RUTGERS

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Experiment Station

Proceedings of the Eighteenth Annual Rutgers Turfgrass Symposium

January 12, 2009

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William A. Meyer, Associate Director

The Center for Turfgrass Science

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Proceedings of the Eighteenth Annual Rutgers Turfgrass Symposium

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Director's Opening Remarks:

Welcome to the eighteenth Annual Rutgers Turfgrass Symposium at the School of Environmental and Biological Sciences/NJAES. The Symposium was established in 1991 to provide Rutgers faculty, students, and staff with an annual forum for the exchange of ideas on a wide range of topics in turfgrass science. This format was expanded to include presentations by colleagues at other institutions. I would like to thank Dr. Rich Hurley for presenting this year's special topic, as well as Dr. Lane Tredway (North Carolina State University), Dr. David Kopec (University of Arizona) and the Turf Center faculty and students who have agreed to present their research at this year's meeting. I would also like to recognize the Symposium Planning Committee comprised of Dr. Jim White (Chair), Dr. Joseph Heckman, Dr. Jim Murphy and Ms. Barbara Fitzgerald (co-editor of the Symposium Proceedings) for their hard work in the preparation of this year's program. Without their efforts, this year's Symposium would not have been possible.

The faculty and students in the Turf Center continue to be recognized for excellence in research, teaching, and outreach. In 2008, Dr. Jim Murphy received the Hall of Fame Award and Dr. Bingru Huang the Recognition Award from the NJ Turfgrass Assn. for their dedication and contribution to the turfgrass industry, the Turf Center was recognized with the President's Award from the New Jersey Golf Course Owner's Assn., and Dr. Brad Hillman was named a fellow of the American Phytopathological Society and Dr. Barbara Zilinskas a fellow of the American Association for the Advancement of Science. Our graduate students in the turf program also received several major awards for their research accomplishments. At the International ASA-CSSA-SSSA meeting in Houston, TX, seven of our graduate students (Jon Bokmeyer, John Inguagiato, Matt Koch, Patrick McCullough, Emily Merewitz, Robert Shortell, and Yan Xu received awards for their research and poster presentations, and Dr. Jo Anne Crouch (who graduated from our program in May) received the Gerald O. Mott Scholarship Award from the Crop Science Society of America.

Turf Center faculty and staff continue to provide excellent undergraduate, graduate, continuing professional education and service programs in support of students and turfgrass professionals throughout the United States. We have forged a close partnership with the turfgrass industry who have donated their time and resources (over 4.4 million dollars in grants and gifts over the last 35 years) to support our efforts. This includes more than $85,000 in scholarships awarded each year to deserving students enrolled in our turfgrass program. We are indeed fortunate to have such a close partnership with the Turfgrass Industry and look forward to working with them to improve turf pest and stress tolerance

Thank you for coming to this year's symposium. I hope that you will find it an enjoyable and worthwhile experience.

Sincerely,

Bruce B. Clarke

Bruce B. Clarke, Director
Rutgers Center for Turfgrass Science and
Ralph Geiger Endowed Chair in Turf Science

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EIGHTEENTH ANNUAL RUTGERS TURFGRASS SYMPOSIUM

School of Environmental and Biological Sciences, Rutgers University

January 12, 2009

Foran Hall, Room 138A

Monday, January 12, 2009

8:30 - 9:00 AM **Registration, Coffee and Donuts**

9:00 - 10:00 AM **SESSION I: TURFGRASS MOLECULAR BIOLOGY**
(Moderator: Dr. James White)

9:00 – 9:20 **Dr. Thomas Gianfagna** (Department of Plant Biology and Pathology, Rutgers University) *Evaluation of Transgenic Creeping Bentgrass with the IPT Gene for Cytokinin Synthesis Under Nitrogen or Phosphorus Deficiency*

9:20 – 9:40 **Dr. Bingru Huang** (Department of Plant Biology and Pathology, Rutgers University) *Identification of Proteins and Genes Associated with Heat Tolerance in Agrostis Species*

9:40 – 10:00 **Ms. Emily Merewitz** (Department of Plant Biology and Pathology, Rutgers University) *A New Model of Genomic Relationships Among Creeping, Colonial, and Velvet Bentgrasses Based on Nuclear and Plastid DNA Sequence Analysis*

10:00 - 10:30 AM **Discussion and Coffee Break**

10:30 – 12:00 PM **SESSION II: SUSTAINABLE TURFGRASS MANAGEMENT**
(Moderator: Dr. Joseph Heckman)

10:30 – 10:50 **Dr. James Murphy** (Department of Plant Biology and Pathology, Rutgers University) *Assessing Wear Tolerance of 'Greenwich' Velvet Bentgrass*

10:50 – 11:10 **Dr. Lane Tredway** *(Department of Plant Pathology, North Carolina State University)* Demystifying Fairy Rings in Golf Course Putting Greens

11:10 – 11:20 Discussion session

11:20 – 12:00 **Special Topic: Dr. Richard Hurley** (Adjunct Professor, Department of Plant Biology and Pathology, Rutgers University) *The Bayonne Golf Club: A Case Study for Building a World-Class Golf Course on a Land Fill in New Jersey*

12:00 - 1:00 PM **Lunch and Poster Session**

1:00 – 2:00 PM **SESSION III: GRASS IMPROVEMENT AND UTILIZATION**
(Moderator: Dr. Thomas Molnar)

1:00 – 1:20 **Dr. David Kopec** (Department of Plant Science, University of Arizona) – *Growth Habit Evaluation of Inland Saltgrass (Distichlis spicata) for Potential Domestication as a New Turf Species*

1:20 – 1:40 **Dr. Stacy Bonos** (Department of Plant Biology and Pathology, Rutgers University) *Breeding Perennial Grasses for Biofuel in New Jersey*

1:40 – 2:00 **Dr. William Meyer** (Department of Plant Biology and Pathology, Rutgers University) *Grasses That Can be Eaten and Walked On*

2:00 – 2:30 PM **Discussion and Coffee Break**

2:30 – 3:30 PM **SESSION IV: PEST BIOLOGY, ECOLOGY AND MANAGEMENT**
(Moderator: Dr. Stephen Hart)

2:30 – 2:50 **Joseph Roberts** (Department of Plant Biology and Pathology, Rutgers University) *Recent Advances in Determining the Influence of Cultural Practices on Anthracnose Disease of Annual Bluegrass Putting Green Turf*

2:50 – 3:10 **Dr. Donald Kobayashi** (Department of Plant Biology and Pathology, Rutgers University) *From Field of Genes to Field of Dreams: How Genomic Approaches are Improving Biological Controls for Turfgrass Diseases*

3:10 – 3:30 **Jonathan Bokmeyer** (Department of Plant Biology and Pathology, Rutgers University) *Inheritance Characteristics of Brown Patch Resistance in Tall Fescue*

3:30 - 4:00 PM **Discussion/Closing Remarks**

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PLENARY PRESENTATIONS

Evaluation of Transgenic Creeping Bentgrass with the *IPT* Gene for Cytokinin Synthesis under Nitrogen or Phosphorus Deficiency

Yan Zhang, Cuiyue Liang, Yan Xu, Thomas Gianfagna and Bingru Huang

Department of Plant Biology and Pathology, Rutgers University

Nutrient deficiency has been associated with leaf senescence. Cytokinins (CKs) play critical roles in delaying leaf senescence. The objective of the study was to determine whether over-expression of a gene from *Agrobacterium tumefaciens* controlling CK biosynthesis, isopentenyl transferase (*ipt*), in a cool-season grass species, creeping bentgrass (*Agrostis stolonifera* L.), would delay or suppress leaf senescence under nitrogen (N) and phosphorus (P) deficiency. The *ipt* gene was ligated to a senescence-associated promoter, SAG12, and transferred into creeping bentgrass using *Agrobacterium*-mediated techniques. Plants of a *SAG12:ipt* transgenic line (S41) and a null transgenic line (control) were grown in nutrient solutions with all essential nutrients or without N or P for 21 d. Significant declines in leaf photochemical efficiency (Fv/Fm) and chlorophyll content of mature leaves were detected in both the control line and *SAG12:ipt* plants exposed to N deficiency, but not in the P deficiency treatment. Compared to the control line, *SAG12:ipt* plants had higher levels of Fv/Fm, chlorophyll content, and cytokinins (ZR, DHZR, and iPA) content in leaves under either P or N deficiency. These differences between the control line and *SAG12:ipt* line became more pronounced with treatment duration. Higher levels of nitrate reductase and acid phosphatase activities in leaves and roots were maintained in *SAG12:ipt* line compared to the control line at 21 d of treatment, suggesting that the transgenic plant was able to use the limited nutrients more efficiently. Despite an almost 50% decline in total leaf protein 7 d after the onset of nutrient deficiency treatments, *ipt* expression continued in the *SAG12:ipt* plants. The changes in *ipt* gene expression in the transgenic line were correlated with leaf chlorophyll content, and indicated that the expression of *SAG12:ipt* in creeping bentgrass was associated with delayed leaf senescence under nutrient deficiency.

Identification of Proteins and Genes Associated with Heat Tolerance in *Agrostis* Species

Bingru Huang, Chenping Xu, Jiang Tian, Jichen Xu, and Faith Belanger

Department of Plant Biology and Pathology, Rutgers University

Summer Bentgrass Decline caused by heat stress is a common problem in creeping bentgrass (*Agrostis stolonifera*) greens. The current commercially available cultivars do not have adequate heat tolerance. Therefore, there is an urgent need to develop high quality creeping bentgrass with improved heat tolerance. The genetic mechanisms of heat tolerance in turfgrasses are not clear. Two independent studies, differential gene expression analysis and proteomic profiling, were conducted to explore the molecular mechanisms of higher heat tolerance of *A. scabra* vs. creeping bentgrass at both RNA and protein levels. Our research on the differential heat response of two *Agrostis* grass species indicated that *A. scabra*, the thermal *Agrostis* species closely related to creeping bentgrass, was able to maintain much higher quality and physiological functions for extended periods of time compared to creeping bentgrass under high soil temperatures. A total of 143 unique genes with higher expression level in heat-stressed thermal *A. scabra* were identified using differential display (DD-PCR) or suppression subtractive hybridization (SSH) approaches. Proteomic profiling of heat-stressed vs. un-stressed *A. scabra* and creeping bentgrass has revealed 70 different proteins. A large portion of identified genes have functions involved in protein and carbon metabolism, signaling / transcription, and stress / defense and , therefore, are potentially associated with the higher heat tolerance of thermal *A. scabra*. Several common genes with known functions in heat tolerance were identified from both gene expression and proteomic profiling, including those encoding for proteins involved in carbon metabolism (fructose 1,6-bisphosphate aldolase), stress and defense responses (phenylalanine ammonia-lyase, disulfide isomerase, and glutathione S-transferase). All of these genes have suggested functions involved in heat stress tolerance by either increased glucose utilization or higher level of signaling molecules biosynthesis. In addition, four heat-responsive genes encoding heat shock protein 70 (HSP70), heat shock protein 16 (HSP16), cysteine protease (AsCP1), and expansion (AsEXP1) were selected based on their heat inducible patterns in both gene expression and Western analyses, and availability of the full length cDNA sequences for a higher chance of primer design. These genes may play critical roles in heat tolerance, and could be used as potential candidate genes for marker development.

A New Model of Genomic Relationships among Creeping, Colonial, and Velvet Bentgrasses Based on Nuclear and Plastid DNA Sequence Analysis

David Rotter and Faith C. Belanger

Department of Plant Biology and Pathology, Rutgers University

Creeping, colonial, and velvet bentgrasses (*Agrostis stolonifera* L., *A. capillaris* L., and *A. canina* L., respectively) are commercially important turfgrass species often used on golf courses. Velvet bentgrass is a diploid and creeping and colonial bentgrasses are both allotetraploids. A model for the genomic relationships among these species was previously developed from cytological evidence (Jones, 1956). The genome designations were A_1A_1 for velvet bentgrass, $A_1A_1A_2A_2$ for colonial bentgrass, and $A_2A_2A_3A_3$ for creeping bentgrass. We have used phylogenetic analysis based on DNA sequences of nuclear ITS and protein coding genes and the plastid *trnK* introns and *matK* gene to reexamine these relationships. In contrast to the previous model, the DNA sequence analysis suggested that velvet bentgrass was more closely related to creeping bentgrass than to colonial bentgrass and it may be the maternal parent of creeping bentgrass. Phylogenetic analysis of some conserved nuclear genes revealed a close relationship of the velvet bentgrass sequences with the A_2 genome sequences of creeping bentgrass. We therefore propose that velvet bentgrass be designated as having the A_2 genome, rather than the A_1 genome as in the previous model.

Reference

Jones, K. 1956. Species differentiation in *Agrostis*. Part II. The significance of chromosome pairing in the tetraploid hybrids of *Agrostis canina* subsp. *montana* Hartmn., *A. tenuis* Sibth. and *A. stolonifera* L. J. Genet. 54:394-399

Assessing Wear Tolerance of 'Greenwich' Velvet Bentgrass

James A. Murphy, T.J. Lawson, and Hiranthi Samaranayake
Department of Plant Biology and Pathology, Rutgers University

Bentgrasses (*Agrostis* spp.) are widely grown as golf course turf with creeping bentgrass (*A. stolonifera* L.) having received more study and use than velvet bentgrass (*A. canina* L.). Golf course putting greens and fairways are subjected to traffic stresses including wear and compaction. Bentgrass cultivars are typically more susceptible to wear stress than compaction, particularly under well-drained putting green conditions (Samaranayake et al., 2009). Velvet bentgrass produces a very high density turf (Beard, 1973) that, if not managed properly, can become to be too soft (Greenfield, 1962) with a strong thatching tendency (Beard, 1973), characteristics which could influence wear tolerance. Yet, Newell et al. (1997) and Samaranayake et al. (2008) reported that recent velvet bentgrass cultivars have improved performance under wear compared to many creeping bentgrass cultivars. Thus, a more comprehensive understanding of the durability of velvet bentgrass putting green turf would be useful. The objectives of these field research trials were to (1) evaluate the wear tolerance of velvet bentgrass putting green turf subjected to four rates of summer N fertilization and (2) calibrate the modified sweepster wear simulator (aka – slapper) to actual foot traffic with soft-spike golf shoes.

Trial I (objective 1). This trial was conducted on 'Greenwich' velvet bentgrass mowed daily at 3.2 mm and used a 4 x 5 factorial strip-block design with 6 replications. The first factor was summer N rate (4.9, 9.8, 19.6, and 39.2 kg ha^{-1} over 6 to 8 week periods) applied as a NH_4NO_3 spray solution of 4.9 kg ha^{-1} of N approximately every 1, 2, 4, or 8 weeks. The second factor was foot traffic (0, 100, 200, 300, and 400 rounds of golf) applied by students wearing golf shoes walking in straight line passes across N treatments on 13 June and 25 July 2008; only 100, 200 and 300 rounds of golf shoe traffic were applied on 20 September 2008. Plots were visually rated on a 1 to 9 scale (5 = minimally acceptable) for turf quality (9 = none, 5 = minimally acceptable) and color to document damage and recovery from wear.

As expected, golf shoe traffic reduced turf quality of 'Greenwich' velvet bentgrass maintained at 3.2 mm mowing height. The reduction in turf quality increased as the amount of traffic increased from approximately 100 to 400 rounds of traffic. Turf quality was nearly always acceptable across all intensities of traffic; lower than acceptable quality was only observed shortly after 300 and 400 rounds of simulated traffic on 13 June when cumulative N fertility was low for all N treatments. Increased N fertility improved turf quality and the lowest N fertility treatment [4.9 kg ha^{-1} approximately every two months] was unable to maintain acceptable ($\geq$ 5) turf quality throughout 2008. The two greatest N treatments (39.2 and 19.6 kg ha^{-1} approximately every two months) always provided acceptable turf quality. Similar results were observed for turf color response to treatments.

Trial II (objective 2). This trial also was conducted on 'Greenwich' velvet bentgrass mowed daily at 3.2 mm. The slapper wear machine was used to simulate wear. Simulated wear was applied to Greenwich velvet bentgrass in 2008 at 10, 20, 30, and 40 passes on 13 June 2008; 8, 16, 24, and 32 passes on 28 July, and 6, 12, and 18 passes on 19 September. Wear also was applied by students wearing golf shoes walking in straight line passes; 32, 64, 96, and 128 passes on 13 June and 28 July, and 32, 64, and 96 passes on 19 September. Thirty-two passes of golf shoe traffic approximates wear stress caused by 100 rounds of play within areas of concentrated traffic on a putting green. Plots were visually rated on a 1 to 9 scale (5 = minimally acceptable) for turf quality and color to document damage and recovery from wear. All plots were rated at 4 positions (subsamples) within each 20-feet long plot. Subsample data were pooled for each traffic plot and analyzed by linear regression. The linear regression equation was used to estimate the amount of golf shoe traffic and wear machine traffic needed to produce a mid-range value of turf quality or color at three to four days after traffic was applied. These estimated amounts of traffic were then used to determine the equivalency ratio of golf shoe traffic to wear machine traffic.

Wear damage increased as the intensity of golf shoe traffic increased for all three traffic events in 2008. The equivalency ratio of golf shoe traffic to wear machine traffic averaged 11.6 for turf quality (Table 1). The equivalency ratio of golf shoe traffic to wear machine traffic averaged 9.2 for turf color. Thus, it appears that the wear machine can be calibrated and used to simulate foot (golf shoe) traffic.

Table 1. Estimate amount of traffic needed to produce a mid-range quality or color rating 3 or 4 days after traffic with either golf shoe traffic or the wear machine in 2008. Estimated amounts were then used to calculate an equivalency ratio of the two forms of traffic.

Rating Date	Days After Wear	Rating Parameter	Mid-range Value	Estimated Number of Golf Shoe Passes	Estimated Number of Wear Machine Passes	Ratio of Golf Shoe to Wear Machine
16 Jun	3	Quality	5	130	11	11.8
16 Jun	3	Color	4	187	18	10.4
31 Jul	3	Quality	6	112	9	12.4
31 Jul	3	Color	6	89	10	8.9
23 Sep	4	Quality	8	37	3.5	10.6
23 Sep	4	Color	7	37	4.4	8.4

Work in 2009 will focus on repeating these trials as well as initiating a third trial to assess the ability of the wear machine to simulate daily (repeated) traffic representative of golf course turf.

Literature Cited

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Greenfield, I. 1962. Turf culture. Leonard Hill. London.

Newell, A.J., F.M.E. Crossley, J.C. Hart-woods, C.E. Richards, and A.D. Wood. 1997. STRI report to turfgrass breeders 1997. Sports Turf Research Institute, Bingley, West Yorhshire, U.K.

Samaranayake, H., T. J. Lawson and J. A. Murphy. 2008. Traffic stress effects on bentgrass putting green and fairway turf. Crop Science. 48:1193-1202.

Demystifying Fairy Rings in Golf Course Putting Greens

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Fairy ring is an aggressive disease problem in golf course putting greens, especially those that are constructed of sand-based root zones. This disease is caused by basidiomycete fungi that infest the soil and thatch and indirectly cause symptoms ranging from increased growth to necrosis and death. Many golf course superintendents have reported inadequate control of fairy ring in recent years. The cause of these control difficulties can be traced back to a lack of knowledge about the disease, including the identity of causal fungi, the soil temperatures at which they are most active, and their sensitivity to fungicides. The objectives of this research were to identify the most common fairy ring pathogens in the Southeastern United States and develop specific recommendations for prevention of the disease. Fairy ring pathogens were identified based on morphological characteristics of fruiting bodies and sequences of the ITS and b-tubulin loci. Puffball fungi belonging to the genera *Vascellum* and *Bovista* have been most common in sand-based putting greens, whereas the mushroom fungi, *Marasmius* and *Coprinus*, have been most common in "push-up" greens or on older sand-based greens. A series of field experiments were performed to develop effective fungicide programs for prevention of *Vascellum* and *Bovista* species. Experiment 1 was designed to determine the optimal rate and timing of DMI fungicide applications for fairy ring prevention. Preventative applications were initiated in spring 2007 and 2008 when 5-day average soil temperatures (2 inch depth) reached 50°F, 55°F, 60°F, 65°F, 70°F, or 75°F. On each application date, a single application of the following treatments were made: Bayleton (triadimefon; 1 oz/1000 ft^2), Bayleton (2 oz/1000 ft^2), Lynx (1 fl oz/1000 ft^2), and Lynx (tebuconazole; 2 fl oz/1000 ft^2). Both Lynx and Bayleton were most effective when soil temperatures were 55°F, but were also effective at soil temperatures up to 70°F. No significant differences in control efficacy were observed between the high and low rates, but the high rates have been observed to produce unacceptable phytotoxicity in some cases. Experiment 2 was designed to compare the DMI fungicides (metconazole, myclobutanil, propiconazole, triadimefon, triticonazole) for fairy ring prevention. Each fungicide was applied twice on a 28-day interval at a low rate beginning in late March. Bayleton, Trinity (triticonazole), and Tourney (metconazole) provided effective control in both locations, whereas Banner Maxx (propiconazole) and Eagle (myclobutanil) were moderately effective. Finally, Experiment 3 was designed to determine the effect of wetting agents and irrigation timing on DMI fungicide performance for fairy ring prevention. Bayleton and Triton (triticonazole) were applied in March and April, and were either applied alone or mixed with the soil surfactant Revolution (6 fl oz/1000 ft^2). In addition, these treatments were either watered-in immediately or 10 hours later with 0.25" of water. Tank-mixing with Revolution significantly reduced the effectiveness of both Bayleton and Trinity; however, the timing of post-application irrigation had no significant impact on fungicide performance. Based on these results, we currently recommend two applications of Bayleton, Trinity, or Tourney at the low label rates on a 30 day interval beginning when 5-day average soil temperatures teach 55°F. These fungicides should not be tank-mixed with a soil surfactant, but should be watered-in with 1/8" to ¼" of irrigation within 10 hours of application to drive the active ingredient into the thatch and soil for best results.

The Bayonne Golf Club: A Case Study for Building a World-Class Golf Course on a Land Fill in New Jersey

The Bayonne Golf Club Construction Time Line: 1996 - 2008

Richard Hurley

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Initiation of the New York Harbor Dredging Project…

- In order to compete globally, the harbor must be dredged to 50 feet to allow large international vessels to enter the port areas, including Elizabeth and Newark.
- Ongoing $1.6 billion harbor dredging project being funded by the Port Authority and the Corps.
- The project involved removing silt and rock from harbor channels to increase the depths of the waterways, but also cleaning up the soils and transferring them to places where they can be used to build new projects, such as the Bayonne Golf Club.

Bayonne Golf Club Conceived as a Result of the Harbor Dredging Project…

Located in Bayonne, NJ, the course is located on a three-mile peninsula bordering New York Bay, Newark Bay and the Kill Van Kull. The setting provides a panorama of views, including the skyscrapers of lower Manhattan, Brooklyn's waterfront, the Verrazano Narrows Bridge and the Statue of Liberty.

- Private club with 250 local and 250 international / out of area members
- Opened May 2006, clubhouse completed in spring of 2008.
- Par 71 - 7,120 yards - traditional linksland design.
- Thirteen golf holes are either on the water or have Hudson River and New York City views.
- The definition of Scottish / Irish linksland: "A strip of non agricultural land that provides the link between the sea, bay or estuary, and land suitable for agriculture."
- Today, it is not possible to construct a "natural" links golf course in an urban city setting near a metropolitan area and harbor such as New York. With this in mind, the Bayonne Golf Club is one of the best examples of a man made links course.

- Only traditional linksland grasses were used to seed the roughs, mounds and slopes. The seed mixture included varieties of Chewings, creeping red, hard and sheep fescue.

- During the planning and construction of the Bayonne Golf Club, Eric Bergstol and Richard Hurley made yearly trips to Scotland and England to evaluate and play links courses. Some of the courses included St. Andrews, Turnberry, Royal Troon, Royal Berkdale, Lytham and St. Annes, Hoylake, Prestwick, North Berwick, Royal Aberdeen, Dornoch, Nairn with over 40 courses played in total. These golfing trips to Scotland provided a wealth of creative ideas, many of which were subsequently used in the design and construction of the Bayonne Golf Club.

Bayonne Golf Club Project Team…

- Eric Bergstol, Golf Course Developer and Architect
- Richard Hurley, Agronomist
- Jim Coady, Club Secretary
- Alan Hicks, Construction Manager
- Bob Wolverton, Golf Course Superintendent
- Chris Drake, Assistant Golf Course Superintendent
- Don Elsworth, Shaper
- Lacoma McMillan, Shaper / Contractor
- Steve Kristoff, Plant Specialist
- Ron D'Argenio, Legal Counsel
- Mark Unger, Financial
- Maser Consulting, Matawan, NJ – *Site Planning, Integrated Pest Management Plan*
- Sadat Associates, Trenton, NJ - Engineering & Environmental Science – *Waterfront Development Permit.*
- Princeton Hydro, Ringos, NJ – Wetlands Mitigation, *Best Management Practices.*
- Margie Ruddick Landscape, Philadelphia, PA – *Walkway Design*

Bayonne Golf Club Property Acquisition and Regulatory Approvals Time Line…

- 1996 – Negotiations with PSGE (Public Service Gas and Electric) to lease /purchase 450 acres (above and below the water line).
- 1996 – At same time, negotiations started for the purchase of 40 acres from the Bayonne Municipal City Land Fill.
- 1999 – 2007 - Developed and Filed for Site Plan Approvals

  - City of Bayonne Planning Board

  - Hudson County Soil Conservation District

  - Bayonne Municipal Utilities Authority

  - Passaic Valley Sewer Commission (water allocation and treatment of leach ate).

  - Federal Energy Regulatory Commission (PA, NJ, MD power grid)
- 1998 – Completed agreement with Army Cops of Engineers and NJ DEP for both "Waterfront Development Permit" and "Remedial Action Work Plan"
- 1999 – OENJ (Orion Elesepth New Jersey). Two Danish Brownfield investors obtained.
- 1999 – Eric Bergstol and OENJ entered into a contract for Mr. Bergstol to design and build a golf course (with a 200 year lease).
- 1999 – Under RAWP (Remedial Action Work Plan) fill material was brought on to the site (with approved materials).

  1. Amended dredge – 8% Portland cement.
  2. Fly ash
  3. Water treatment residual
  4. Screenings from construction material including soil with concrete, rock and bricks.
  5. Ground glass
- 2001 - Mr. Bergstol started negotiations with PSE & G to purchase and close a 61 megawatt peaking plant (powered by two 747 jet engines and one 1,000,000 kerosene holding tanks). Purchased and dismantled in 2006. NJ Board of Public Utilities and Federal Energy Regulatory Commission transfer of utility to private property.

Bayonne Golf Club Construction Time Line…

- 1999 – 2004 - A total of 7 ½ million cubic yards of approved materials was brought to the site.
- 1999 - 2004 - Total of 2 million cubic yards of dredge was brought to the site.
- 1999 – 2003 - Built slurry wall around 130 acres of upland. Slurry wall made of dredge mixed with Bentonite.
    - Slurry wall and cap made an upside down bathtub to retain leachate.
    - Installed a methane gas venting system – multiple vents and one flare.
    - Installed leachate collection system
- 2001 - Cherokee Investment Partners purchased OENJ.
- 2002 - A 14 acre wetlands mitigation area constructed (adjacent to holes 2, 8, 14, and 16).
- 2004 - Stopped taking harbor dredge except for clean fill and sand (for capping fairways).
- 2004 - 2005 - Golf Course shaping and construction of greens, tees, fairways, bunkers and roughs.
- 2005 - Seeding greens, tees, fairways and roughs. Building bunkers and planting plants.
- 2005 - Eric Bergstol bought out the lease and purchased the property.
- 2006 – In May golf course open for play.
- 2006 – Clubhouse construction started.
- 2006 – Short game practice area constructed and seeded.
- 2007 - Fish habitat study netted 12,000 fish (adjacent to holes 2, 8, 14, and 16). In 1997 prior to construction of the wetlands mitigation area, a fish habitat study netted 189 fish.
- 2007 – NFA letter received (No Further Action – Completed the RAWP or Remedial Action Work Plan). This is a covenant from the state of NJ DEP which states that all of the remedial work has been successfully completed and legal suit against the developer cannot take place (for non performance).
- 2008 - In April clubhouse construction completed and opened for use by the club members.
- 2008 – The Bayonne Golf Club identified as one of the "BEST NEW PRIVATE GOLF CLUBS IN AMERICA" and ranked as number 32 for Golfweek's modern list of the "TOP 100 GOLF COURSE BUILT SINCE WW II".

Growth Habit Evaluation of Inland Saltgrass (*Distichlis spicata*) for Potential Domestication as a New Turf Species.

David M. Kopec

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Inland saltgrass is a native C4 grass which, as a true halophyte, tolerates high soil salinity, alkalinity or both. It is dioecious and produces robust rhizomes deep in the soil. Collections of clonal plant accessions were initially made on the Front Range of the Rocky Mountains where this grass is often maintained as an unidentified natural turf on roadsides, parks and, on occasion, golf courses. One hundred accessions of saltgrass were mowed three times per week at 5.5 cm as replicated propagules in two greenhouse trials. Test 1 was conducted using a soilless media, while test 2 utilized a medium sand irrigated with a 12,000 ppm of a ½ strength Hoagland solution and sodium chloride. Clones were evaluated for number of shoots per plant, visual estimates of innate density, leaf width, percent pot cover, turfgrass quality, color and percent straw retention of the foliage. Estimates of Broad Sense Heritability (BSH; Repeatability) were calculated from the combined analysis of variance using the formula BSH = Vg / (Vg + Vg *Ve + Ve), where Vg, Ve, and Ve * Vg are estimates of the genetic, environment and interaction variance components derived from expected mean squares, respectively. BSH values for percent pot cover and visual turfgrass quality were 72% and 74% over the two environments, while BSH estimates of visual plant density were greater in the saline environment. Leaf angle and the presence of leaf hairs were not phenotypically correlated with growth habit. About 10% of the screened collection proved to have had high shoot densities and short leaf internodes, which produced a more attractive turf-type growth habit. Plant growth habit was not related clone sex type.

Breeding Perennial Grasses for Biofuel in New Jersey

Stacy A. Bonos, Eric N. Weibel, Robert Shortell, Mathew Koch and Laura Cortese

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The Energy Policy Act of 2005 (EPACT 2005) issued a mandate for the use of up to 7.5 billion gallons of renewable fuel in gasoline by 2012 (Farrell et al., 2006). This amount will likely increase in the future as we shift our energy demands away from foreign oil supplies. One concern is whether sufficient amounts of biomass can be supplied without impacting the cost of agricultural land, competing with food production and harming the environment. The national strategy is to produce bioenergy crops on marginal cropland (Vogel, 1996) where there will be no competition with food production. Although perennial grasses such as switchgrasses are expected to be used as a biofuel crop on marginal land, there has been little to no extensive research to evaluate their performance on marginal land. Therefore, it is unknown how perennial grasses will perform on marginal land. This knowledge is critical to the successful development of biofuels so that enough biomass can be generated domestically to offset foreign oil dependency.

To date, most if not all of the research and selection of perennial grasses including switchgrass for biofuel production has been conducted by scientists on prime farmland on their research farms. The cultivars developed by these breeding programs are expected to perform well in all environments. However, biomass yield is considered a complex trait that is controlled by many genes and influenced by the environment (Sleper and Poehlman, 2006). The interaction of genes with the environment results in different biomass yields in different environments. Additionally, Parrish and Fike (2005) reported that cultivars originating in Eastern North America were less productive when grown in the Great Plains and vice versa. Breeding switchgrass and other 'locally' adapted native grasses on marginal land for maximum biomass productivity on a regional basis will improve the quality/productivity of grass crop feedstocks used in biomass energy programs/processes.

Our project is investigating 1) the optimum selection technique to identify the best performing switchgrass plants on marginal land in the Northeast; 2) the germplasm/cultivars of switchgrass that perform best on marginal land; 3) development of new cultivars with improved biomass production for biofuels on marginal land in the Northeast US.

Trials have been established in paired fields (prime vs. marginal) in nine locations throughout the country in 2008 and 2009. Additionally, paired fields in three locations in NJ are also being evaluated. Biomass characteristics including tiller number, plant height, establishment and biomass yields will be collected on these trials to determine the effect of marginal soils on biomass characteristics. This information will be used to identify optimum selection techniques to develop new cultivars with high productivity on marginal land for the Northeast US.

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Grasses That Can Be Eaten and Walked On

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In the fall of 2008, a pasture trial was seeded at the Adelphia Research Center, Freehold, New Jersey. This trial was seeded in 5 x 7 ft plots with 40 different species and mixtures of forage grasses. This trial was maintained at a 2.5 in. mowing height and mowed weekly. In June of 2008, a wear strip was applied to one-half of each plot using the 3 ft wide Rutgers wear simulator. During the period from June 19th to September 16th, a total of 20 passes were applied to this trial.

Kentucky bluegrasses (*Poa pratensis*), and combinations of Kentucky bluegrass, orchardgrass (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), and colonial bentgrass (*Agrostis capillaris*) provided the highest level of wear tolerance. The tall fescues and tetraploid ryegrass (*Lolium perenne*) were intermediate for wear tolerance. The mixtures of forage grasses sold commercially and orchardgrass cultivars were below the mean for wear and the three Timothy (*Phleum pratense*) cultivars were the poorest for wear tolerance.

A breeding program was initiated to breed for tall fescues and Kentucky bluegrasses that have potential for pastures and hay. Two synthetics of tall fescue and a few cultivars of Kentucky bluegrass were found to have better forage potential.

Five cultivars of Teff (*Eragrosis tef*) were seeded in forage trials at the Adelphia Research Center on May 30, 2008. This trial was harvested on July 29, 2008, with the cultivar Tiffany producing over 3 tons of dry hay per acre. The biggest disappointment in this hay crop was the low levels of protein (6.7%) in this bright, attractive hay. However it was perfect for adult horses that have only maintenance requirements and could easily be balanced with supplemental concentrates fed to horses with higher nutritional needs.

A collection trip was conducted by Melissa Wilson and Pieter den Haan in June 2008 in the mountains of Italy and France. They collected over 150 orchardgrasses with improved resistance to stem and stripe rust. These will be evaluated in New Jersey in the fall of 2009.

Recent Advances in Determining the Influence of Cultural Practices on Anthracnose Disease of Annual Bluegrass Putting Green Turf

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Anthracnose is a destructive disease of annual bluegrass (*Poa annua*) and bentgrass (*Agrostis stolonifera*) putting green turf throughout the United States. The disease begins as small areas of yellowed turf (2.5 to 5 cm in diameter) with individual leaf blades eventually senescing. As neighboring plants are affected, the condition worsens resulting in an overall thinning of the turf canopy which greatly influences the aesthetics and playability of putting green turf. The frequency and severity of anthracnose outbreaks on golf course putting greens has increased over the past decade. While the reason for greater anthracnose severity is not fully understood, it has been suggested that management practices employed to improve playability and increase ball roll distance on putting greens are partly responsible. Previous work at Rutgers indicated that management practices such as mowing, rolling, verticutting, fertilization, growth regulators and sand topdressing have significant effects on anthracnose development on annual bluegrass putting green turf.

An evaluation of irrigation quantity effects on anthracnose was initiated in 2006. This study was arranged as a randomized complete block design with five replications. Treatments of 100, 80, 60 and 40% replacement of reference evapotranspiration (ET_o) were chosen to represent irrigation quantities ranging from excessively wet (100% ET_o) to severely dry (40% ET_o). Treatments were calculated daily using onsite weather data and the Penman-Monteith equation. Irrigation was applied using a hand-held watering wand equipped with a flow-meter. Deficit irrigation (40% daily ET_o replacement) generally had the greatest amount of disease compared to plots with higher soil water content over the three years of this trial. Disease tended to decrease as irrigation quantity increased; that is, 60% ET_o replacement had less disease than 40% $ET_{o,}$ and 80% ET_o had less disease than 60% ET_o. Replacing 100% ET_o daily (excessive soil water) produced levels of disease similar to 40% ET_o treatments in August 2006 and 2008. While this relationship was not observed in 2007, turf quality of 100% ET_o plots was greatly reduced by August in all three years of study. Irrigation at 80% ET_o often resulted in the least disease and best turf quality. In 2008, an additional study was initiated to evaluate the effect of mowing timing relative to daily irrigation at 100% ET_o. Disease severity was the same regardless of whether irrigation was applied before or after mowing.

Light-weight rolling can be effectively used to increase ball roll distance (aka, green speed) on putting green turf. A study to follow-up on previous work was initiated as a 2 x 3 factorial in 2006 and compared the effect of light-weight roller type as well as area of equipment operation (center vs. perimeter of a putting green) on anthracnose severity. The first factor of location was blocked as either the center or perimeter of the study. The center block received straight-line passes of rolling and mowing equipment while the perimeter received additional traffic associated with the turning of equipment as well as clean up passes from mowing. The second factor of roller type (i.e., sidewinder, triplex mounted vibratory and non-rolled) consisted of one pass oriented as strips perpendicular to the location blocks; rolling was performed every other day after the mowing in the morning. Both roller types reduced disease compared to non-rolled turf under moderate disease pressure in 2007 and 2008. The heavier sidewinder roller had less disease than the triplex mounted vibratory roller on 4 of 13 rating dates over two years. Turf areas where rollers changed direction of travel and received a “clean-up” mowing (i.e., at the edge of a putting green) had less disease compared to the area where rollers traversed with no clean-up mowing on 6 of 13 rating dates. It should be noted that clean-up mowing was done in straight passes which may not produce as much wear stress as a mower travelling along a curved path.

Previous studies at Rutgers reported that routine sand topdressing can effectively reduce anthracnose severity. A study was initiated in 2007 to evaluate the effects of sand topdressing and foot (golf shoes) traffic on anthracnose severity. This experiment used a 2 x 2 factorial arrangement with 4 replications. The first factor of traffic consisted of 0 or 64 passes of foot (golf shoe) traffic each day, 5 days wk^{-1}. Foot traffic was initiated from 16 different starting positions at the ends of each plot so that footsteps were distributed evenly over each plot. Traffic resulted in 323 footsteps m^{-2} which is representative of traffic experienced around the hole of a putting green that receives ~200 rounds day^{-1}. The sub-plot factor of topdressing consisted of 0 or 0.3 L m^{-2} of sand applied weekly. Unexpectedly, foot traffic decreased anthracnose regardless of whether or not sand topdressing was applied. Sand topdressing initially increased anthracnose in 2007; however, continued weekly topdressing reduced anthracnose severity by August 2007 and throughout 2008. The combination of daily foot traffic and weekly sand topdressing resulted in the lowest disease severity and best turf quality at the end of 2007 and 2008.

Adequate N fertility to sustain moderate growth has been previously reported to reduce anthracnose severity. A study was initiated in 2007 to determine the effect of variable rate and frequency of soluble-N fertility during mid-season on anthracnose severity. This was a preliminary study to support a larger N fertility study that will examine spring and autumn N fertilization (granular) in combination with soluble-N (foliar) fertilization. Treatments of 4.9 kg ha^{-1} every 1, 2, 4, and 8 wk and 9.8 kg ha^{-1} every 2 and 4 wk were applied as a foliar spray to plots arranged in a randomized complete block design with 3 replications. The latter two treatments were included to determine the feasibility of increasing intervals between sprays by increasing the rate of application in an effort to reduce costs, while maintaining the same level of disease suppression. The date of initiating mid-season fertilization (mid-May vs. mid-June) was also evaluated by replicating all treatments over two start dates. Nitrogen applied at 4.9 kg ha^{-1} every 7-d or 9.8 kg ha^{-1} every 14-d had the greatest reduction in anthracnose severity throughout both years. Preliminary analysis indicates that the response is linear within the range of treatments studied. Nitrogen applied at 4.9 kg ha^{-1} every 14-d was the lowest N treatment to reduce disease severity. Mid-season soluble-N fertilization at 4.9 kg ha^{-1} every 28- or 56-d had the greatest anthracnose severity. Initiating N fertilization before the onset of disease (May) reduced anthracnose severity compared to fertilization begun at the onset of disease (June). Essentially, treatments initiated in May applied more N than treatments initiated in June before disease was present. These results were used to determine the appropriate soluble-N treatments to be used in a more comprehensive N study initiated in September 2008.

From Field of Genes to Field of Dreams: How Genomic Approaches are Improving Biological Controls for Turfgrass Diseases

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For more than two decades, biological control has been recognized for its potential as an environmentally safe method for turfgrass disease control. While significant effort has gone into developing microbial agents for this purpose, only a few have been developed commercially. The lack of products largely reflects efficacy problems that have evolved from our failure to better understand biocontrols and how to use them effectively.

Rapid advances in the field of genomics have been made over the past decade and researchers are now turning to these modern molecular methods to advance our understanding of biocontrol. Two areas of practical benefit arising from genomics research involve genome mining, and the capacity to pursue a systems biology approach for studying biocontrol systems. Both can contribute to gene discovery as well as provide insights into ecological aspects that are useful for improving biorational approaches for plant disease control. Currently, the genomes of at least two bacterial biocontrol agents that display efficacy toward turfgrass diseases have been sequenced. The genome sequence of *Pseudomonas fluorescens* Pf-5, which has been shown to suppress dollar spot and Drechslera leaf spot, was completed and published in 2005 (Paulsen et al. 2005. Nat. Biotechnol. 23:873-878). The genome sequence of *Lysobacter enzymogenes* C3, which has shown promise in controlling Bipolaris leaf spot, summer patch, dollar spot and gray leaf spot, has also been recently completed and its analysis is currently in progress. Analyses of both bacterial genomes have already identified novel genes predicted to contribute to fungal antagonism; these genes are now being evaluated for their role in biological control. We are also utilizing transcriptomic and proteomic approaches to evaluate whole genome expression patterns during bacterial interactions with fungal hosts. Results from these studies are expected to improve our understanding of turfgrass biocontrol and help devise new biorational approaches for turfgrass disease control.

Inheritance Characteristics of Brown Patch Resistance in Tall Fescue

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Tall fescue (*Festuca arundinaceae* Schreb.) is a cool-season turfgrass widely utilized throughout the United States transition zone for home lawns, athletic fields, parks, and low maintenance situations. Brown patch (caused by the fungus *Rhizoctonia solani* Kühn) is one of the most devastating fungal pathogens that affect tall fescue during the summer months. Symptoms of the disease range from small lesions on individual leaf blades to large circular patches of discolored turf (Smiley et al., 2005). These symptoms compromise the overall quality and aesthetics of the turf stand and during periods of intense disease pressure can result in the reduction of stand density

As more emphasis is placed on developing management strategies that reduce the amount of chemicals applied to the turf, alternative strategies for controlling disease development must be identified. The development of genetic resistance through classical and modern breeding techniques would provide a stable long-term solution for controlling brown patch in tall fescue. Very little information regarding the genetic inheritance of brown patch resistance in tall fescue is known. The objectives of this project were to (*i*) estimate the broad-sense heritability of brown patch resistance in tall fescue and (*ii*) determine inheritance characteristics of brown patch resistance in tall fescue.

Broad-Sense Heritability

A study was initiated in 2005 evaluating 230 tall fescue genotypes comprised of experimental breeding material as well as germplasm recently collected from Europe. Field trials were established at the Rutgers Turf Research Farm at North Brunswick, NJ and at the Rutgers Turf Research Farm in Freehold, NJ. Both field trials were arranged in a randomized complete block design with six replications. Each field trial was inoculated with two isolates of *R. solani* and visual disease ratings were taken when brown patch symptoms were present during the summers of 2005 and 2006. A continuous phenotypic response to brown patch was observed among the tall fescue genotypes. A moderate broad-sense heritability estimate was calculated from this study indicating that while there is a genetic component to resistance, there is also a significant environmental influence.

Inheritance Characteristics

To understand the type of gene effects that are contributing to brown patch resistance in tall fescue, controlled crosses were made between resistant and susceptible plants. Three resistant and three susceptible parents were crossed in a diallel design in the spring of 2006. Seed was harvested from each parent used in the crosses, germinated, and then 100 seedlings were randomly selected to be planted in a field study. A field trial was established at the Rutgers Turf Research Farm in Freehold, NJ in the fall of 2006. The field trial was arranged in a randomized complete block design with four replications (each replication consisting of 25 progeny harvested form each parent and six clonal replications of each parent plant). The field trial was inoculated with a single isolate of *R. solani* and visual disease ratings were taken while disease symptoms were present during the summers of 2007 and 2008. Narrow-sense heritability and both general and specific combining ability estimates indicate that additive gene effects are relatively more important than nonadditive gene effects in the phenotypic expression of brown patch resistance in tall fescue. From this study it was also estimated that 1-4 genes for resistance were segregating in the different populations.

Brown patch resistance in tall fescue appears to be quantitatively inherited and strongly influenced by the environment. This research project has shown that the most effective selection program for brown patch resistance in tall fescue is to screen plants over multiple locations and years. Because of the environmental influence, progeny testing of resistant plants must be done to identify parents with high general combining abilities that can be incorporated into a breeding program.

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POSTER PRESENTATIONS

Development of Molecular Typing Methods to Identify Shifts in Races and Species in Turfgrass Rust Populations

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Rust is a common disease of cultivated turfgrass that can result in extensive damage or even plant death in heavily infested areas. Current control methods rely on improving turf vigor and planting resistant cultivars. Over the past six years, turfgrass breeders have observed a shift in susceptibility among certain Kentucky bluegrass (*Poa pratensis* L.) cultivars, particularly the 'Midnight' types. Once highly resistant to rust, these cultivars now experience a greater incidence of disease. It has been proposed that newly emerging fungal races or species may be responsible for this recent upsurge in turfgrass rust susceptibility, but the genetic information required to test this prediction is lacking. In this study, we are developing molecular methods to evaluate the species and populations that contribute to rust diseases in turfgrass hosts. To date, 114 rust infested samples have been collected from eighteen states and four countries from Kentucky bluegrass and ten additional warm- and cool-season grass hosts in the family Poaceae. Using rust spores that have been directly harvested from infested host tissue, a reliable protocol for rust DNA extraction has been developed. Initial broad screening of the rust samples through phylogenetic analysis of sequence data from the ribosomal internal transcribed spacer region (ITS) is currently underway. The data generated through these investigations will be used as a foundation for the targeted development of species and race specific molecular markers that can be used by pathologists and breeders for rapid detection and identification of turf rust pathogens from the field in real-time.

Characterization of Bioenergy Traits in Ten Populations of Switchgrass Grown in New Jersey

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Switchgrass (*Panicum virgatum*) is a perennial warm-season grass (C4) native to most of the United States with the exception of some Northwestern States. Switchgrass has been used in restoration, buffer strips, as a forage crop, and as an ornamental, but is now emerging as source of alternative energy due to its native status, perennial life cycle, ability to grow on marginal land, and high yields with little inputs (Vogel, 2004). The Northeast/ Mid-Atlantic region is the most populated and urbanized region of the United States and is responsible for a large amount of the fossil fuels consumed in this country. It is unlikely that it will be economically feasible to transport biomass from far distances to utilize as renewable energy in the Mid-Atlantic and Northeast. Therefore it will be important to identify and breed germplasm specifically for this environment if biomass is going to be a significant source of renewable energy for this region. In addition, researchers have identified the importance of breeding for improved biomass production for specific sites (Fike et al., 2006; Lewandowski et al., 2003; Vogel and Jung, 2001) and suggest that no single switchgrass cultivar is likely to have yield advantages across all locations (Casler et al., 2004; Hopkins et al., 1995).

Switchgrass is an outcrossing, self-incompatible polyploid species with a high degree of genetic diversity. Switchgrass ecotypes are grouped into Upland and Lowland types, which differ in growth habit and habitat adaptation. Upland types are typically octaploids (Hopkins et al., 1996), have shorter, finer stems, are better adapted to drier habitats (Lewandowski et al., 2003), and mature earlier than lowland types (Parrish and Fike, 2005). Lowland types are generally tetraploid (Hopkins et al., 1996), taller, and more robust than upland types. They have courser stems, a more bunch type growth habit and are adapted to wetter sites (Lewandowski et al., 2003). They typically mature later than Upland types and require a longer growing period. In general the two types are genetically incompatible (Barnett and Carver, 1967) due to difference in ploidy level (Missaoui et al., 2005) and have significant differences in chloroplast DNA (Hulquist et al., 1996). The two types have also been found to have different biomass yields in different environments. Upland ecotypes are better adapted to mid-northern latitudes and Lowland types are better adapted to lower latitudes (Parrish and Fike, 2005).

Ten switchgrass populations were planted in a spaced-plant nursery in the spring of 2006 at the Rutgers University Plant Biology Research and Extension Farm at Adelphia, NJ. Plants were evaluated for two years in 2007 and 2008 for several characters contributing to biomass including: winter injury, disease, lodging, maturity, tiller density, plant height, and biomass yield. In general, Lowland cultivars were taller and matured later than Upland ecotypes. Northeast ecotypes had less winter injury and showed better disease resistance and tiller density than Midwest ecotypes. Northeast ecotypes generally were taller with less lodging, supporting the concept of locally adapted ecotypes. Typically, Lowland populations had greatest biomass yields, while the Upland populations had the lowest biomass yields. Timber, an Eastern Lowland ecotype had one of the highest mean biomass yields with tall plants and medium winter injury, tiller density, maturity, disease, and lodging, making it a promising population for biomass production in the Northeast/Mid-Atlantic region of the United States.

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Characterization of Anthracnose Disease of Switchgrass (*Panicum virgatum*) Caused by a Novel Species of the Fungus *Colletotrichum*

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Perennial grasses such as native switchgrass (*Panicum virgatum*) are a renewable energy resource that may ultimately play a role in reducing U.S. dependence on foreign fossil fuels and minimizing emissions of greenhouse gases and other environmental toxins, as well as providing a new source of income for American farmers. Because switchgrass is grown in perennial monoculture on a long-term basis, disease susceptibility may become a serious problem for this plant if potential pathogens are not considered during the breeding process. Forty two species of fungi have been reported to cause disease in switchgrass in the United States (Farr *et al*, 1985), although smut caused by *Tilletia maclaganii* is presently the only disease where reductions in biomass yields have been reported.

In June 2008, anthracnose symptoms were observed from several accessions of 3-yr old switchgrass plants grown in the evaluation plots at the Rutgers University Adelphia Research Center, Adelphia, NJ. Cultivars 'Cave-in-Rock', 'Brooklyn' and 'NSU 200101' were all symptomatic. Leaf lesions were found on several plants from each cultivar; they were tan coloured and elongate, with sharply tapered ends and reddish brown borders. Masses of black setae (93-127 µm x 1.3-3.5 µm) diagnostic for the genus *Colletotrichum* were frequently present in the center of the lesion, often arranged in long rows running parallel to the leaf veins. Comparison of the diseased leaf tissue with herbarium samples of switchgrass anthracnose collected during the years 1886-1962 showed that the leaf lesions were consistent with anthracnose disease. The 'Brooklyn' cultivar also had irregularly shaped lesions on the stalks; here the lesions possessed a slightly discolored center bordered by a reddish brown margin, with many of the lesions coalescing. In contrast to the leaf lesions, those observed on stalks had rounded ends, setae were infrequently observed, and small black circular regions were found in the center of lesions, sometimes coalescing with adjacent spots. Microscopic observation identified the presence of sharply falcate-shaped mitotic spores with apices acute (34.2-40.5 µm x 2.8-5.0 µm) characteristic of *Colletotrichum* species that cause anthracnose disease in other grass hosts. No sexual structures were present.

Lesions from leaves and stalks were plated onto potato dextrose agar (PDA) supplemented with antibiotics. Several pale grey to cream colored fungal cultures similar to species of *Colletotrichum* associated with other warm-season (C4) grasses were isolated from both leaf and stalk lesions. Like many species of *Colletotrichum* associated with C4 hosts, no setae or spores were initially produced in culture, nor were sexual structures present. Under high light and temperature, sporulation and production was induced, with size and shape of the structures consistent with those observed *in planta*. In moist growth chambers, hyphal appressoria were induced in response to a glass surface, confirming that the fungal isolates were members of the genus *Colletotrichum.* These melanized structures were ovoid to obovoid, lobate or multi-lobate (10.0-14.8 µm x 7.3-9.5 µm). Together, these morphological characteristics do not specially conform to the descriptions of any of the 14 species of *Colletotrichum* characterized from grass hosts (Crouch *et al*., 2009a), nor are they consistent with the species names applied to the switchgrass anthracnose herbarium samples (*i.e.*, *C. graminicola*, *C. lineola*, *C. sanguineum*). Given that host association is tightly linked to species boundaries in the C4 grass associated *Colletotrichum* (Crouch *et al*, 2009b), in combination with the distinct morphological characteristics observed, it is likely that switchgrass anthracnose is caused by a novel species of *Colletotrichum*. Multilocus DNA sequence analysis as previously described for species determination in the graminicolous *Colletotrichum* (Crouch *et al*, 2009b) is currently underway to provide additional information about the identity of this fungus. Experimental confirmation of pathogenicity is also underway in a controlled environment growth chamber (30ºC, 16-h day, 60% RH) using 8-wk-old seedlings of *P. virgatum* cultivar 'Brooklyn' inoculated with 5-ml conidial solution (5 x 10^4 conidia ml^{-1} in 0.1x potato dextrose broth [PDB]); sterile 0.1x PDB solution serves as the negative control.

Although anthracnose disease was present on several switchgrass accessions in the present study, there was no indication that overall plant vigor was affected, and it is unknown whether biomass was reduced due to disease. Susceptibility of other cultivars is also unknown but needs to be investigated. Switchgrass anthracnose has been documented since 1886, but has never been considered an important disease of this plant. Nevertheless, given the increasingly important role that switchgrass will play in the U.S. economy in the upcoming years, gaining a comprehensive understanding of the pathogens that could potentially limit production is vital. This is particularly true for anthracnose disease, as there are two recent examples of destructive outbreaks of anthracnose epidemics in grasses that emerged in the past 30 years: the corn anthracnose epidemics of the 1970-80s, and the anthracnose epidemics of cool-season turfgrass hosts (*i.e.* annual bluegrass [*Poa annua*] and creeping bentgrass [*Agrostis stonlonifera*) which began during the 1990s and persist to this day.

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Does Movement of *Colletotrichum cereale* from Natural Grasses and Cereal Crops Promote Turfgrass Anthracnose Disease?

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Anthracnose disease caused by *Colletotrichum cereale* is one of the most destructive maladies of golf course turfgrasses. The fungus has also been identified from numerous natural grasses and cereal crops, although disease symptoms are rarely observed. In this research we investigated the role of ecosystem (turf, cereal crop or prairie) and the impact of natural grass/cereal strains on turf anthracnose. Genotypic signatures from 4 nuclear genes and 22 microsatellite markers were used to analyze an extensive sample from the North America, Europe and Japan. Eleven major populations were identified, structured according to ecosystem type: three turfgrass groups, seven prairie/cereal groups and one diverse group comprised of both turf and non-turf isolates. The turfgrass populations were further defined according to host species: two groups almost entirely limited to *Poa annua*, the third to *Agrostis stolonifera*. In cereal/prairie populations, a similar pattern was observed, dividing wheat and oat isolates into discrete groups. Extreme differentiation between locally-adapted populations suggests asymptomatic grasses are unlikely reservoirs of infectious disease particles that could serve to fuel disease in turf. But gene flow between the generalist founder population and specialized genotypes provides a mechanism for genetic exchange between otherwise isolated populations. These findings demonstrate that while disease occurrence and spread is currently localized to the turfgrass environment, introgression between *C. cereale* ecotypes can lead to the expansion of anthracnose disease into new ecosystems.

Capacity of Grass Seeds to Hold Mineral Nutrients

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Planted seeds often respond positively to fertilization because seed nutrient reserves are easily exhausted by the rapidly dividing and growing cells. Also, developing root systems have limited contact with nutrients from the soil. Phosphorus may be an especially limiting nutrient because P is resupplied to the root surface from the soil solution by the very slow process of diffusion.

In our previous soil test calibration studies (Hamel, S.C. and J.R. Heckman. 2006. Predicting need for phosphorus fertilizer by soil testing during seeding of cool-season grasses. HortScience. 41:1690-1697.), we observed that Kentucky bluegrass requires higher levels of soil P availability to achieve its early growth potential as compared to either perennial ryegrass or tall fescue. This variation in responsiveness to soil fertility levels has shown that it is risky to extrapolate soil fertility recommendations from one crop to another. One of the potential explanations for why species can vary markedly in growth rate during establishment from seed is that plant species vary substantially in seed size and therefore in inherent capacity to store mineral nutrients needed for a plant's early growth phase. The present study was therefore conducted to measure the mineral nutrient contents stored in grass seeds.

Four varieties each of Kentucky bluegrass (SR2284, Brooklawn, Alpine, and Award), tall fescue (Tarheel 2, Sixpoint, Mustang 3, and Falcon IV), and perennial ryegrass (Repell, Panther, RNS-M146-5, and Palmer) seeds were collected from commercial sources. The fresh and dry weight of 100 seeds was measured. Seeds were placed in an oven at 70°C for 24hrs, and ground with a Wiley mill to pass a 1 mm screen. The samples were sent to Brookside Laboratories, New Knoxville, OH for determination of tissue mineral concentrations. The results, presented in Table 1, are expressed both in nutrient concentration per unit dry weight of seed tissue and as nutrient content per seed.

Seed weights on average showed that tall fescue seeds are 6.3x and perennial ryegrass seeds are 4.6x larger than Kentucky bluegrass seeds. For concentrations of mineral nutrients in seeds, only K, Ca, and B were different among species. However, the content of mineral nutrients on a per seed basis was markedly different for nutrients as may be expected due to the large difference in seed size. For example, the concentrations of P in grass seed tissue was very similar among species, but because of differences in seed size there was a highly significantly reduction in amount of P contained in the seeds of Kentucky bluegrass. This large difference in capacity to store P may partly account for why new plantings of Kentucky bluegrass grow much slower at a given soil test P level than either perennial ryegrass or tall fescue.

Table 1. Analysis of Kentucky bluegrass (KB), tall fescue (TF), and perennial ryegrass (PRG) seeds for fresh and oven dry weight, water and mineral content, and concentrations of minerals in the seed tissue.

	Seed Properties			Mineral Content Per Seed										
Species	Fresh weight	Dry weight	Water	N	P	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn
	mg/seed			µg/seed										
KB	38.0	35.0	3.0	703	144	150	92	60	83	0.3	3.9	2.2	0.3	1.4
TF	240.6	217.5	23.2	4803	906	1565	397	412	480	0.9	16.0	10.4	1.6	6.8
PRG	180.8	163.1	17.7	3891	681	940	312	262	404	0.5	13.2	6.5	1.8	5.9
Species	0.0001	0.0001	0.0001	0.0002	0.0006	0.0001	0.003	0.0001	0.0002	0.002	0.002	0.005	0.038	0.0005
$LSD_{0.05}$	30.0	27.2	3.2	1059	235	207	125	74	104	0.2	4.9	3.7	1.1	1.7
CV%	11.4	11.3	12.6	19.5	23.5	13.5	27.2	17.4	18.6	25.9	25.6	33.8	52.3	20.9
				Mineral Concentrations in Seed Tissue										
			%							mg/kg				
KB			8.0	2.03	0.38	0.44	0.27	0.17	0.24	7.7	113	64	9.4	40
TF			9.6	2.23	0.38	0.72	0.18	0.19	0.22	4.1	74	48	7.2	32
PRG			9.8	2.37	0.37	0.58	0.19	0.16	0.25	2.9	80	40	12.1	36
Species			0.14	0.30	0.93	0.001	0.02	0.08	0.25	0.001	0.09	0.34	0.55	0.08
$LSD_{0.05}$			NS	NS	NS	0.089	0.058	NS	NS	1.7	NS	NS	NS	NS
CV%			12.8	12.7	14.4	8.9	15.8	9.2	8.1	19.6	25.1	43.3	62.6	11.6

Phenolic, Flavonoid and Antioxidant Profiling for Cool-Season Grasses With and Without Endophytes

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Previous studies on green leaf chemistry profiles of turfgrass species showed that differences in profiles between species have potential as an identification tool for different genotypes; nevertheless, endophytic infection was not considered. Symbiotic plants have been reported to increase exudation of phenolic-like compounds in the rhizosphere under phosphorous nutrient deficiency. In addition, the increase of plant phenolic compounds in symbiotic grasses has been suspected of acting synergistically with ergot alkaloids to deter predation by plant-parasitic nematodes. *Poa ampla* (big blue grass) infected by the endophyte *Neotyphodium typhinum* has been reported to produce bioactive flavonoids, with possible impact on the symbiotic association. We evaluated four different extraction methods to study the phenolic and flavonoid profiles in endophyte-infected and endophyte-free grasses. We applied HPLC with photodiode array detection to analyze total phenols and total flavonoids in plant material of three cool-season grass species with and without endophytic infection. *Poa ampla.*, *Festuca arundinacea* and *Festuca rubra ssp. commutata*, and *Lolium perenne* leaf material with (E+) and without (E-) endophyte were studied. Extraction methods were used were: Method 1: 80% Methanol + 0.1% Acetic acid. Method 2: 80% Acetone + 0.1% Acetic Acid. Method 3: 80% Alcohol + 0.1% Acetic Acid and Method 4: 100% Methanol. For each sample's chromatogram, phenolic and flavonoids peaks were identified by their characteristic UV spectra and standards rutin and chlorogenic acid. Total phenolic compound analysis and total antioxidant activity (TAA) was also performed by spectrophotometric methods.

Methanol (100%) performed the best for phenolic and flavonoids extraction. E+ plants presented higher total phenolic content in *P. ampla* (49.04 ug/g fresh weight, ±6.44) and higher total flavonoid content in *F. arundinacea* (133.62 ug/g fresh weight, ±28.7), *L. perenne* (22.5 ug/g fresh weight, ±2) and *F. rubra ssp. commutata* (54.93 ug/g fresh weight, ±8.94). The correlation between TAA and total phenolic content based on spectrophotometric results had a correlation coefficient of R^2=0.71. The presence of endophytes changed the phenolic and flavonoid profiles in all plant species. Endophytic presence was related to both increase and decrease in particular phenolics/flavonoid concentrations. In general increases in phenolics and flavonoids correlated with total antioxidant activity. Further studies are necessary to explain the mechanism by which endophytes alter leaf chemistry in the plant

Topdressing Incorporation and Sand Particle Shape Effects on Anthracnose Severity of Annual Bluegrass

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Sand topdressing is a common practice of putting green turf to minimize thatch accumulation, smooth the surface and modify soil. However, wounding and abrasion of host tissue associated with this practice is suspected to enhance anthracnose (caused by *Colletotrichum cereale* Manns sensu lato Crouch, Clarke, and Hillman). This study evaluated topdressing incorporation method (none, vibratory rolling, soft bristled brush and stiff bristled brush) and sand shape (none, round and sub-angular) for effects on anthracnose severity in 2006 and 2007. A split-plot design with incorporation method as the main plot factor and sand shape as the subplot factor was used on a 5-yr-old annual bluegrass (*Poa annua* L.) turf mowed at 3.2 mm. Topdressing was applied at 0.3 L m^{-2} every 14 d from 27 June to 5 Sept. 2006 and 14 May to 24 Sept. 2007, respectively. Disease severity was measured as the percent turf area infested using a line-intercept grid count method. The sand shape main effect was the only significant source of variation in both years. Both sand shapes initially increased disease severity 4 to 14% compared to non-topdressed turf in July 2006; whereas neither sand shape increased severity compared to non-topdressed turf in July 2007. Sub-angular and round sands reduced anthracnose 8 to 29% and 7 to 29%, respectively, compared to non-topdressed turf during August and September of 2006 and 2007. Anthracnose was less severe in plots topdressed with sub-angular sand than round sand in July 2006 and July through September 2007. The topdressing incorporation methods did not affect anthracnose severity and cumulative sand topdressing reduced disease severity particularly with sub-angular shaped sand. These results support other research recently conducted by the authors indicating that subtle wounding or bruising associated with routine topdressing is not a significant factor affecting anthracnose severity.

Genetic Improvement of Switchgrass as a Biofuel Feedstock

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The ongoing project is to develop an *Agrobacterium*-mediated transformation system for superior high yielding ecotypes of switchgrass that grow on marginal lands in the northern geographical region of the United States and southern Canada for the production of stable transgenic plants. The germplasm that we are using is that identified by Dr. Stacy Bonos and colleagues at Rutgers University to be adapted to the temperate region of the US and which exhibits increased biomass, modified/lower lignin content and improved tolerance to poor soils, disease and lodging. We were generously provided with mature seeds harvested from five different ecotypes, namely Carthage, Contract, Kanlow, NSU and Timber. We compared these ecotypes with regard to ease of initiation of callus and propagation of embryogenic callus cultures. We found that Timber BBL-9 5316-1, a selection from a lowland ecotype, and NSU EB-6 9184-4, a selection from an upland ecotype, were most amenable for establishment and proliferation of callus in culture. In the past six months, we defined optimum conditions for production of embryogenic callus from these two ecotypes. In addition, we have empirically determined the ideal conditions for regeneration of plantlets from these callus cultures. We are in the process of defining the culture medium for production of the type of callus that has previously proven most successful for *Agrobacterium*-mediated transformation of turfgrass species. We are also performing a kill curve with callus produced from these two ecotypes to determine the appropriate concentration of the herbicide to be used as a selection agent for transformed cells. When we have amassed a sufficient amount of callus, we will conduct transformation trials using protocols that have been developed in our laboratory and modified as necessary for the species in hand. The ultimate goal is to genetically modify switchgrass cultivars, improved by Dr. Bonos and her colleagues through conventional breeding, for use as a biofuel feedstock.

Response of Kentucky Bluegrass Cultivar with the Use of Mesotrione at Fall Establishment.

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Field experiments were conducted Fall of 2007 and Fall of 2008 to evaluate the response of mesotrione to new established seedlings of *Poa pratensis* cultivars. *Poa pratensis* cultivars that were evaluated in the experiment were, Avalanch, Kingfisher, America, Bedazzal, Thermal, P105, Award, Washington and Midnight II. Each *P. pratensis* cultivar was seeded at a rate of ½ lb per 1000 sq. ft. Experimental layout of the field included 2 m strips of the cultivars within three randomize blocks that were 16.45 meters in length. Mesotrione was applied to the *P. pratensis* cultivars at rates of 8, 16, 32, 64 oz/A either as a single application or with sequential applications. Sequential applications were applied 4 WAT with a NIS at 0.25 % v/v. Treatments were applied to 0.9 x 16.45 m plots with a single-nozzle CO_2 backpack sprayer system utilizing a 9504 EVS nozzle tip which delivered 187 L/ha of spray solution at 222 Kpa. Experimental design was a randomized complete block with 4 replications per treatment in all experiments.

Field experiments that were conducted in Fall of 2007 after a 4 WAT cover rating showed that mesotrione at 8 oz/A caused no significant damage to the turf, but provided complete control of winter annuals. At 64 oz/A, mesotrione caused significant damage resulting in whiting of the tips and thinning of the turf. A rating was taken 8 WAT to evaluate injury from the four sequential treatments. Injury that was observed included whiting on the tips of the cultivars and thinning of the turf.

The last rating of the trial that was conducted in Fall of 2007 was taken in April, 2008. From this rating, mesotrione at 8 oz/A either as a single or sequential application produced the best turf quality and gave almost complete control of *P. annua*. The results from this study indicate that applying 8 oz/A of mesotrione either as a single or sequential application will provide control of *P. annua* and winter annuals, and is safe to use on turf.

Creeping Bentgrass Putting Green Tolerance to Bispyribac-sodium

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Bispyribac-sodium is an efficacious herbicide for annual bluegrass (*Poa annua*) control in creeping bentgrass (*Agrostis stolonifera*) fairways, but turf tolerance and growth inhibition from applications may be exacerbated on closer mowed putting greens. To test this hypothesis, field and greenhouse experiments investigated creeping bentgrass putting green tolerance to bispyribac-sodium. In greenhouse experiments, creeping bentgrass discoloration from bispyribac-sodium was exacerbated by reductions in mowing height from 24 to 3 mm, but mowing height did not influence clipping or root weight. In field experiments, discoloration of creeping bentgrass putting greens was greatest from applications of 37 g/ha every ten days compared to 74, 111, or 222 g/ha applied less frequently. Chelated iron effectively masked discoloration of creeping bentgrass putting greens from bispyribac-sodium, while trinexapac-ethyl inconsistently masked these effects. Overall, creeping bentgrass putting greens appear more sensitive to bispyribac-sodium than higher mowed turf, but chelated iron and trinexapac-ethyl can mask discoloration.

Effects of Endemic and Released Entomopathogenic Nematodes on Annual Bluegrass Weevil Populations in Golf Course Fairways

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The annual bluegrass weevil (Listronotus maculicollis Kirby) (ABW) is a highly destructive pest of golf course turfgrass in the Northeastern United States. Turf managers primarily manage ABW with preventive applications of synthetic insecticides against the overwintered adults in spring. In an effort to develop less toxic management options for ABW, we investigated the impact of endemic and released entomopathogenic nematode (EPN) populations on ABW population dynamics. Field surveys between 2005 and 2007 demonstrated that two EPN species, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, regularly infect many weevil stages and increase in density in response to increasing weevil densities. However, variable generational mortality and sensitivity to environmental extremes suggest that EPNs cannot reliably keep ABW populations below damage thresholds. Laboratory assays and field releases of commercial species of EPNs targeting fourth and fifth instar larvae have shown promise. In the laboratory, S. feltiae and S. carpocapsae demonstrated high levels of control of ABW larvae (97 and 100%, respectively). Fifth instar larvae were less susceptible than fourth instars, suggesting that application timing will be critical for effective control. Field trials using endemic and commercial EPN strains indicate that high control levels can be achieved with several species (S. feltiae, S. carpocapsae, H. bacteriophora). However, control was variable between trials and years and seemed to be greatly affected by ABW densities.

Perennial Plants for a Sustainable Future: Progress and Perspectives at Rutgers

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Research into the understanding, development and implementation of underutilized perennial crops for food, feed, bioenergy and environmental enhancement was initiated and has been ongoing at Rutgers since 1996. A decision was made early on to focus primarily on nut producing tree species adapted to northern temperate regions. The principles followed in the establishment of this program were based on comprehensive methods proven highly successful in the Rutgers turfgrass breeding program. Obviously, due to their different breeding behaviors and propagation requirements, working with tree crops is quite different than working with perennial turfgrasses. However, many of the breeding fundamentals remain the same. Similar to the turf program, a major focus of the nut tree program has been obtaining and rigorously evaluating a very large collection of diverse germplasm. Access to superior and diverse genetic resources are especially important when working with these long generation cycle perennial species. It is necessary to collect, evaluate, and identify the best available genetic resources for use as parents in controlled hybridization programs to produce superior offspring in as short a period as possible. Our early efforts were to assemble and evaluate a diverse germplasm collections of walnuts (*Juglans spp.*), hazelnuts (*Corylus spp.*), pecans, hickories, and hicans (*Carya spp.*), and several others including chestnuts (*Castanea spp*.), oaks (*Quercus spp*.), almonds (*Prunus dulcis* [Mill.] D.A.Webb), and apricots (*Prunus armeniaca* L.). The commercial production regions of many of these species are currently limited due to climatic restrictions, length of growing seasons, and/or susceptibility to major diseases; or the species are not grown commercially and have only been harvested from the wild. Our first step was to start very wide with both the number of species evaluated and the diversity of each species established at Rutgers. From these collections, our objective was to ultimately identify the species best adapted to our region that also showed the most potential for rapid genetic improvement. From there we would initiate more intensive germplasm collection efforts, systematic evaluations, and a focused genetic improvement program to greatly enhance the species' usefulness for New Jersey and the eastern U.S.A.

Since 1996, our nut tree program has expanded in size yearly. Our research nurseries are planted across five Rutgers research farms located in Cream Ridge, Adelphia, and North Brunswick, New Jersey. Currently our collection holds almost 18,000 trees, all of which are undergoing systematic and rigorous evaluations. Our collection increases by about 3,000 trees each year. However, we have also been culling the same or an even greater number of trees from our nurseries yearly, as individuals prove poorly adapted to our climate or they are inferior and not useful for breeding purposes. Therefore, the total number of trees in our holdings now remains almost consistent from year to year. To develop our large collection of germplasm, we have made trips every year since 1997 to many parts of the former Soviet Union and Eastern Europe for the collection of Persian walnuts and hazelnuts, as well as almonds and apricots prior to the restriction of importation of *Prunus* to the U.S. due to the Plum Pox virus. Our travels have resulted in the amassing of a large amount of valuable plant genetic resources previously unavailable for evaluation in North America. Our travels have also allowed us to develop priceless contacts and improved relationships with friendly scientists and institutions in many previously inaccessible parts of the world, including Ukraine, Russia, Kyrgyzstan, Uzbekistan, Tajikistan and Kazakhstan. Part of our mission has been to help improve communications with and spread knowledge of the various institutions we have collaborated with in the former Soviet Union. One method to accomplish this has been to publish detailed manuscripts in U.S. journals about the history, scientific activities, and germplasm resources of the organizations we have worked with over the years (Mirzaev et al., 2003; Mavlyanova et al., 2005; Yezhov et al., 2005; Ibragimov et al, 2008).

From our collection efforts we have acquired clonal (grafted) cultivars and selections, as well as diverse seedling populations of hazelnuts (*Corylus avellana*) and Persian walnuts (*Juglans regia*) originating from Kyrgyzstan, Uzbekistan, Kazakhstan, Tajikistan, Russia, Ukraine, Belarus, Moldova, Estonia, Latvia, Lithuania, and Poland. Much of the material we obtained from Central Asia has never been grown previously in the U.S. We are currently evaluating this material for its adaptation to New Jersey's climate and soils. In addition to nut species, our efforts have facilitated the useful exchange of other species, including grasses, cotton, fruit, vegetables, melons, legumes, medicinal plants and forbs. We have also helped arrange visits of Central Asian scientists, administrators, and students to Rutgers and other institutions throughout the U.S.

Our most advanced work to date is with hazelnuts. Reasons for this advanced state includes hazelnut's relatively short generation time (4-5 years from seed to seed), their small plant size relative to the other nut tree species, the huge genetic diversity available and wide inter-fertility of *Corylus*, and the ease of obtaining large numbers of hybrid seed. Currently, 99% of the U.S. production of hazelnuts occurs in the Willamette Valley of Oregon. Our goal with hazelnuts is to develop well-adapted and reliably-productive commercial quality hazelnut cultivars for New Jersey and the northeastern U.S. that are highly resistant to the fungal disease eastern filbert blight (EFB), caused by *Anisogramma anomala*. This disease is the number one limiting factor of hazelnut culture in our area, and it is the primary reason we do not grow hazelnuts here commercially. As such, much of our research efforts center on identifying and developing resistance to this disease. One direction we have headed to achieve this goal is to make hybridizations between *Corylus avellana*, the European hazelnut of commerce, and our native species *C. americana*. While our native species generally produces small, thick-shelled nuts, it is naturally adapted to our climate and many individual plants are highly resistant to EFB. By making hybridizations between selected *C. americana* plants and improved *C. avellana* cultivars that produce high-quality, thin-shelled, large size nuts, it is possible to recover offspring that are resistant to EFB and are sufficiently cold hardy, while also retaining the superior nut quality of the European parent. In 2009, we will be establishing our first multi-location replicated yield trials to evaluate superior hazelnut selections developed and identified from our breeding and research efforts.

While nut trees are our current focus, we plan to include the genetic improvement of additional perennial food and bioenergy species as our long-term program is expanded in the future. We feel the development and widespread use of numerous highly productive perennial food and bioenergy species will be of great help in diversifying agriculture, eliminating hunger, and improving health, prosperity, self-reliance, and productivity in many regions of the world.

Perennial crops can be grown in an environmentally sustainable manner on steep, rocky slopes and other lands not suitable for cultivated annuals, as discussed in detail by J. Russell Smith (1950). Ultimately, we feel the development of perennial crops for lands not suitable for traditional annual crop production will greatly increase the world's food and bioenergy production capacity. This increased production will be desperately needed as the world's population reaches a projected nine billion in 2050, while clean water and petroleum resources dwindle and increasing amounts of level, fertile farmland are used to produce annual energy crops. Through our underutilized perennial crops genetic improvement program, and other similar programs we may help inspire, it is our goal to make a significant contribution towards enhancing the sustainability and longevity of agricultural systems in the U.S. and around the world.

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Traffic Tolerance of Tall Fescue in 2008

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Wear tolerance and adaptation to the transitional zone between the cool- and warm-humid regions of the United States are important reasons to use tall fescue (*Festuca arundinacea* Schreber) on sports fields and other recreational sites in New Jersey. Evaluation of tall fescue cultivar performance under traffic stresses (wear and compaction) during specific seasons (spring, summer, and fall) is needed since new cultivars continue to be released and sports field use is often season-specific. The objective of this field research trial was to determine whether seasonal differences in traffic tolerance and recovery exist among tall fescue cultivars and experimental selections.

Tall fescue cultivars and experimental selections, including the 113 entries comprising the 2006 National Turfgrass Evaluation Program (NTEP) tall fescue test, were seeded in September 2006 on a Nixon loam in North Brunswick NJ. Also included in the trial were 'Titanium', four (4) experimental selections, and two (2) commercially available blends: Pennington's Best ('Forte' [33%], 'Prospect' [33%], and 'Signia' [33%]); and Water Saver ('Labarinth' [34%], 'Aztec II' [24%], 'Focus' [20%], and 'Rendition' [20%]).

The experimental design for this trial was a randomized complete block with three replications. Wear was applied using a modified Sweepster that permitted control of both operating speed (4.0 km h^{-1} [2.5 mph]) and paddle rpm (250 rpm). Sixteen wear passes were applied to the tall fescue trial over two days in summer 2008 (8 passes on 22 July and 8 passes on 23 July). Visual ratings of the fullness of turf cover (0-100%) were taken before wear (C_{BW}) on 21 July and after 16 passes of the wear simulator (C_W) on 23 July. The ability of an entry to retain turf cover after was calculated using the following equation: C_W-C_{BW}; and will be referred to as cover retention. Recovery from wear was rated as fullness of cover at 12 days after wear (C_{12DAW}), which was used to calculate the change in cover as C_{12DAW}–C_W. Ten (10) passes of a 1264-kg (2784-lb) vibratory pavement roller were applied to the worn portion of plots on 4 August (12 DAW) to compact the soil. Visual ratings of the fullness of turfgrass cover were taken on 13 August (9 days after compaction [C_{9DAC}]). The change in turfgrass cover compared to pre-wear ratings was calculated as C_{9DAC}-C_{BW}. Turfgrass recovery after traffic was assessed at C_{18DAC} and C_{18DAC}-C_{9DAC}; C_{30DAC} and C_{30DAC}-C_{9DAC}; and C_{60DAC} and C_{60DAC}-C_{9DAC}.

Analysis of variance was performed and revealed a significant entry effect for C_{BW}, C_W, C_W-C_{BW}, C_{12DAW}, C_{12DAW}–C_{BW}, C_{30DAC}-C_{9DAC}, C_{60DAC} and C_{60DAC}-C_{9DAC}. Cultivars and selections that had the greatest C_W after 16 wear passes and retained the most cover after wear (C_W-C_{BW}) were 'Falcon V', 'Shenandoah III', 'Hemi', 'Talladega', RK 6, SC-1, 'Finelawn Xpress', 'Speedway', RK 5, PST-5WMB, 'Turbo', IS-TF-138, K06-WA, 'Bullseye', 'Jamboree', 'Falcon NG', CE-2, JT-42, 'Essential', 'Aggressor', 'Mustang 4', PSG-85QR, 'SR 8650', 'Traverse SPR', and BGR-TF1.

Entries that exhibited poor C_W (<50%) after wear yet had the greatest cover retention after wear (C_W-C_{BW}) included 06-WALK, Pennington's Best (blend), BAR Fa 6363, and ‘Plato’. This suggests these entries were unique in that they exhibited good wear tolerance in the absence of superior canopy density. It may useful for turfgrass breeders to examine these entries for a unique trait(s), which may be governing wear tolerance.

The following cultivars and selections were among the highest ranked for C_{60DAC} on 3 October and exhibited the least cover change (C_{60DAC} – C_{9DAC}): Shenandoah III, RK 6, Speedway, ‘Raptor II’, CE-2, SR 8650, Mustang 4, ‘Escalade’, IS-TF-138, IS-TF-152, PST-5HP, ‘Firenza’, IS-TF-159, Hemi, ‘3rd Millennium SRP’, BGR-TF1, PSG-85QR, Falcon NG, and PST-5WMB. Thus, these entries required a lesser amount of recovery to achieve the greatest cover at 60 DAC.

Several entries had the lowest turfgrass cover C_{60DAC} and exhibited the poorest recovery (C_{60DAC} – C_{9DAC}) on 3 October including BAR Fa 6363, Water Saver (blend), PSG-TTST, ‘Silverado’, Plato, ‘Toccoa’, Pennington's Best (blend), Aristotle, ATF 1328, and ‘Kentucky 31’. Because of this poor performance, the use of these entries should be limited to utility turfs or other sites not subject to intense traffic.

Traffic (wear and compaction) will be applied to this test in spring 2009 to assess the spring traffic tolerance and recovery of tall fescue. Data generated from this trial will assist turfgrass managers in selecting tall fescue cultivars for highly used sports fields and other recreational areas.

LC-MS-MS Identification and Quantification of Phenolics in Symbiotic Tall Fescue.

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An estimated 20 to 30% of grass species are associated with systemic fungal endophytes. Endophytic fungi have been demonstrated to confer benefits to the host plant through production of alkaloids and related fungal metabolites leading to protection of the host from herbivory by mammals and insects that results in improved plant performance. Most studies of the chemistry of the grass-endophyte symbiosis have been focused on fungal metabolites such us ergot alkaloids and anti-herbivore functions. However there is little understanding of the effects of the symbiosis on plant metabolites. Plant phenolics are a diverse group of aromatic molecules playing key roles in signaling between plants and microbes, in defense as antimicrobial agents, feeding deterrents, UV protection, and oxidation protection. HPLC coupled electrospray mass spectrometry in tandem mode (LC-ESI-MS-MS) with negative ion detection was used to generate a phenolic profile that was identified and quantified using standards, mass fragmentation patterns including mass spectra in full scan mode (MS), and MS/MS (i.e. product ion, precursor ion and neutral loss). Flavonoid concentrations in E+ plants (133.62 ug/g fresh weight±28.7) was higher than in E- plants (58.92ug/g fresh weigh±3.69), flavonoid composition was also different. Identified compounds included (1) 3-caffeoylquinic acid, (2) 5-caffeoylquinic acid, (3) 4-caffeoylquinic acid, (4) p-Coumaric acid-O-glucoside, (5) Caffeicacid-O-glucoside, (6) Quercetin-3-diglucoside, (7) Quercetin-3-glucoside, (8) Quercetin-3-rutinoside, (9) Quercetin-3-dirutinoside, (10) Luteoline-7-O-galactoside, (11) Luteoline-7-O-glucoside, (12) Kaempferol-O-glucoside, (13) Triacin, (14) apigenin C arabinosyl-O-glucoside (15) dimetoxy-luteolin-glucosyl-diferulate and other unidentified flavonoids and phenolic acids. Deprotonated aglycones were formed by loss of galactoside, glucoside and caffeoyllate residues from their glycosides. Fragmentation patterns demonstrated several derivatives; aglycons and phenolics provided characteristic ions for each flavonoid family. Further studies will need to determine the final structural elucidations.

Proteomic Response to Drought Stress in Kentucky Bluegrass Cultivars Differing in Drought Tolerance

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Drought is one of the major limiting factors of plant production worldwide. Understanding genetic variations and mechanisms in turfgrass drought tolerance would facilitate breeding and management programs to improve turf quality under drought stress. The objective of this study was to investigate protein changes associated with drought tolerance in two Kentucky bluegrass (*Poa pratensis* L.) cultivars. Plants of 'Brilliant' and 'Midnight' were subjected to drought stress by withholding water for 15 days in growth chambers. The leaves were harvested at 5, 10, and 15 days after drought treatment. Midnight maintained higher relative water content and photochemical efficiency, and lower membrane leakage than Brilliant at 15 d of drought stress. Proteins were extracted and separated by differential gel electrophoresis. Ninety four leaf protein spots were differentially accumulated in response to drought stress in at least one cultivar. The sequences of 45 protein spots were analyzed using mass spectrometry and 39 spots were identified. The results revealed that Brilliant exhibited more severe protein degradation than Midnight. Many protein spots identified as enzymes (glycine hydroxymethyltransferase and aminomethyltransferase) involved in amino acid metabolism were reduced by drought in both cultivars. One protein for ascorbate peroxidase decreased in abundance only in 'Brilliant', indicating antioxidant enzymes were down-regulated by drought stress, which may weaken the antioxidative scavenging capacity for 'Brilliant' under drought stress. One protein with putative function as heat shock protein 70 was found to increase in its abundance under drought only in 'Midnight', suggesting that heat shock proteins may contribute to superior drought tolerance in 'Midnight'.

Identification of Heat Tolerance Genes in *HSP-ipt* Transgenic Creeping Bentgrass

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Creeping bentgrass (*Agrostis stolonifera*) is a widely used turfgrass in cool climatic regions. Heat stress is one of the limiting factors for the turf management of this grass species. Transgenic creeping bentgrass carrying the adenine isopentenyltransferase (*ipt*) gene driven by a heat shock promoter (HSP) was generated in our group. A higher cytokinin synthesis was observed in the *ipt* transgenic plants. The objective of this study was to identify differentially expressed genes associate with heat tolerance in *HSP-ipt* transgenic creeping bentgrass due to heat-induced overexpression of *ipt*. Plants of a *HSP-ipt* transgenic line (H31) and a null transgenic line (control) were subjected to 35^{o}C heat stress or a 20^{o}C normal environment for 10 days. Higher level of *ipt* expression was detected in H31 subjected to 35^{o}C, suggesting the activation of heat shock promoter under heat stress, which drove the overexpression of *ipt*. After exposed to 35^{o}C for 10 days, H31 had significantly lower shoot electrolyte leakage compared to the control line, which indicated the *HSP-ipt* transgenic creeping bentgrass had better growth with improved heat tolerance. To identify heat stress associated genes, four subtraction cDNA libraries are under construction from shoots or roots of transgenic H31 and control plants subjected to 35^{o}C at 2 days and 10 days. Both heat-tolerance and heat-sensitive genes will be identified using forward and reverse subtraction strategies, respectively. The expression of selected heat stress related genes will be further studied in *HSP-ipt* transgenic and control plants grown under normal and high temperature to discover the molecular basis of *ipt* associated heat tolerance. The confirmed heat tolerance genes will be used to develop molecular markers to generate creeping bentgrass adapted to a high heat environment.