Title: Evaluation and Utilization of Allelopathic *Festuca rubra* Turfgrass Cultivars for Alternative Weed Management Strategies

Project Leader: Leslie A. Weston, Department of Horticulture, Cornell University

Project Cooperators: Cecile Bertin, graduate student in Horticulture; Andrew F. Senesac, extension educator, Long Island Horticultural Research and Extension Center, Riverhead NY; Dr. Frank Rossi, turf specialist, Department of Horticulture; Peder Weibull, Swalof Weibul Seed Company, Sweden.

Type of Grant: Alternative Pest Management Strategies, Pest Resistant Crops

Project Locations: Ithaca NY; Riverhead NY; Swalof, Sweden.

Abstract:

Recent experiments in Ithaca and Riverhead NY have shown that fine fescue cultivars Intrigue, Reliant and Oxford were most suppressive in field and laboratory settings due to their allelopathic activity and their ability to establish readily in a field setting. The cultivars Treasure and Boreal were least weed suppressive in both sites in 2 years of replicated trials. Mode of action studies would suggest the inhibitor(s) in the root exudate act upon photosynthesis or respiration or possibly both. Current anatomical studies suggest root exudates are produced in actively dividing root cells and are stored until extrusion from roots in osmiophilic inclusions in root cells. The structural elucidation studies performed using GC and HPLC coupled to mass spectrometry suggest that the components of the root exudates may be steroidal in nature. Purification and structural elucidation work is ongoing.

Background and Justification:

The development of alternative weed management strategies in landscape and turf settings involves the use and implementation of novel bio-control practices that can provide effective control over the course of the growing season. Use of pathogenic organisms to control weeds has not proven particularly effective, due to problems in obtaining consistent control and difficulty in formulation of biocontrol organisms. Organically derived products, such as corn gluten or cramby meal, have also not provided consistent or inexpensive control, especially in commercial settings such as golf courses, parks and athletic fields where complete control is often desirable. One novel approach that shows very strong potential is the selection, development and use of allelopathic or weed suppressive turfgrasses or groundcovers to naturally control annual weeds in the landscape, without the use of herbicides. Fescues, especially *Festuca rubra* and other related spp., produce secondary products, known as allelochemicals, with potent ability to suppress weed seed germination and growth.

A collection of fescues was established as part of the NTEP trials at Cornell's turf farm in 1998. Quality and weed suppressive ability were evaluated by C. Bertin in 1999 - 2001. Of the 80 cultivars evaluated, nine cultivars were identified that provided greater than 90% weed suppression as compared to other cultivars in 1999. In 2000, seven of these same cultivars were also extremely weed suppressive, showing that despite variability in seasonal weather patterns, the weed suppressive trait remains active in

selected cultivars. Further laboratory and field research was conducted to determine which fescue cultivars were consistently most weed suppressive in these settings and the mechanisms of weed suppression. Our findings have shown that in the field, cultivars are most suppressive because they establish rapidly and also exhibit allelopathic potential in suppressing weeds. At this point, we propose to further study the site of production of root exudates in fescue root tips and determine anatomically and intracellularly the exact site of exudate production and release. In addition, our collaboration with Swedish colleagues will allow us to initiate further selection of diverse fescue germplasm in the field and laboratory in an attempt to develop a more highly weed suppressive fescue cultivar for use in the home landscape, commercial landscapes and athletic fields.

Objectives: 1. To compare a diverse collection of weed suppressive accessions of fescue for establishment and weed suppressivity in field and laboratory screens in both Ithaca NY and Swalof Sweden. 2. To further characterize the chemical constituents present in fescue root exudates and their cellular site of production. 3. To compare the weed suppressive ability and chemical constituents of exudates of a diverse group of fine and creeping fescues for selection of most suppressive germplasm. 4. To utilize these findings as the basis for selection of a superior weed suppressive cultivar of fine leaf fescue for the home and commercial landscape.

Procedures:

Field plots were established in Ithaca NY and also Swalof Sweden to evaluate a diverse collection of 10 top performing fine leaf fescue cultivars for their weed suppressive abilities using NTEP trial specifications. We also included 2 coarse fescue selections for comparative purposes. We know from past work that fescue cultivars exhibit great differences in their ability to suppress weeds. At this time we wish to assess the world's most diverse collection of fescue for weed suppressive abilities in the laboratory and field. Germplasm from the Nordic gene bank in Sweden, the origin of fine leaf fescues, will be screened along with commercially strong cultivars, including Intrigue, Oxford and Reliant. Plots were approximately $1m^2$ each and were replicated 4 times for each cultivar. Included in each evaluation will be closely and distantly related material available from the Nordic gene bank. In this manner, we can continue to evaluate how the various genotypes are ranked individually for their ability to suppress weeds over time and utilize most suppressive genotypes in our future breeding programs. Weed suppressivity ratings will be collected for each accession at 4 week intervals during the 2004 growing season, using visual ratings of total weed infestation (based on a 0-100% infestation scale) and separate weed species infestations, including large crabgrass, white clover, annual bluegrass and dandelion. A weed suppressive index will be determined for each accession, based on total turfgrass biomass produced and level of weed infestation present. At the same time, the genotypes will be assayed for their ability to produce allelopathic or weed suppressive root exudates directly using a gel bioassay system, where several seedling fescues are allowed to establish, produce toxic root exudates, and their ability to suppress the growth of weed seedling indicator seeds are measured. This system has proven valuable in allowing us to measure the toxicity, length of persistence and selectivity of root exudates upon the growth of a variety of common weed seedlings. The selections that show greatest suppression in field and laboratory evaluation will be used for further breeding experiments.

A capillary mat system (Czarnota et al, 2001) was designed to collect large quantities of fescue root exudates. Fescue seed is placed on the sterile mat system and allowed to germinate for a period of 2 weeks. During this time, fescue seedlings develop an extensive root system which is supported between layers of cheesecloth and absorbent fabric. After 14 days, the roots are harvested by removal with a razor blade. Roots are extracted for bioactive root exudates in methylene chloride or water. Each accession can be produced separately on this capillary mat system. Exudates of the 5 least and most suppressive accessions (10 total) will be collected using this system and subjected to bioassay to test for inhibitory activity. Extracts will be tested at concentrations of 0.125 to 2.0 mg/ml using a standard petri dish assay to assess seed germination and seedling growth over a 72 hour period. Extracts will also be evaluated for their chemical constituents by thin layer chromatography with various silica plates and by HPLC (Waters Reliance system, reverse phase, C18 Nova pak column, Waters Inc.) using an acetonitrile: water gradient mobile phase at 2 mls/minute with detection at UV 254 nm using a photodiode array detector. We have developed a sensitive bioassay system to assess inhibitory activity. Using thin layer chromatography and HPLC, we have purified the root exudates and have determined that certain constituents are associated with inhibitory activity. We plan to compare activity and active constituents present in each accession's exudate. We also are working to identify active constituents within each exudate. The use of mass spectrometry and nuclear magnetic resonance spectroscopy will enable us to attempt structural elucidations for each constituent. Light microscopy and transmission electron microscopy will be utilized to further evaluate the root tips of fine leaf fescue, the site of exudate production. Currently exudate is produced in a swollen root tip containing osmiophilic inclusions containing the root exudate. We wish to pinpoint production of the exudate within cellular organelles and examine the mode of release or exudation by root tip cells.

Once chemical structures of these root inhibitors are identified, we plan to evaluate the quantity of these constituents present in each accession's exudate. It is likely that weed suppressivity will be linked to the presence of greater quantities of bioactive constituents in the exudate. Most toxic exudates as determined by laboratory analysis will be evaluated for chemical fingerprinting or similarity using HPLC. Once structural determination of these inhibitors is completed, we can attempt to assess their biosynthetic pathways of production in the higher plant and characterize the genes that control the exudation process.

The diverse collection of fescue germplasm will help us to develop a chemotaxonomic base for intra and interspecies relationships. This will prove useful for selection of weed suppressive traits for further attempts to breed an attractive highly weed suppressive fine fescue turfgrass, with stress tolerance and disease resistance. After further field selection, the parental lines exhibiting greatest weed suppressivity over time will be selected for crossing. Using standard outcrossing of parental lines, and further selection of F1 and F2 generations with our Swedish fescue breeding colleague, we believe we will have the ability to release genetic material for cultivar development within 5 to 7 years, exhibiting pest resistance, good color and aesthetic appeal and demonstrating enhanced ability to suppress weeds.

Results and Discussion:

This project is the focus of Cecile Bertin's PhD thesis within the Department of Horticulture at Cornell University, which is now just underway. Results will be utilized to

potentially secure additional funding from USDA NRI (proposal now under consideration) and BASF Ag in Limburgerhof Germany for support of molecular and chemical characterization studies, in an attempt to develop a more weed suppressive group of quality turfgrasses for use in the home and commercial landscape. BASF's seed subsidiary in Sweden Swalof Weibul is now cooperating with us to provide a large grouping of fescue accessions for further evaluation. After 2 more years of additional field and laboratory evaluation, a select grouping of fescue genotypes will be used as the basis for parental breeding lines for cultivar development for enhanced weed suppressive ability. Our collaboration with Swalof Weibul has resulted in great interest in the company in working on this longterm project and additional potential for funding by the seed marketing division and the Swedish government which currently supports environmentally relevant projects.

At this time, we have established field plots in Ithaca and Sweden to evaluate more promising selections for weed suppressivity and ability to establish rapidly in cooler season climates. Plots were established in the fall of 2003 in Ithaca. In addition, demonstration trials of weed suppressive cultivars were established in Suffolk County on the municipal golfcourse for fairway trials to also demonstrate ability to establish and weed suppressivity.

Laboratory analyses have been initiated and demonstrated some chemical differences among cultivars, with several related compounds being identified in each cultivar's root exudates. However, quantity and chemical composition varied with each cultivar. In working in association with Dr. Meinwald's laboratory group at Cornell University, and utilizing both HPLC and GC coupled to mass spectrometry, we have identified several novel but complex steroid type compounds contained in bioactive root exudates. Root exudates were more active when collected using aqueous extraction versus solvent extraction. Bioassay-directed purification is still underway, with structural elucidation to follow. Protocols for sectioning and tissue preparation for electron microscopy of root tissue have been developed. A joint collaboration with faculty at the University of Wisconsin Osh Kosh campus is now underway and studies utilizing their exceptional electron microscopy facility will be performed in March 2004 by Cecile Bertin. We anticipate completion of these studies at the end of 2005.

Current references:

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