRHIZAL FUNGI *GLOMUS INTRARADICES* AND *GLOMUS ETUNICATUM* AFFECTS ESTABLISHMENT OF LANDSCAPE TURF WITH NO IRRIGATION OR FERTILIZER INPUTS

Sophie Pelletier and Julie Dionne*

Abstract

Arbuscular-mycorrhizal (AM) symbiosis confers numerous benefits to host plants, including improved tolerance to abiotic and biotic stresses. Although the majority of grasses form an AM symbiosis, little is known of the mycorrhization of turfgrass species. This study was conducted to determine whether two mycorrhizal species, *Glomus intraradices* Schenck & Smith and *G. etunicatum* Becker & Gerdemann, affected the establishment of a lawn mixture of Kentucky bluegrass (*Poa pratensis* L.), red fescue (*Festuca rubra* L.), and perennial ryegrass (*Lolium perenne* L.). Turfgrass inoculated with *G. intraradices* at rates between 40 and 60 mL m^{-2} established more quickly than turfgrass inoculated with *G. etunicatum* when inoculated at time of seeding, with no irrigation or fertilization inputs.

Arbuscular-mycorrhizal symbiosis confers numerous benefits to host plants, including improved plant growth and mineral nutrition (Raju et al., 1990; Marschner and Dell, 1994), tolerance to disease (Trotta et al., 1996, Matsubara et al., 2000, 2001) and abiotic stresses such as drought (Subramanian and Charest, 1995), chilling (El-Tohamy et al., 1999), and salinity (Ho, 1987). The majority of grasses form an AM symbiosis (Newman and Reddel, 1987). However, very few studies have been conducted on mycorrhization of turfgrass species, because it was generally believed that because they grow under high maintenance conditions, these plants are less dependent on mycorrhizae. Recent work has shown that many species of mycorrhizae are associated with highly maintained turf of creeping bentgrass [*Agrostis stolonifera* L.] and velvet bentgrass (*A. canina* L.) (Koske et al., 1997a,b). Also, benefits associated with AM symbiosis in crop species have been recently documented in turfgrass grown under low phosphate conditions. Charest et al. (1997) and Gemma et al. (1997a) reported that Kentucky bluegrass and creeping and velvet bentgrasses produced more aboveground biomasses over time when inoculated with mycorrhizae compared with the uninoculated control. Moreover, Gemma et al. (1997b) showed that creeping bentgrass inoculated with the AM fungus *G. intraradices* tolerated drought conditions for longer periods and recovered more quickly from wilting than did nonmycorrhizal turf.

Most studies on mycorrhizal turfgrass have been obtained under controlled conditions. To determine if AM fungi function efficiently as growth promoters in the field, as they do in controlled conditions, they must be studied under field conditions. The objective of the present study was to determine whether two AM species, *G. intraradices* and *G. etunicatum*, affected the establishment of a seed mixture of Kentucky bluegrass, red fescue, and perennial ryegrass planted as landscape turf with no irrigation or fertilization inputs.

S. Pelletier, Centre de Recherche en Horticulture., Dép. de Phytologie, Univ. Laval, Sainte-Foy, QC, Canada, G1K 7P4; J. Dionne, Dep. of Plant Agric., Univ. of Guelph, Guelph, ON, Canada, N1G 2W1. Received 20 Feb. 2002. *Corresponding author (jdionne@uoguelph.ca).

Published in Crop Sci. 44:335–338 (2004).

677 S. Segoe Rd., Madison, WI 53711 USA

Materials and Methods

The experiment was conducted at Laval University, Québec City, QC, Canada (46°47′ N, 71°19′ W; elevation ≈70 m), in the summer of 2000, on a Sillery series shaly loam. Experimental plots measured 1.5 × 2.0 m and were separated from each other by 25-cm paths. There were five mycorrhizal treatments: *G. etunicatum* inoculum at 60 mL m^{-2}; *G. intraradices* inoculum at 40, 60, and 100 mL m^{-2}; and an uninoculated control. On 17 May 2000, plots were inoculated with the AM fungi supplied as spores in a mixture of peat moss and plant roots produced by Premier Tech, Rivière-du-Loup, QC, Canada. This mixture is not available commercially. On the same day, plots were seeded with a standard commercial lawn seed mixture composed of 30% Kentucky bluegrass, 40% red fescue, and 30% perennial ryegrass (Centre de Jardin Hamel, Ancienne Lorette, QC, Canada). Both mycorrhizal inoculum and seed were uniformly sprinkled by hand over the surface plots and mixed with a rake into the top 1 cm of the soil. Turf plots were not fertilized nor treated with pesticides during the establishment period. During the establishment period, no cultural or pest problems occurred, weeds were manually removed once a week and plots were not mowed. They were watered with rainfall only (Normal rainfall 1971–2000 for the month of June = 111.7 mm). The experimental design was a randomized complete block with four replications.

Percentage of plot area covered by turfgrass was evaluated by visually dividing each plot in four sections of equal size, and by estimating the surface covered by turfgrass. These evaluations were made weekly by the same person, from 5 June to 25 June. To assess mycorrhizal colonization of roots, five root samples, each containing ≈10 to 15 single plants, were collected randomly in each plot ≈4 wk after seeding and again on 7 wk after seeding (5 July). Roots were washed with water to remove all soil. No attempt was made to separate the different species of turfgrass, as the mixture used was a standard lawn seed mixture. Roots were then digested in a 10% KOH solution, and stained in Trypan blue (Phillips and Hayman, 1970). Percentage mycorrhizal colonization was then assessed on 100 root intersections by the gridline intersect method of Giovanneti and Mosse (1980).

Abbreviations: AM, arbuscular-mycorrhizal.

Table 1. Mycorrhizal colonization rate of turfgrass roots on 14 June and 5 July 2000 at Laval University, QC, Canada.

	14 June 2000		5 July 2000	
Mycorrhizal treatments	Rate	Mean ± SE	Rate	Mean ± SE
	mL m^{-2}		mL m^{-2}	
Control	0	5.50 ± 3.63	0	19.50 ± 2.45
G. etunicatum	60	4.00 ± 4.32	60	15.50 ± 2.92
G. intraradices	40	5.50 ± 3.63	40	11.50 ± 2.45
G. intraradices	60	4.75 ± 3.63	60	15.25 ± 2.45
G. intraradices	100	10.25 ± 3.63	100	21.50 ± 2.45

Statistical Analysis

Analysis of variance was performed with the General Linear Model Procedure of SAS statistical software (SAS Institute, 1990). When the *F* tests for main effects were significant ($P < 0.05$), least square means tests were used to compare mycorrhizal treatment effects on the percentage of plot area covered by turfgrass and on root mycorrhizal colonization. Linear and quadratic contrasts were made to compare the efficiency of mycorrhizal species and mycorrhizal inoculation rates for the establishment experiment.

Results

Mycorrhizal Colonization of Field Roots

There was no significant difference in the mycorrhizal colonization rate of roots between the treatments (Table 1). On 14 June 2000, one month after mycorrhizal inoculation, the average root colonization rate of all treatments varied from 4 to 10% (6% average), and by 5 July it increased to an average of 17% (12 to 22% colonization).

Turfgrass Establishment

Turfgrass coverage increased to an average of 90% on 25 June for all treatments (Table 2). Establishment differed between mycorrhizal treatments after 3 and 4 wk of growth (11 and 18 June, respectively) but not on the final rating date (25 June). On 11 and 18 June, percentage of turf coverage was higher for the two lower rates of *G. intraradices* than for the *G. etunicatum* treatment. Plots inoculated with *G. intraradices* had an average of 47% turfgrass coverage on 11 June and 75% on 18 June, while plots inoculated with *G. etunicatum* had an average of 39% turfgrass coverage on 11 June and 66% on 18 June. We also observed a significant quadratic effect for the inoculation rate of *G. intraradices* (Fig. 1). On 11 June the plots inoculated with 60 mL m^{-2} of *G. intraradices* had 55% turfgrass coverage. This was significantly higher than the plots that received the 100 mL m^{-2} inoculation rate, which had 40% turfgrass coverage, and it also tended to be higher than the 0 and 40 mL m^{-2} inoculation rate, which had 44 and 46% turfgrass coverage, respectively. On 18 June the 40 mL m^{-2} inoculation rate with 80% turfgrass coverage was significantly higher than the 0- and 100-mL m^{-2} rate, which had 71 and 68% turfgrass coverage, respectively. The 60-mL m^{-2} inoculation rate was close to the 40-mL m^{-2} inoculation rate, with 78% turfgrass coverage.

Discussion

No prior information was available about the ability to colonize turfgrass roots of the experimental inoculum produced by Premier Tech. In the present experiment, the increase of root colonization rates throughout the establishment period shows that the inoculum is able to colonize turfgrass seeding roots. Our results show significant differences among mycorrhizal species for turfgrass establishment. At two different dates, the *G. intraradices* treatments showed a higher turfgrass coverage percentage compared with the *G. etunicatum* treatments (Table 2). This suggests that *G. intraradices* is more efficient in increasing the speed of establishment

Table 2. Effects of *Glomus* mycorrhizal species and inoculation rates on turfgrass establishment (percentage of coverage of plots by turfgrass) in June 2000 at Laval University, QC, Canada.

		Date			
Treatment†	Rate	5 June	11 June	18 June	25 June
	mL m^{-2}				
Control		31	44abc‡	71bc	91
G. etunicatum	60	24	39c	66d	88
G. intraradices	40	31	46ab	80a	91
G. intraradices	60	34	55a	78ab	91
G. intraradices	100	29	40bc	68cd	89
LS means		ns§	$P < 0.0183$	$P < 0.0017$	ns
Linear contrast					
G. intraradices vs. *G. etunicatum*		ns	**	***	ns
Quadratic contrast					
G. intraradices 0–40–60–100 mL m^{-2}		ns	*	**	ns

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.
† Plots were seeded and inoculated on 17 May 2000. Visual evaluation of percentage of coverage of plots by turfgrass were made weekly from 5 June to 25 June.
‡ Means in a column followed by the same letter are not statistically different ($P < 0.05$) according to least square means test.
§ ns, nonsignificant.

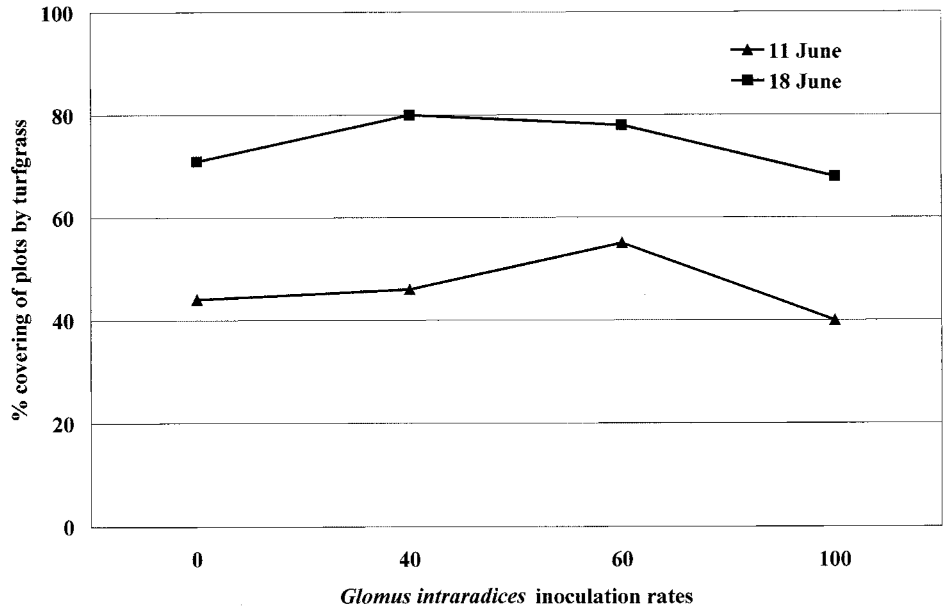

Fig. 1. Quadratic effect of *Glomus intraradices* inoculation rates on turfgrass establishment in June 2000, Laval University, QC, Canada. Quadratic contrast significant at $P < 0.05$ on 11 June and at $P < 0.01$ on 18 June.

of turfgrass seedlings than *G. etunicatum*. Such differences among mycorrhizal species have also been reported on mineral acquisition and biomass production in plants (Plascencia et al., 1997; Clark et al., 1999). Moreover, a significant quadratic effect was observed with inoculation rates of *G. intraradices*. The most effective rates to increase turfgrass establishment speed were 60 mL m^{-2} on 11 June and 40 mL m^{-2} on 18 June. On these dates, these respective rates performed better and were significantly different than the highest rate of 100 mL m^{-2}. Trépanier (1998) reported similar results with *G. intraradices* on the growth of two species of shrubs, and Plascencia et al. (1997) showed that plants inoculated with *G. intraradices* had the largest response in height gain and biomass production when root colonization percentage was low. The increase in establishment of grasses is an important benefit to golf course superintendents and landscape owners. First, there are economic benefits to have a golf course available even a few days earlier to golfers. Second, faster establishment is of environmental interest for landscape owners and professionals, as it improves the aesthetics of lawns, covering the surface and increasing the quality of grasses by reducing weed development. A dense, weed-free cover may require less fertilizer and herbicide. Finally, earlier establishment reduces the need for water as growers can stop irrigation sooner.

It is important when inoculating with mycorrhizal fungi to carefully chose a species that is efficient. The results presented here indicate that the most efficient inoculum is cheaper to produce as it is not the most concentrated. This may be explained by the need of the mycorrhizal fungi for C when establishing a symbiosis. If the amount of inoculum is too high, an important quantity of C is drained from the host plant, which may then become C deficient, thus reducing its growth (Harley and Smith, 1983). A balance between both plant and mycorrhizal fungi needs must be achieved to have the optimal plant growth and colonization rates. This indicates the importance of establishing the optimal inoculation rate to reach the optimal plant response.

We conclude that AM fungi *G. intraradices* at rates between 40 and 60 mL m^{-2} is more efficient than *G. etunicatum* in increasing the speed of establishment of a standard turfgrass lawn seed mixture when inoculated at time of seeding.

Acknowledgments

The authors thank Eric Dugal, Marie-Pierre Lamy, and Annie Duval for their excellent technical assistance. This research was supported by Premier Tech, Rivière-du-Loup, QC, Canada, and the Natural Sciences and Engineering Research Council of Canada.

References

Charest, C., G. Clark, and Y. Dalpé. 1997. The impact of arbuscular mycorrhizae and phosphorus status on growth of two turfgrass species. J. Turfgrass Manage. 2:1–14.

Clark, R.B., R.W. Zobel, and S.K. Zeto. 1999. Effects of mycorrhizal fungus isolates on mineral acquisition by *Panicum virgatum* in acidic soil. Mycorrhiza 9(3):167–176.

El-Tohamy, W., W.H. Schnitzler, U. El-Behairy, and M.S. El-Beltagy. 1999. Effect of VA mycorrhiza on improving drought and chilling tolerance of bean plants (*Phaseolus vulgaris* L.). J. Appl. Bot. 73: 178–183.

Gemma, J.N., R.E. Koske, E.M. Roberts, and N. Jackson. 1997a. Enhanced establishment of bentgrasses by arbuscular mycorrhizal fungi. J. Turfgrass Sci. 73:9–14.

Gemma, J.N., R.E. Koske, E.M. Roberts, N. Jackson, and K.M. de Antonis. 1997b. Mycorrhizal fungi improve drought resistance in creeping bentgrass. J. Turfgrass Sci. 73:15–29.

Giovanneti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.

Harley, J.L., and S.E. Smith. 1983. Mycorrhizal symbiosis. Academic Press, New York.

Ho, I. 1987. Vesicular-arbuscular mycorrhizae of halophytic grasses in the Alvord Desert of Oregon (USA). Northwest Sci. 61:148–159.

Koske, R.E., J.N. Gemma, and N. Jackson. 1997a. Mycorrhizal fungi associated with three species of turfgrass. Can. J. Bot. 75:320–332.

Koske, R.E., J.N. Gemma, and N. Jackson. 1997b. A preliminary survey of mycorrhizal fungi in putting greens. J. Turfgrass Sci. 73:2–8.

Marschner, H., and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102.

Matsubara, Y., Y. Kayukawa, M. Yano, and H. Fukui. 2000. Tolerance of asparagus seedlings infected with arbuscular mycorrhizal fungus to violet root rot caused by *Helicobasidium mompa.* J. Jpn. Soc. Hortic. Sci. 69(5):552–556.

Matsubara, Y., N. Ohba, and H. Fukui. 2001. Effect of arbuscular mycorrhizal fungus infection on the incidence of fusarium root rot in asparagus seedlings. J. Jpn. Soc. Hortic. Sci. 70(2):202–206.

Newman, E.I., and P. Reddel. 1987. The distribution of mycorrhizas among families of vascular plants. New Phytol. 72:127–136.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158–161.

Plascencia, E., O. Francisca, J.J. Vargas Hernandez, R. Ferrera Cerrato, and V.A. Gonzalez Hernandez. 1997. Effect of vesicular-arbuscular mycorrhiza on growth and biomass allocation of eucalyptus seedlings. Terra (Mexico) 15(1):7–14.

Raju, P.S., R.B. Clark, J.R. Ellis, and J.W. Maranville. 1990. Effects of species of VA-mycorrhizal fungi on growth and mineral uptake of sorghum at different temperature. Plant Soil 121:165–170.

SAS Institute. 1990. GLM-VARCOMP. p. 891–1686. *In* SAS/STAT user's guide. SAS Inst., Cary, NC.

Subramanian, K.S., and C. Charest. 1995. Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress. Mycorrhiza 5:273–278.

Trépanier, M. 1998. Effets des champignons endomycorhiziens sur le bouturage et la croissance de plantes ligneuses ornementales. M.S. Thesis. Univ. Laval, QC, Canada.

Trotta, A., G.C. Varese, E. Gnavi, A. Fusconi, S. Sampò, and G. Berta. 1996. Interactions between the soilborne root pathogen *Phytophthora nicotianae* var. *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant Soil 185:199–209.