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

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Abstract: The development of alternative weed management strategies in landscape and turf settings involves the use and implementation of novel bio-control practices which 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 which shows 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 both 1999 and 2000. 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. Laboratory and field research was conducted to determine which fescue cultivars were consistently most weed suppressive in field and laboratory settings and the mechanisms of suppression. Results from the laboratory and field trials, along with new plots recently established in Ithaca and Riverhead NY will determine which fescue cultivars can be successfully established and maintained with fewest invasive weeds in Central NY and Long Island NY growing conditions. A weed suppressive index will be determined for the materials under evaluation, based on growth measurements obtained. Longterm studies are now underway to evaluate the allelochemicals, secondary metabolic pathways and genes involved in this suppressive trait in selected fescue cultivars. Recommendations will be developed for cultivar and species selection, seeding or planting rate and mowing height.

Objectives: 1. To compare weed suppressive and non-weed suppressive accessions of fescue for establishment and weed suppressivity just after seeding and throughout the growing season in 2 different NY production sites.
2. To further characterize weed suppressivity of these accessions in the laboratory using plant based assays and assay of exudates.
3.To finish identification of the chemical constituents present in fescue root exudates and

determine activity of each constituent.

Materials and Methods:

Field plots were established in fall 2000 in Ithaca NY and Riverhead NY, according to NTEP trial specifications. Earlier in 1999 and 2000, eighty fine fescue accessions were evaluated in trials in Ithaca; based on these findings and laboratory results, 5 of the least suppressive and 5 most weed suppressive accessions were selected for further evaluation in Ithaca and Riverhead. Plots were approximately $1m^2$ each and were replicated 4 times for each cultivar. Different levels of weed management will be applied to each accession in an attempt to determine the weed suppressive ability of each accession. Treatments will include: complete weed control provided throughout the season (hand weeding and herbicide application), early season weed management provided only and finally, no weed suppression provided at all. In this manner, we can evaluate how the various accessions may be ranked individually for their ability to suppress weeds immediately after establishment or later in the season, when well established. Weed suppressivity ratings will be collected for each accession and weed treatment at 4 week intervals during the 2001 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, creeping bentgrass and dandelion. A weed suppressive index will be determined for each accession, based on total turfgrass biomass produced and level of weed infestation present.

Laboratory evaluations of most and least suppressive festuca accessions are now being conducted. Two bioassays were designed to assess the impact of fescue seedlings of various accessions and their root exudates upon large crabgrass and curly cress seedlings. Fescue accessions were separately established in sterilized sand placed in sterile well plates and then moistened with 350 micro-liters of autoclaved water. Water was added every 2 days to maintain moisture. Large cress and crabgrass seed was then introduced after 1 week, when fescue seedlings were newly established. After 10 days of growth, seedling biomass of seed indicators was measured. Roots and shoot weights were collected separately. In a separate bioassay, magenta boxes were filled with 50 mls of sterile 0.2 % water agar. Twenty fescue seedlings of each accession were established in each box and allowed to grow for 14 days in a growth chamber under 12 hours of light and 12 hours darkness photoperiod at 25 degrees C. After 14 days, an equal number of large crabgrass seeds were placed in each box and returned to the growth chambers. After 10 days, crabgrass germination and seedling biomass was measured. All treatments will be replicated at least 5 times and experiments will be repeated in time. We are currently finishing these evaluations and repeating selected experiments, after determining that these 2 bioassay systems were reliable and provided uniform and repeatable results.

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. 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 mass spectrometry and nuclear magnetic resonance spectroscopy will enable us to attempt structural elucidations for each constituent.

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. 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 which control the exudation process.

Results:

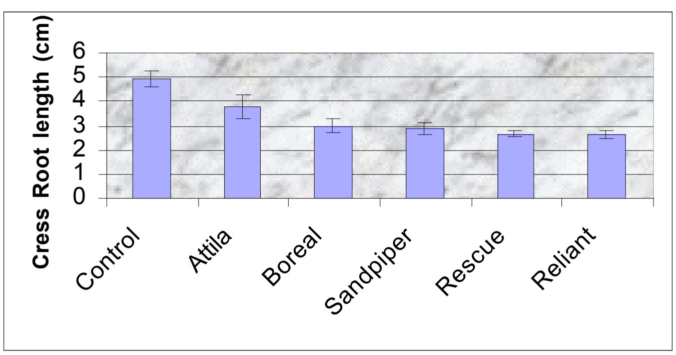

Figure 1. Inhibition of curly cress root growth when interseeded with various cultivars of fescue.

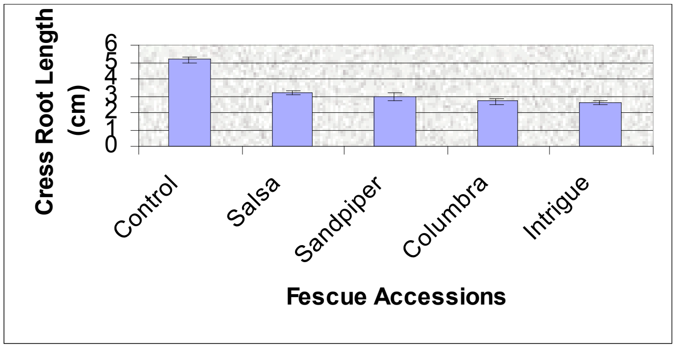

Figure 2. Inhibition of curly cress root growth in laboratory assays as influenced by fescue cultivar after fescue was removed from the growth media .

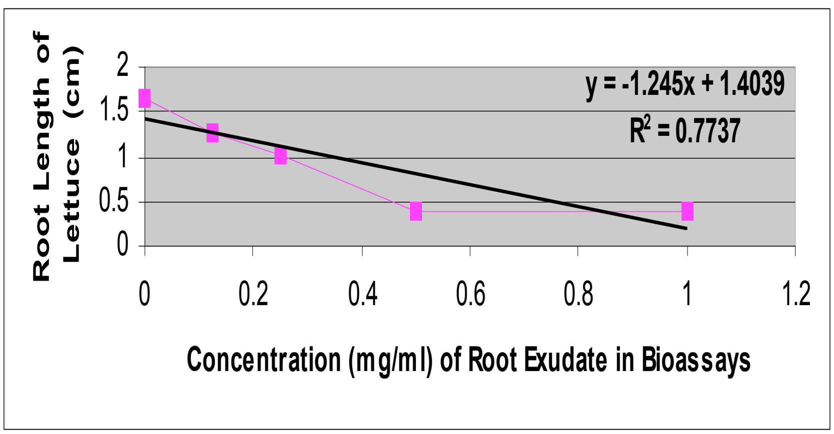

Figure 3. Reduction in lettuce root growth with increasing concentrations of fescue root exudate collected from cultivar Intrigue roots.

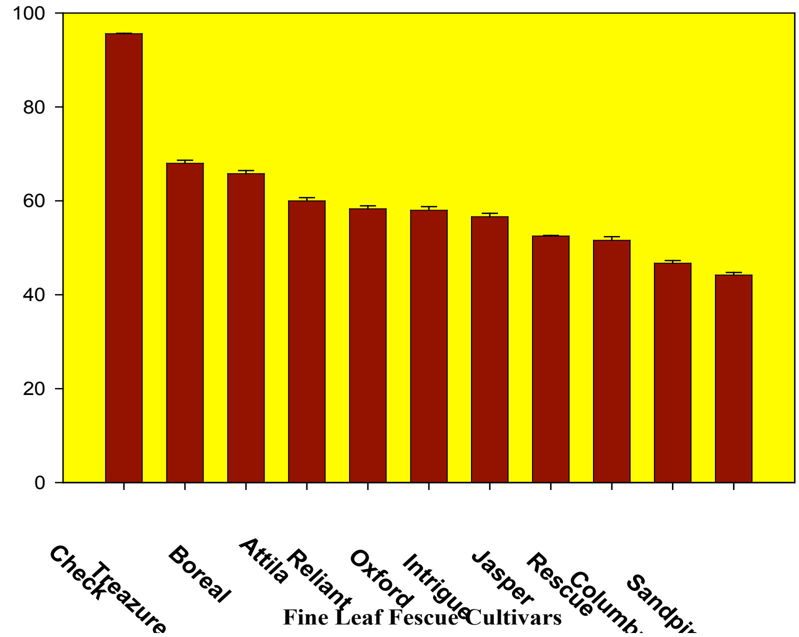

Figure 4. Influence of various fescue cultivars on weed biomass in 9/01 in Ithaca NY, 12 months after seeding under weedy (unweeded) conditions.

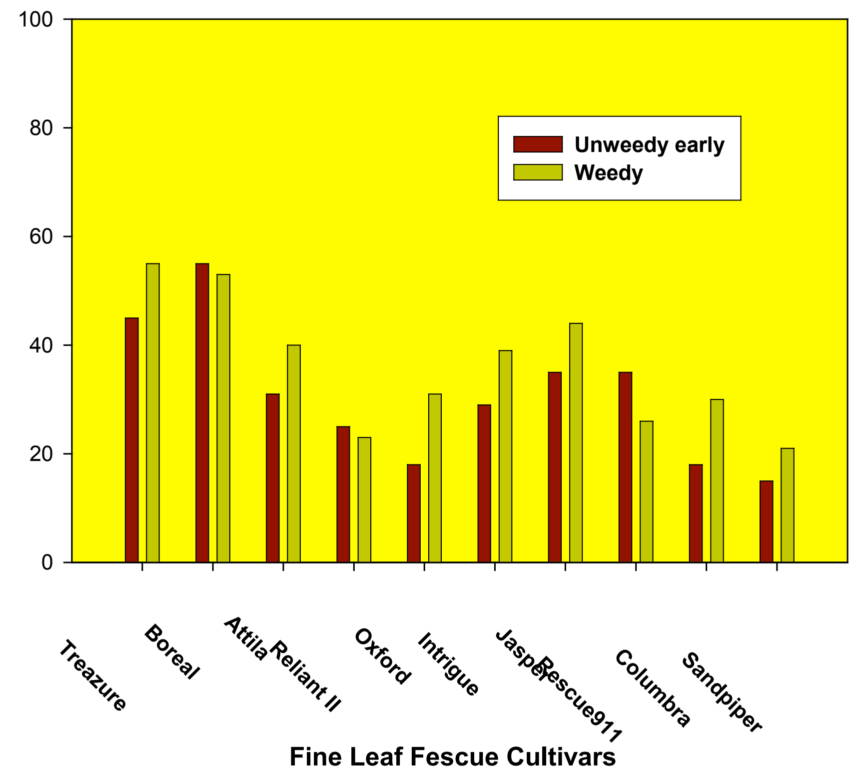

Figure 5. Influence of fescue cultivar on weed biomass in 9/01 in Riverhead NY under weedy (unweeded) and weeded conditions.

Discussion:

The data collected in the laboratory assays confirmed that living roots of fine leafed fescue cultivars produce a root exudate which is released from actively growing seedling roots and dispersed into growth media, whether the media is sand or agar based. The growth of seedling weeds and sensitive crop species is affected by significant concentrations of the fescue root exudate; seedlings show initial chlorosis and stunting followed by reductions in root and shoot growth, with root growth being most affected. Eventual death of seedlings may occur within 2 to 3 weeks following interplanting with living fescue seedlings (figure 1) . The agar media itself retains the fescue root exudate following removal of the fescue seedlings (figure 2). Therefore, sensitive seedlings are inhibited by planting into growth media which had contained living fescue seedlings, even though removed previously. This is a strong indication that bioactive chemical inhibitor(s) are produced by living fescue seedlings and the inhibitors are present in small concentrations which persist for several weeks following fescue seedling removal.

Certain cultivars of fine leafed fescues produce more exudate than others, when seedling roots were collected and extracted for quantification using a capillary mat for growth of large quantities of seedling roots. The cultivar Intrigue generally produces a potently active seed germination inhibitor which can be collected in significant quantities for further purification (figure 3).This difference in inhibitory growth potential may be due to differences in overall quantity of root exudate produced, or chemical differences within the extract that lead to more or less activity. In any case, all cultivars tested produced significant levels of exudate but certain cultivars such as Intrigue, Rescue, Reliant, Columbra and Sandpiper were generally more inhibitory in both field and laboratory assays than others such as Treazure and Boreal. This was evident in agar assays in the laboratory with greatest root inhibition noted with Reliant, Intrigue, Columbra or Sandpiper cultivars (figures 1 and 2). HPLC analysis of the root exudate using gradient analysis with a reverse phase system showed that root exudates generally contained up to 20 components with one major component which appeared similar among cultivars. Currently, purification and identification of the active components are underway.

Field experiments established in 2000 after 2 years of previous data showed again that certain fescue cultivars exhibited greater weed suppression than others in 2 separate locations, Ithaca NY and Riverhead NY, where climatic differences among locations were significant (figures 4 and 5). In particular, difficulties were encountered in obtaining initial establishment of fescues in Ithaca due to a cold fall and late spring. By August of 2001, after repeated mowing and an herbicide application to minimize crabgrass competition in one end of the experiment after seeding crabgrass previously, a thick stand of fescue was established. In September of 2001, ratings obtained for weed suppression by cultivars in Ithaca and Riverhead were uniform and consistent. Data is presented from only the September date as no other data was collected in Ithaca in 2001 due to the time required for dense establishment. Interestingly, cultivar differences were significant at both locations and moderately consistent as well. In Ithaca and Riverhead, the cultivars Boreal and Treazure were less suppressive, while the cultivars Columbra, Sandpiper and Rescue had less weed biomass and were most suppressive, with up to 2 times less weed biomass encountered in these plots. Differences appeared to be related to cultivar establishment, growth and perhaps ability to produce allelochemicals. Although establishment density may initially play a role in suppression, over time all cultivars appear to establish at similar densities from visual analysis. Further work under field conditions must be performed to determine the exact mechanism(s) involved in weed suppression over time.

This work has strong application in the turf and landscape industries as many managers and stakeholders are seeking alternative methods for weed management, other than synthetic herbicides. In addition, the green industry in NY State is expanding, with greater numbers of golf courses, athletic fields and lawns recently established in turf. The use of a weed suppressive fescue or turfgrass cultivar which offers visually appealing turf, resistance to disease and natural weed suppression due to allelopathy could be very important now and in the future, as non-chemical alternatives for weed management are mandated.