UNIVERSITY OF RHODE ISLAND

THE USE OF MYCORRHIZAE IN ESTABLISHMENT AND MAINTENANCE OF GREENS TURF

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This report covers research completed through Oct., 1992. The project consists of several related studies: identifying the species of mycorrhizal fungi that are associated with velvet and creeping bentgrass and Poa annua in New England, culturing the dominant/most promising species of fungi, and testing the ability of the fungi to promote establishment of greens turf in a sand medium, minimize application of P fertilizers and water, and offer protection against root pathogens. The research combines laboratory, greenhouse, and field experimentation.

In addition to testing fungi isolated from turf, we also are investigating the potential benefits of species associated with naturally occurring sand media, i.e., vegetated sand dunes.

MAJOR RESULTS

Very promising results have been achieved, providing strong evidence that inclusion of mycorrhizal fungi during construction of sand greens will result in a superior turf with lower maintenance requirements. The following report summarizes work from the first two years of the study and includes details of experimentation during the most recent year.

I. SURVEY OF TURFGRASS SOILS FOR MYCORRHIZAL FUNGI

The species of mycorrhizal fungi associated with *Agrostis palustris* cv. Penncross, *A. canina* cv. Kingstown, and *Poa annua* were determined by monthly sampling of field plots over an 18-mo period. In addition to turf plots at the University of Rhode Island, sand greens at several New England golf courses have been sampled for mycorrhizal fungi, although not on a monthly basis. Fungi were isolated directly from the soil and identified, and pot cultures were established and examined for additional species.

Twenty-nine species of mycorrhizal fungi have been identified (including 15 new, undescribed species) as being associated with greens turf. Different seasonal trends in spore abundance and species composition were identified for each species of turfgrass.

DOMINANT VA MYCORRHIZAL FUNGI ISOLATED FROM TURFGRASSES GROWING IN LOAMY SOILS

Scutellospora erythropa	Scutellospora calospora
Scutellospora pellucida	
Glomus lamellatum	Glomus mosseae
Glomus #3347	
Acaulospora #3347	Acaulospora #3382
Entrophospora infrequens	

II. MYCORRHIZAL FUNGI FROM SAND DUNE SOILS

Mycorrhizal fungi growing in association with native grasses in sand dunes at Cape Cod National Seashore and at several coastal locations in southern Rhode Island were identified for possible use in sand greens.

DOMINANT SPECIES OF FUNGI ISOLATED FROM NATIVE GRASSES GROWING IN SAND DUNE SOILS

Scutellospora erythropa	Scutellospora calospora
Scutellospora pellucida	Scutellospora persica
Gigaspora gigantea	Gigaspora albida
Acaulospora scrobiculata	Acaulospora 7034
Glomus clarum	

III. ISOLATION AND ESTABLISHMENT OF MYCORRHIZAL FUNGI IN POT CULTURE

Eighteen species/isolates of VAM fungi from turf or dunes have been established in pot culture. Of these, six species (8 isolates) have been amenable for use in inoculum production:

Scutellospora erythropa*,**	Scutellospora pellucida*,**
Scutellospora persica**	Glomus clarum**
Gigaspora gigantea**	Gigaspora albida**

note: * indicates species isolated from turfgrasses growing in loamy soils.
** indicates species isolated from grasses growing in sand dune soils.

IV. MYCORRHIZAL INOCULATION EXPERIMENTS:

1. GREENHOUSE:

We have carried out numerous experiments testing the interaction of various species of mycorrhizal fungi (isolated from turf plots or from dune soils, or the species that was commercially available), type of peat (sphagnum or sedge), levels of available phosphorus, drought, pathogens, and bentgrass species growing in the sand green medium. The following results were obtained:

A. Promotion of growth and vigor

Mycorrhizal benefit is most pronounced at low phosphorus levels when plants are grown in the sphagnum/sand mix. Plant growth in the low P/inoculated conditions equalled or exceeded that recorded for 2X (15 ppm) and 4X (30 ppm) concentrations in the absence of mycorrhizal fungi.

Growth response was apparent in newly seeded turfs (= enhanced establishment) and in re-growth from mature (6-mo old) minigreens after mowing.

Fig. 1. Enhanced establishment of creeping bentgrass by inoculation with mycorrhizal fungi. Turf was grown from seed for 8 weeks before clipping. A complete nutrient solution was applied every other week and contain P at 7.5 or 30 ppm.

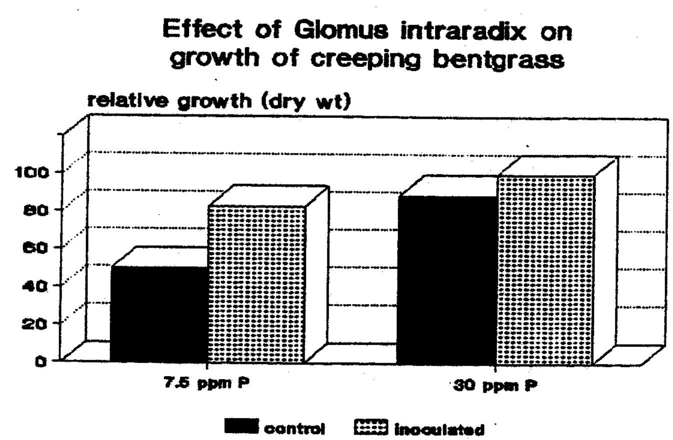
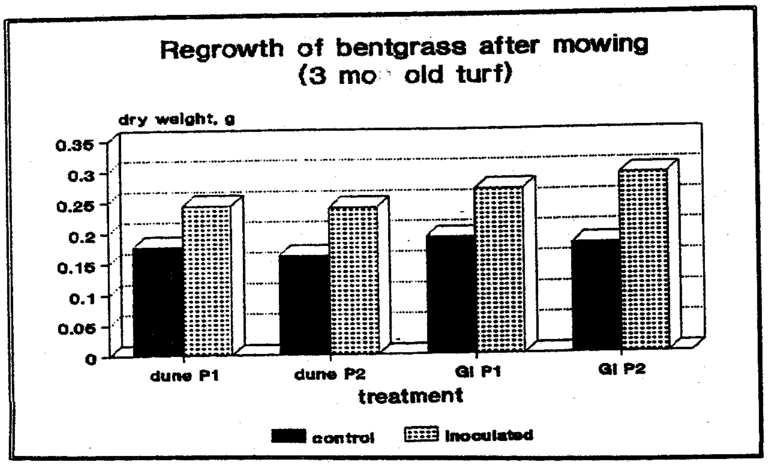
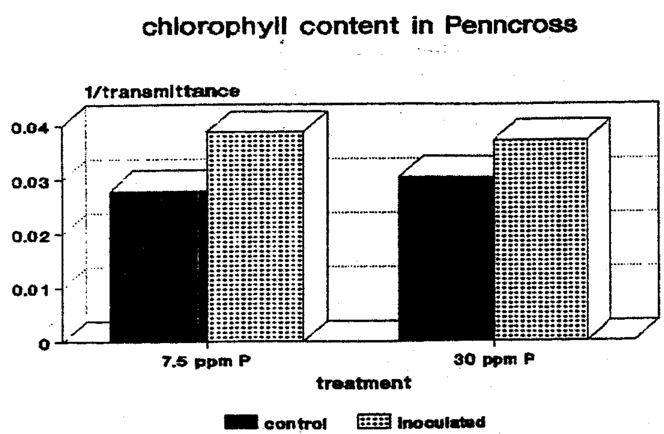


Fig. 2. Enhancement of re-growth of a mature turf of creeping bentgrass at two P levels. Leaf dry weight production over a two week period was measured. Similar responses were measured in subsequent weeks. Two inocula were used, a sand dune soil ("dune") and <u>Glomus intraradix</u> ("GI"). Fed as in Fig. 1.



In addition, inoculated plants were greener than were the control plants, containing more chlorophyll (on a dry weight basis). The effect was most striking under conditions of low phosphorus. Addition of phosphorus at 4X the low concentration did not result in chlorophyll content as high as in the low P, inoculated plants.

Fig. 3. Increase in chlorophyll content in creeping bentgrass inoculated with mycorrhizal fungi.



B. Sedge peat vs. sphagnum peat

Overall, the sphagnum and sand mix is more conducive to growth of both bentgrasses than is the sedge/sand mix. Enhancement of growth by mycorrhizal fungi also is greater in the sphagnum mix than in the sedge mix.

C. Phosphorus effects of the symbiosis

In general, increasing levels of P in the soil are correlated with decreased levels of colonization of turfgrass roots by mycorrhizal fungi.

D. Source of most effective fungi

Fungi isolated from sand dune soils are more effective than are fungi isolated from turf plots in promoting growth of Penncross when grown in the USGA sand green medium.

E. Enhancement of drought tolerance

Inoculation of Penncross plants in the sand green medium results in a turf that is more resistant to drought than is an uninoculated turf and one that also is capable of making much more rapid recovery (producing new green leaves) when drought conditions cease.

Minigreens (3" diam x 11" deep) of Penncross were grown with or without mycorrhizal fungi for 2-6 months (time enough for root colonization to reach 35 - 50%). Water was withheld from the greens for up to 14 days. Plant water stress was measured using a J-14 hydraulic press. Inoculated plants were significantly more drought tolerant than were control plants (see Fig., next page), indicating that a "missed" watering of a young, inoculated green would not have the same consequences as would occur in an uninoculated green.

Fig. 4. Water potential in creeping bentgrass in response to drought. Note difference between inoculated and control plants after 4 days without water.

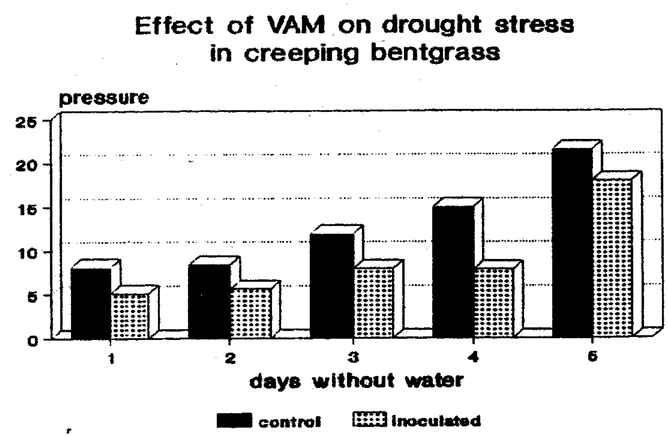
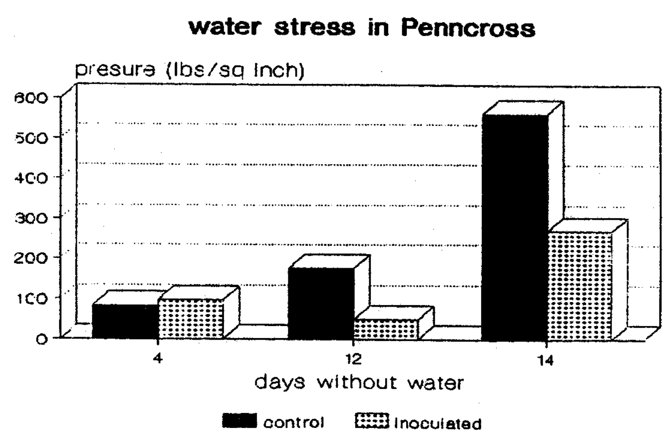
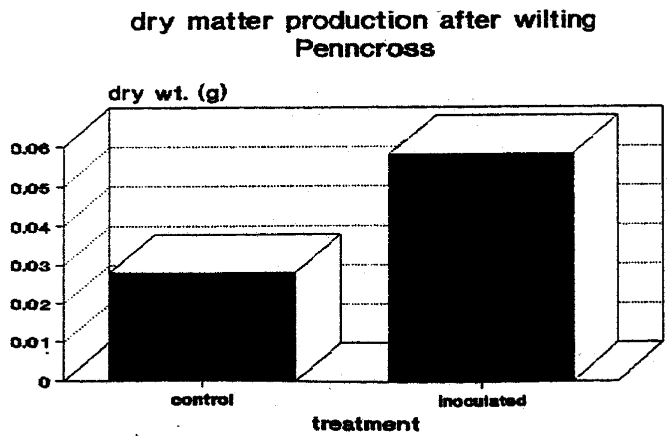


Fig. 5. Effect of drought on leaf water potential in creeping bentgrass. Water was withheld from plants growing in the greenhouse for 14 days. The method used to determine the end point was different from that used in FIg. 4, so that pressure values in the two experiments are not directly comparable. Cool, humid conditions between days 4 and 10 permitted the plants to survive for longer than expected.



Recovery from drought was measured by collecting the amount of new growth made by plants that had been severely stressed. Inoculated plants produced more than twice as much growth during the recovery period.

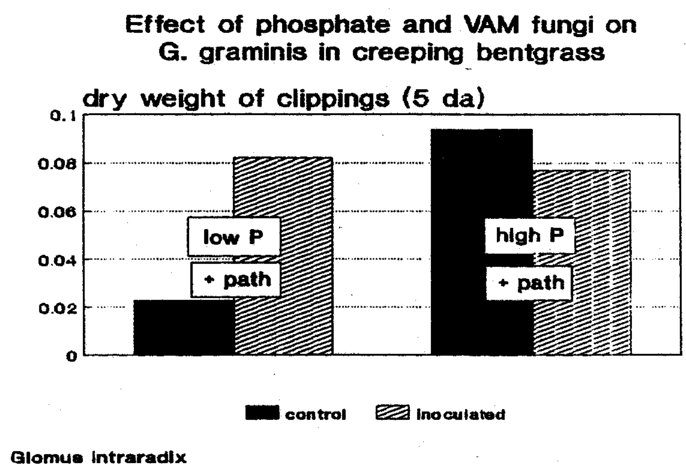
Fig. 6. Re-growth from drought-damaged creeping bentgrass. After a 14-day drought, turf was clipped once and watered daily. The new growth that was made in the 5 days after the drought was collected and weighed.



F. Resistance to pathogenic fungi

Minigreens (see above) were grown for 4 months to allow plants to become mycorrhizal. Greens were fertilized with a complete nutrient solution containing P at 7.5 or 30 ppm. Half of the greens were inoculated with <u>Gaeumannomyces graminis</u>, and half received an equivalent amount of carrier lacking the pathogen. Disease was assessed by visually rating the greens and by measuring the amount of leaf tissue produced by inoculated and control minigreens over a 5-day period. At the low P concentration, VAM fungi provided some protection from the pathogen, but none at the high P concentration (see next page).

Fig. 7. Effect of VAM fungi on losses of turf to the take-all fungus at two different P levels.



G. Resistance to invasion by Poa annua

Minigreens (see above) grown for 4 months with or without mycorrhizal fungi and a two P levels were overseeded with Poa annua. Emergence and growth of Poa were to be monitored. Dense growth of bentgrass in all treatments essentially prevented any establishment of Poa. The experiment will be repeated using younger, less crowded minigreens.

2. FIELD EXPERIMENTS:

A. A small sand green (USGA specifications) divided into 288 square-foot plots was installed in the field in late September, 1990. The effect of mycorrhizal inoculation with Glomus intraradix and four different levels of phosphorus on creeping bentgrass and velvet bentgrass is being evaluated.

As of Oct., 1992, few differences have been noted between inoculated and uninoculated plots. The fungus used in the study has since been found to be very sensitive to soil phosphorus (in the greenhouse) and to be ineffective when P exceeds 25 ppm. In subsequent greenhouse studies, other species have been identified that are not so sensitive and provide benefits over a broader range of soil P concentrations.

The population of spores of the VAM fungus was measured in the inoculated plots in June, 1992. There was a significant negative correlation between spore abundance and P, confirming that high P suppresses mycorrhizal activity.

Sampling of uninoculated plots revealed that the fungus had spread from the inoculated plots, apparently through gaps in the wooden separator boards.

B. A second field planting was set up in June, 1992, using 12" plastic pots buried in the soil with the top even with the surrounding turf. Pots were filled with the USGA sand green mix and inoculated with either Glomus intraradix or a mixed-species sand dune "cocktail." Appropriate controls were incorporated into the study. Two levels of P were used in the fertilization regime.

Roots were invaded by the VAM fungi by September, and some differences in color were apparent. The study will be monitored for dry weight production and chlorophyll content in the Spring of 1993. Resistance to drought and to G. graminis also will be investigated in this trial.

C. Application of certain fungicides can reduce the efficacy of VAM fungi in stimulating the vigor of turfgrass. To determine the effects of frequently used compounds, we surveyed the populations of VAM fungal spores in experimental plots (loams) that had a long history of fungicide use. Three plots treated with each of the following fungicides were assessed: daconil, bayleton, dyrene, chipco, vorlan. In addition, three plots each treated with a combination of bayleton and each of the four other fungicides were examined. Spore populations were significantly reduced only when bayleton was present (singly or in combination).

However, preliminary studies in the greenhouse suggest that even a single application of daconil on an established turf temporarily, but significantly, eliminates the benefit of inoculating Penncross with some species of VAM fungi. Additional studies of the effect are in progress.

D. Construction began in August on a full size green. The green will be seeded in Spring, 1993. We will inoculate various parts with VAM fungi and monitor the performance. The new inoculum will be tested, as will a mix of VAM fungi from a local sand dune.

3. LABORATORY:

A. Synthesis of mycorrhizae in vitro

A method of inoculating bentgrass plants with mycorrhizal fungi under monoxenic conditions in liquid culture was developed. Attempts to establish root organ cultures of bentgrasses have not succeeded

Recent experiments in the laboratory have suggested that it may be possible to grow some isolates of VAM fungi in agar culture in the presence of certain root exudates. If VAM fungi can be readily cultured, inexpensive, high grade inoculum for field use would be achieved.

B. Viability studies of inoculum

Inoculum produced in the greenhouse is being tested for viability and dormancy in laboratory tests. Methods of shortening the dormancy period of spores of Gigaspora gigantea are being investigated.

V. INOCULUM PRODUCTION TECHNIQUES

We have had to develop a new source of inoculum since writing the original proposal. The commercially available inoculum, an isolate of Glomus intraradix from Florida, is no longer produced in the U.S. However, another supplier has recently been located (see below).

Studies performed in the first two years of the project indicated that maximal benefits to turfgrasses could be obtained using fungi isolated from sandy soils, and we have concentrated on developing inoculum production methods for those species.

Methods that were tried and proved successful include:

1. aeroponic culture

Marigold plants grown in aeroponic culture produce numerous roots that become infected with mycorrhizal fungi. These roots can be used successfully as inoculum.

2. calcined clay

Sudan grass plants grown in a calcined clay mix fertilized with a modified Hoagland's solution have been used to produce relatively large amounts of high grade inoculum. Other plant hosts have been investigated, and a patent for the process is being sought by the University of Rhode Island.

3. mixed-species inoculum

To provide the maximum benefits over the widest range of growth conditions, we have begun production of mycorrhizal inoculum that includes a variety of fungal species. Sand dune soils are used as a source of the fungi.

Experiments recently completed using other carriers for the mycorrhizal inoculum (e.g., isolite, solite [an expanded shale], quartz sand, and vermiculite were inconclusive.

Another manufacturer of VAM fungal inoculum in North America has recently been located. The producer uses an isolate of Glomus intraradix (the species that was available commercially from NPI, Salt Lake City, when this proposal was written). The new source of inoculum uses an isolate that was first recovered from a cool, sandy soil, unlike the NPI isolated that was from a warm soil with fairly high clay content. Because our trials have shown that fungal isolates are adapted to particular soil conditions (rather than to particular plant species), we expect that the new isolate will be well suited for use in sand greens in cool season grasses. In addition, the inoculum is sold pre-mixed in sphagnum peat, and thus is in a convenient form for use in building sand greens. We have arranged to use the new inoculum in field and greenhouse trials.

SUMMARY

1. The dominant species of mycorrhizal fungi associated with velvet and creeping bentgrass and Poa annua have been identified, as have the species of mycorrhizal fungi that occur in native grasses growing in sand dunes.

2. Mycorrhizal fungi isolated from sandy soils appear to be more effective than are isolates from loamy soils in stimulating greensturf grown in the USGA sand green medium.

3. Seven species of mycorrhizal fungi have been identified as having potential in terms of enhancing turfgrasses vigor and ease of inoculum production.

4. Response of velvet and creeping bentgrass to mycorrhizal fungi is maximal at low P concentrations.

5. Establishment of young turf is enhanced by inoculation with mycorrhizal fungi, and established turf grows more vigorously when inoculated.

6. Inoculated turf is greener (has a greater chlorophyll concentration) than is uninoculated turf.

7. Inoculated turf is more resistant to drought than is uninoculated turf, and it recovers from drought much quicker.

8. At low levels of soil P, mycorrhizae appear to provide some protection against the take-all fungus, Gaeumannomyces graminis.

9. A sphagnum/sand mix is superior to a sedge peat/sand mix in terms of plant growth and response of plants to inoculation with mycorrhizal fungi.

10. Methods of producing inoculum are being assessed. A large supplier of a sphagnum-based inoculum has been identified.

11. A method was developed to inoculate bentgrass plants with mycorrhizal fungi under sterile laboratory conditions.