Executive Summary

Annual Report
Nov. 1992-Nov. 1993

THE USE OF MYCORRHIZAE IN THE ESTABLISHMENT AND MAINTENANCE OF GREENS TURF

UNIVERSITY OF RHODE ISLAND
Kingston, RI

Drs. Noel Jackson,
R. E. Koske, and J. N. Gemma
Principal Investigators

Grant Awarded 1990: $40,000/yr

This report describes research carried out from Nov. 1992 through Nov. 1993. The original project consisted of several interdependent studies: identifying the species of mycorrhizal fungi that are associated with velvet and creeping bentgrass and Poa annua, culturing these fungi, and testing the fungi to promote establishment of greens turf, minimize applications of P fertilizers, offer protection against root pathogens, enhance resistance of greens to invasion by *Poa annua,* and increase drought tolerance.

Major accomplishments during the period covered by this report include:

1. In field and greenhouse studies mycorrhizal fungi conferred markedly enhanced drought tolerance in creeping bentgrass.
2. Mycorrhizal turf contains significantly more proline, an amino acid implicated in drought tolerance and disease resistance.
3. Three fungal isolates significantly stimulate growth and early establishment of turf.
4. Fertilization of mycorrhizal turf with low levels of phosphorus results in plant growth that is equal or superior to that resulting from higher phosphorus levels
5. Mycorrhizal Penncross turf is greener than nonmycorrhizal turf, possessing nearly 60% more chlorophyll.
6. Benefits of mycorrhizae could be consistently maintained by frequent applications of a complete fertilizer solution containing low concentrations of phosphorus. Established mycorrhizal turf that no longer showed benefits (because of excessive added phosphorus or fungicides) could be easily restored.
7. A commercial source of mycorrhizal inoculum has been found. In addition to providing their own fungal isolate, they are willing to produce inoculum of our sand dune isolates that have shown promise in the sand green medium.

UNIVERSITY OF RHODE ISLAND

THE USE OF MYCORRHIZAE IN ESTABLISHMENT AND MAINTENANCE OF GREENS TURF

1990 Research Grant: $40,000 (4th year of support)

Drs. Noel Jackson
R. E. Koske & J. N. Gemma
Principal Investigators

This report covers research completed through Oct. 31, 1993. The original project consisted of several related studies: identifying the species of vesicular-arbuscular mycorrhizal (VAM) fungi that are associated with velvet bentgrass (*Agrostis canina* cv Kingstown), creeping bentgrass (*A. palustris* cv. Penncross), 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 the USGA sand green medium, minimize application of P fertilizers and water, and offer protection against root pathogens.

In the last year we have focused on the effects of mycorrhizae on creeping bentgrass (Penncross) in four general areas: 1) ability of mycorrhizal fungi to confer drought tolerance, 2) tests of isolates of fungi for abilty to stimulate establishment and quality of creeping bentgrass turf, 3) determining the amount of phosphorus fertilizer to add to achieve consistent benefits from mycorrhizae, and, 4) locating a commercial source of inoculum of our sand dune isolates for use in sand greens. The results from the last year are emphasized below. However, because this is the final year of funding for the project, results from years 1-4 will be summarized at the end of the report.

MAJOR RESULTS

Very promising results have been achieved in field and greenhouse trials, providing strong evidence that inclusion of mycorrhizal fungi into sand greens will result in a superior turf with lower requirements for water and phosphate fertilizer. In addition, we learned that the method to achieve consistent performance from mycorrhizae is to use agronomic practices that favor the mycorrhizal association (i.e., frequent feeding with low levels of P, avoidance of fungicides and pesticides that inhibit mycorrhizal fungi, etc.). A desirable turf can be achieved by concentrating efforts on maintaining optimum conditions for the mycorrhizae. Greens that have been treated in such a way to inhibit mycorrhizae (i.e., excessive P or fungicides) can be restored and again benefit from the presence of mycorrhizae. Finally, a large supplier of suitable mycorrhizal inoculum has been located.

1. Drought tolerance

Preliminary greenhouse studies in 1992 suggested that addition of VA mycorrhizal fungi to the sand green medium dramatically increase the tolerance of the turf to water stress. In 1993 we carried out a field trial and a large greenhouse experiment to monitor changes in several parameters while water was withheld from established mycorrhizal and non-mycorrhizal Penncross turfs.

Field Trial After several weeks of thorough watering, water was withheld from ten 1-year-old plots (5 mycorrhizal, 5 control) during a summer drought. Weather during the experiment was warm and humid. Mycorrhizal turfs were far more resistant to drought than were the controls (Figs. 1,2), and significant differences were apparent after 3 days and were maintained until the completion of the experiment. Leaf water potential was significantly better in mycorrhizal turfs from day 1 to day 14. Soluble sugars in leaves were significantly higher in mycorrhizal turf when water stress was greatest (Fig. 3). In addition, inoculated turfs possessed significantly more chlorophyll(see Fig. 11). Some results from the field study are shown on this page and the next page.

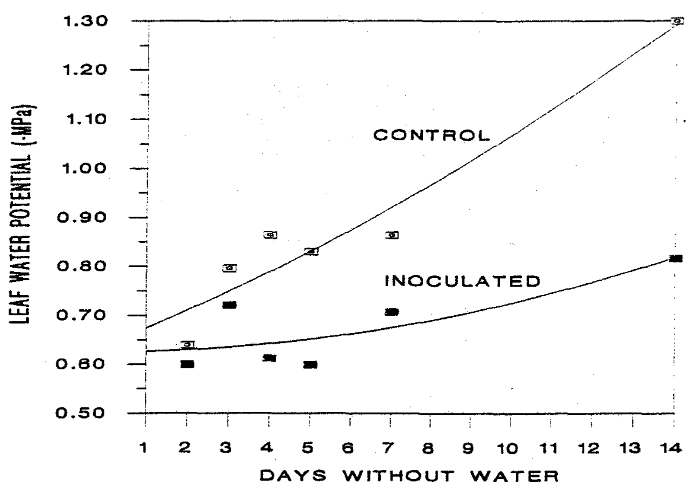

Fig. 1. Water potential in drought-stressed Penncross in a field expt. Values are from a hydraulic leaf press. Low numbers indicate high potential (i.e., sufficient water). 3/4" rain fell on day 10. Greens were inoculated with the fungus *Glomus intraradices.*

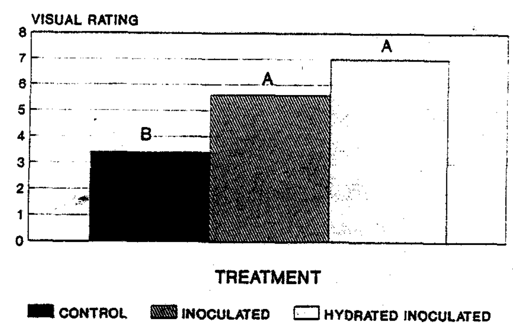

Fig. 2. Visual rating of drought-stressed Penncross turf in the field. Turfs were rated on day 10 of the drought. "Hydrated" turfs were watered daily.

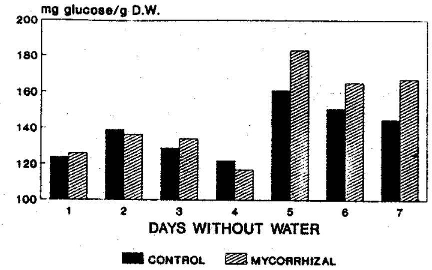

Fig. 3. Soluble sugars in drought-stressed turf in the field. Note increased levels in mycorrhizal plants as stress developed.

Greenhouse Trials Fifty-two 5-month old turfs growing in pots were watered to field capacity with a complete nutrient solution containing either 10 or 40 ppm P. There were 4 treatments (13 pots in each): 1) 10 ppm P + VAM, 2) 10 ppm P - VAM, 3) 40 ppm P + VAM, 40 ppm P - VAM. Water was then withheld for up to 7 days, and various measurements were made daily: percentage of pots with wilted plants, evapotranspiration losses, changes in relative water capacity and leaf water potential, qualitative and quantitative changes in free amino acid pools (by HPLC), stomatal density, leaf phosphorus and total soluble sugars. Mycorrhizal turfs receiving the 10 ppm P were far more resistant to drought than were controls and than both sets of turf at 40 ppm P (Figs. 4, 6). At 10 ppm P, control turfs became wilted about three days before mycorrhizal ones did. Water potential in mycorrhizal plants stayed significantly higher, another indication of lack of water stress. The mycorrhizal benefit was lost at the higher P level, and at 40 ppm P, mycorrhizal turfs were slightly less resistant to drought than were the controls (Fig. 4).

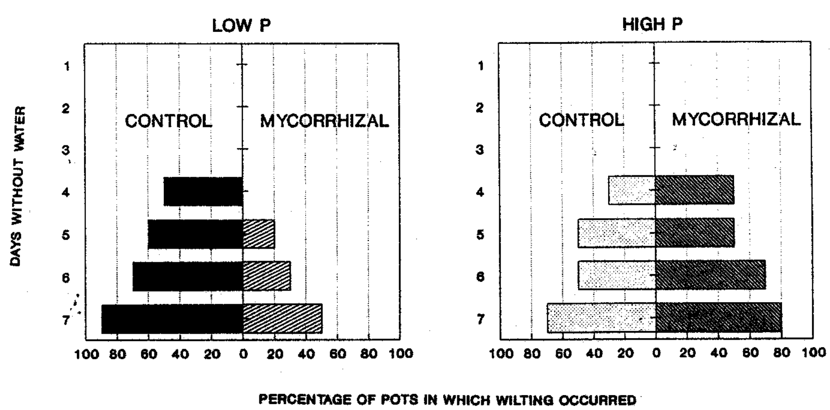

Fig. 4. Greenhouse wilt study. Percentage of pots in which wilting occurred during the drought. No mycorrhizal plants receiving 10 ppm P were wilted on day 4. Note lack of protection by mycorrhizae at 40 ppm P. Measurements of relative water capacity of leaves and water potential corroborated these results. Fungus used was *Glomus intraradices*.

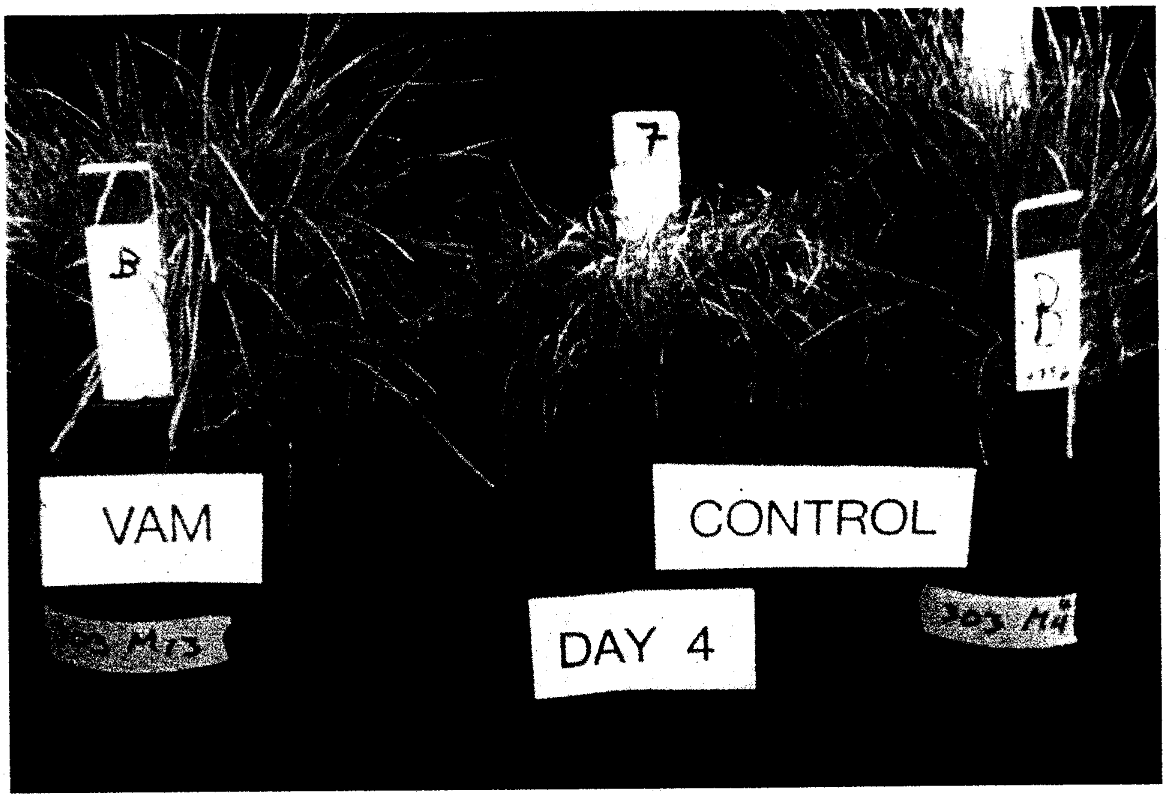

Fig. 5. Greenhouse wilt study, day 4 of drought. Pot on left contains mycorrhizae, pot in center lacks mycorrhizae. Pot on right was watered daily. These plants were on the 10 ppm P regime.

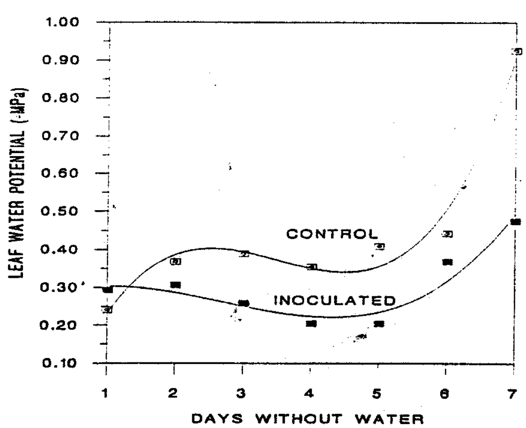

Fig. 6. Greenhouse wilt study. Water potential in drought-stressed plants. Note long lag in inoculated turfs before water potential begins to change. Data shown for 10 ppm P turfs.

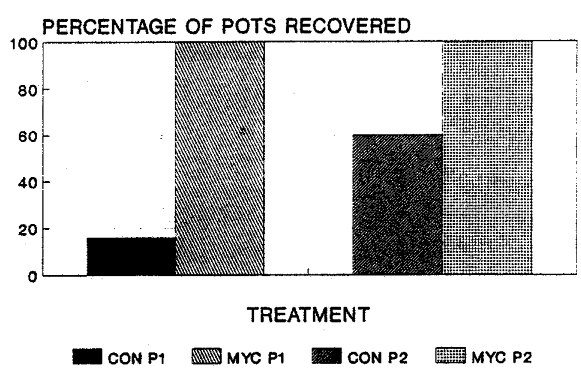

Fig. 7. Greenhouse study. Recovery of turfs after a 5-day long drought. Plants were watered on day 6, and their recovery from wilting was assessed on day 7. Data for 10 ppm P ("P1") and 40 ppm P ("P2") are shown.

After turf had become wilted in this experiment, the mycorrhizal turf recovered significantly more rapidly than did the controls (Fig. 7). In addition, phosphate concentrations in leaves were higher in mycorrhizal plants, both before and after drought stress (Fig. 8).

The analysis of amino acids was undertaken in cooperation with the URI Department of Chemisty and Millipore, Inc. Several new methods of derivitization and separation were utilized in this reverse-phase High Performance Liquid Chromatography technique. Although the HPLC analysis of free amino acid pools in leaves is still in process, initial findings are very exciting. Mycorrhizal plants (at both P concentrations) had markedly higher levels of several amino acids that are known to be involved not only in drought tolerance but in resistance to some fungal pathogens.

In another greenhouse experiment using Penncross turf, free pools of proline were measured during drought stress. Mycorrhizal turf had significantly more proline than did the difference was maintained in the stressed plants (Fig. 9).

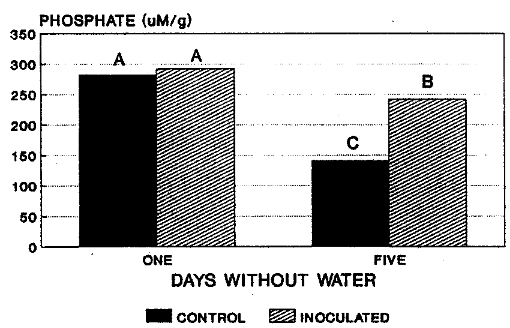

Fig. 8. Greenhouse wilt study. Leaf phosphate changes in turfs exposed to drought. Note large difference by day 5. Data for 10 ppm P are shown.

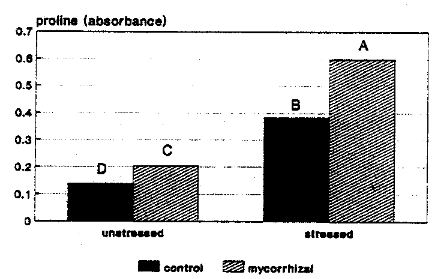

Fig. 9. Effect of presence of mycorrhizae on proline concentration in drought-stressed and unstressed turf.

00400

2. Establishment of new turf

Numerous greenhouse trials were set up using three isolates of mycorrhizal fungi. Turfs were clipped every 7-14 days to measure dry matter production. Phosphorous was added at 10 or 40 ppm. At the low concentration of P, all fungal isolates stimulated growth, with the fungus isolated from the sand dunes (*Gigaspora gigantea*) being most effective in stimulating establishment.

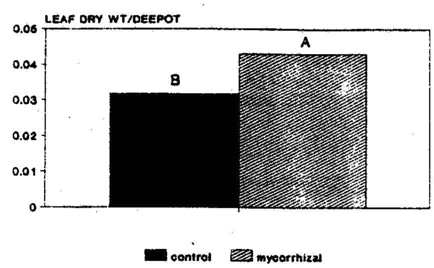

Fig. 10. Early establishment of Penncross in response to inoculation with *Gigaspora gigantea*. Dry weight of leaves produced during the first 25 days after sowing are shown. Fertilizer solution contained 10 ppm P.

In a field trial, inoculated plants were significantly greener than control plants, having nearly 60% more chlorophyll.

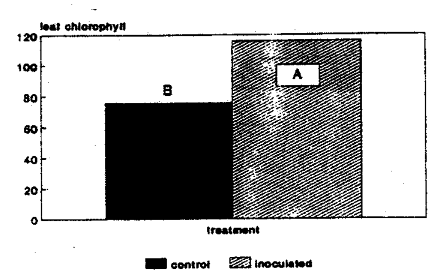

Fig. 11. Amount of chlorophyll in inoculated and control turfs of Penncross in a field study. Fungus used was *Glomus intraradices*.

The rate at which seedlings of Penncross developed mycorrhizal infections was followed in a turf growing in the USGA sand green medium and containing the mycorrhizal fungus *Glomus intraradices* (Premier Peat isolate) first formed mycorrhizae 10 days after sowing. These results agree with our observations that mycorrhizal growth benefits are apparent within the first 3 weeks of growth response

3. Effects of phosphorus fertilization on mycorrhizal response

Too high a concentration of P in the soil solution is reported to inhibit the functioning of mycorrhizae. We examined the effects of P fertilization on mycorrhizae formed between three fungal isolates and creeping bentgrass turfs in greenhouse studies. Turfs were maintained on a strict regime of fertilization and clipping, allowing us to assess responses over a period of 2-4 months. Frequent fertilization with a low P fertilizer (10 ppm) resulted in best performance by mycorrhizal plants. Routinely, the most vigorous turfs were those that were inoculated with mycorrhizal fungi and grown in the low-P regime. At higher P levels, mycorrhizae did not confer growth benefits.

4. Restoration of benefits in mycorrhizal turf

During greenhouse growth experiments, it was frequently noted that mycorrhizae enhanced growth during the first few weeks of establishment. Later, the differences between uninoculated and inoculated turfs often were not significantly different. This observation led to a series of experiments to see if the mycorrhizal benefit could be restored in poorly performing turf.

Pots routinely were fertilized weekly with a complete nutrient solution containing P at 10 ppm. When fertilized three times a week with this dilute fertilizer, the same turfs that had shown a decrease in mycorrhizal benefit under the weekly fertilizing regime then recovered their mycorrhizal advantage. Thus, although the growth benefit was temporarily lost, the mycorrhizae were not. Manipulation of the environment allowed the benefits to reappear. Use of the same fertilizer solution except with P at 40 ppm did not encourage the mycorrhizae, and no growth benefit was detected.

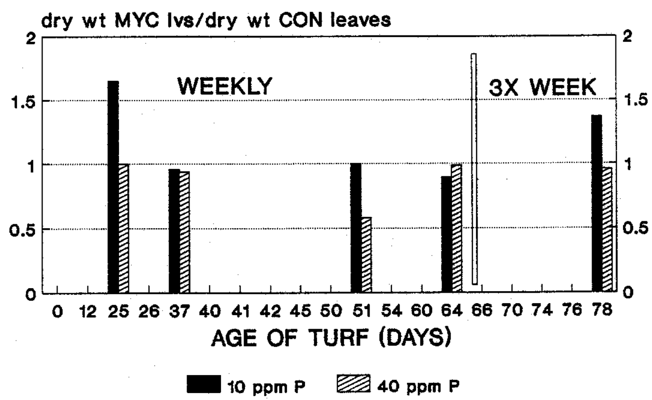

Fig. 12. Effect of fertilization frequency and rate on mycorrhizal growth benefits. The dry wt of leaves or the mycorrhizal turfs was divided by weights from control plants to obtain a Mycorrhizal Performance Index (MPI). A MPI of >1 indicated enhancement by mycorrhizae. Fertilization frequency increased from weekly to thrice weekly when the turfs were 65 days old. Note relatively poor performance at 40 ppm P.

Similar restoration of benefits was noted in turfs that had been treated with Daconil and Diazanon, compounds that inhibited the beneficial function of the mycorrhizae. Cessation of use of these materials allowed the benefits to re-appear.

00402

5. Inoculum production techniques

A rapid culturing system for producing inoculum of mycorrhizal fungi from sand dunes was developed. The high grade inoculum produced by this system will be used for additional trials. Premier Peat, a large manufacturer in Quebec, has been marketing a mycorrhizal product (Mycori-Mix) that contains *Glomus intraradices* dispersed in Sphagnum peat. The inoculum is easily compatible with the USGA recommendations for sand green construction by substituting the fungus-containing sphagnum for unamended sphagnum. Their fungus was originally isolated from sandy soil in Quebec. In 1992/1993 we tested their inoculum in greenhouse studies and found it to be effective. Premier Peat has been extremely cooperative with us, supplying a specially formulated Mycori-Mix to us for use in our field and greenhouse experiments. The company is easily capable of supplying sufficient inoculum for greens construction. In addition, Premier Peat has agreed to produce inoculum of our most potent fungus (the sand dune isolate of *Gigaspora gigantea*). We will be performing trials with their inoculum of this species in the winter.

6. Field Trials

In addition to the drought study that wss completed this year, two additional field trials were initiated in October 1993. Vigor and appearance will be monitored as will incidence of diseases, chlorophyll and phosphorus content, and drought resistance.

7. Miscellaneous studies

During our greenhouse studies, turfs were plagued by collembola, fungal gnats, and a *Fusarium* sp. Attempts to control these pests with chemicals were successful, but the treatments interfered with the mycorrhizal fungi. The mycorrhizal benefits to growth (leaf matter production) were lost when Diazanon (for collembola) or Daconil (for *Fusarium*) was applied. Mycorrhizal fungi were not killed by these treatments, since the mycorrhizal benefits appeared after the chemical treatments ceased. We have been investigating other compounds and treatments to control these and other pests. The biocontrol compound Gnatrol (for controlling fungal gnats) appears to be compatible with mycorrhizal fungi. Trials with Captan also have been promising.

SUMMARY OF ACCOMPLISHMENTS FROM 1990-1993

1. Twenty-nine species of mycorrhizal fungi associated with velvet and creeping bentgrass and *Poa annua* have been identified.

2. Mycorrhizal fungi isolated from sandy soils are more effective than are isolates from loamy soils in stimulating greens turf 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. Sphagnum peat in the sand-peat mix results in a better turf than does sedge peat.

7. Inoculated turf is greener (up to 60% more chlorophyll

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

9. Inoculated turf has significantly greater quantities of some free amino acids that may have importance in protection against drought or pathogens

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

11. Turf that has lost its mycorrhizal benefit because of too little or too much soil P or fungicide applications can be restored.

12. A large supplier of a sphagnum-based inoculum has agreed to produce inoculum of our best sand dune isolate as well as providing their own isolate.

13. A method was developed to inoculate bentgrasses with mycorrhizal fungi under sterile laboratory conditions