Development of High Yielding Turf-type Kentucky Bluegrass Varieties for Non-thermal Management in Washington State

Submitted to: Agricultural Burning Practices and Research Task Force

Funding: $14,000 (November 1, 2003 to June 30, 2005)

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Major Participants:
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Email: rcjohnson@wsu.edu

Grower Cooperators - Washington State grass seed producers to be identified.

Dan Nordquist
Director
Office of Grant and Research Development
Development of High Yielding Turf-type Kentucky Bluegrass Varieties for Non-thermal Management in Washington State

PROPOSAL SUMMARY

Traditionally, seed production management practices have included open-field burning after harvest to remove residue and stimulate seed production the following year. A ban on burning has been implemented in Washington State causing economic stress for grass seed producers.

Currently, growers are bailing post-harvest residue in an attempt to mimic residue removal by open-field burning. A recent 3-year study in eastern Washington by Johnson et al. (2003) showed that, across all the known genetic variation in Kentucky bluegrass, when post-harvest residue was not removed, yield was reduced 63% compared to burned plots. When residue was removed, similar to baling, yield was reduced 27%.

Objective 1 will be to assess the variation in selected bluegrass accessions identified in the previous 3-year study (Johnson et al., 2003) through agronomic and molecular characterization. The selected material will then be planted into seed increase nurseries to obtain sufficient seed to carry out Objective 2.

Objective 2 will be to determine the selection response for yield by testing the resulting selections from Objective 1 for seed production under a residue removed (bale) management system in central and eastern WA over several years. In addition, selections will be evaluated for turf quality to insure this factor is not lost in selection for high seed yield.

The study will consist of 10 entries; eight are PI accessions and two are check cultivars (‘Kenblue’ and ‘Midnight’). Each plot will consist of 28 plants spaced 100 cm apart within a row of seven plants, four rows wide. Research plots will be located at Pullman, WA. Seed production from individual plants will be used to select individual plants from each accession. The selected material will be increased and transplanted into seed increase nurseries to obtain sufficient seed to carry out Objective 2.

In Objective 2, seed production plots will be established at diverse locations (e.g., central WA and eastern WA). The seed production plots will consist of seven, 7-ft rows spaced 7 in. apart, and a 2 ft border between plots. The experimental design will be a randomized complete-block with three replications. There will be of 20 selections, 10 additional entries of remnant seed from the original population, and three check cultivars. Seed yield will be the primary measurement. The experiment will continue for three seasons past the establishment year. If selection was effective, then high and low seed yield selections should have higher and lower seed yield than their parent populations.

Turf plots of the same set of entries will be established and will be evaluated by criteria established by the National Turfgrass Evaluation Program (NTEP). Of most interest will be to determine if, and to what extent, the selection for seed yield affected turf quality and how this interacts with the individual entries.

At this point we are asking only for funding to complete Objective 1 and to establish and harvest the bluegrass seed increase plots. In due course, requests for funding and detailed procedures will be presented to complete Objective 2 and to carry the project forward to the goal of releasing new, high yielding bluegrass varieties with good turf quality for seed production in Washington State without burning.

Findings will be made available to the ABPRTF, public and private turfgrass breeders, and seed producers by presentations at field days, grower meetings, publication of Cooperative Extension Service material, and publication in scientific journals.
PROJECT NARRATIVE

1. Background:
   Burning of Kentucky bluegrass (*Poa pratensis* L.) seed production fields in the fall normally maximizes seed production the following year. With increased regulation of field burning, this practice has been essentially eliminated in Washington State. As will be outlined in “Related and Current Work” genetic variation in bluegrass to improve seed production under non-thermal residue management does exist. To sustain seed production at economically viable levels, new germplasm that enhances yield in non-thermal management systems needs to be identified, selections made, germplasm enhancement carried out, and ultimately high yielding bluegrasses be made available to Washington growers.

2. Related and Current Work:
   Over 75% of all Kentucky bluegrass seed in the U.S. is produced in Washington, Idaho, and Oregon (Ensign et al., 1989). Traditionally, seed production management practices have included open-field burning after harvest to remove residue and stimulate seed production the following year. A ban on burning has been implemented in Washington State, and restrictions on the timing and/or amount of burning are in place in Idaho and Oregon, causing economic stress for regional grass seed producers.

   In 1994-1995, an initial evaluation of 228 Kentucky bluegrass Plant Introduction (PI) accessions and 17 commercial cultivars from the Western Regional Plant Introduction Station at Pullman, WA was completed (Johnston et al., 1997; Johnson et al., 2003). That data was used to develop a core collection using Ward’s cluster analysis to include 20 accessions representing the genetic diversity within the entire bluegrass collection. An additional 16 PI accessions with high yield and high turf quality (based on color and texture) were also identified. These 36 accessions and nine commercial check cultivars were established in plots with three residue management treatments to identify bluegrass germplasm with high seed yield under non-thermal residue management. This set of material was also established in turf trials.

   When post-harvest residue was not removed seed yield was reduced 63% compared to burned plots. When residue was removed, similar to baling, yield was reduced 27% (Johnson et al., 2003). Lamb and Murray (1999) found seed yield response to residue management was cultivar dependent, as we did for PI accessions. When residue was retained, crop development was delayed, the interval from heading to harvest shortened, and harvest date was delayed.

   The reduction in seed yield associated non-burned residue was closely associated with the reduction in panicles per unit area. However, the interaction of residue treatment with germplasm entry was highly significant, indicating that some bluegrass accessions reacted differently to residue treatments than others. In a number of high yielding accessions there was no difference in seed yield in the burned and residue removed (similar to baling) treatments. Thus, in a multi-year study completed with this highly diverse set of germplasm (Johnston et al., 1999), several accessions were identified that had improved seed production compared to check cultivars under non-thermal management systems. Sufficient variation for seed production appears available to encourage development of germplasm for non-thermal management systems.

   Separate, but adjacent, turf plots were also established with the same accessions used in the seed production trial. As expected, turf quality was negatively correlated with seed yield. However, some entries combined good seed yield with turf quality as high, or higher, then the mean of the check cultivars. What is needed now is to determine if, and to what extent, variation within
acccessions is available for yield selection and if turf quality is changed through this process.

Since Kentucky bluegrass is a facultative apomictic species (Huff et al., 1993), with the apomictic aspect dominating reproduction, uniformity is promoted from one generation to the next within a given genotype. However, during the field collection process at diverse locations, different genotypes may have been included in a single population for a variety of reasons. The facultative component of Kentucky bluegrass reproduction allows for some genetic recombination (Huff et al., 1993) and the introgression of new genes, albeit at a relatively low frequency. For reasons not yet understood, we have observed variation in plant type within accessions suggesting there is a potential for improving bluegrass seed yield by selecting individual plants from within accessions.

3. Objectives:

1. Assess the within and among variation in selected Kentucky bluegrass accessions utilizing agronomic and molecular characterization. Select individual plant genotypes divergent for high and low seed production from accessions identified in previous work. Establish, and harvest, bluegrass seed increase plots in central and eastern WA.

2. Determine the selection response for seed yield by testing the resulting selections in Objective 1 for seed production under a residue removed (baled) management system in diverse environments and over years. In addition, at the same time, test the selections for turfgrass quality.

The above objectives have the potential for developing bluegrass germplasm that may be of use for further breeding work in the turfgrass industry. High seed production and good turf quality bluegrasses for non-thermal residue management in the Pacific Northwest may well be attained, but delivering high turf quality outside this area of adaptation will be more difficult. This will be an important issue as the major markets for Kentucky bluegrass seed lie outside the Pacific Northwest.

The facultative apomictic nature of Kentucky bluegrass make breeding for identified traits in a widely adapted parent cultivar much more difficult than in species in which a classical breeding system can be applied. Nevertheless, new methods for gene transfer and efforts to recombine and develop Kentucky bluegrass from crossing are ongoing. Regardless of the difficulties there are clear benefits to pursuing these objectives: 1) there is the potential for developing enhanced bluegrass germplasm with improved seed yield under non-thermal management that will have uses and benefits to Washington growers, 2) the selection response for seed production in Kentucky bluegrass will be understood for future application, and 3) the consequences of seed yield selection on turf quality will be better understood.
4. Approach:

**Objective 1.** Assess the within and among variation in selected bluegrass accessions through agronomic and molecular characterization. Select individual plant genotypes divergent for high and low seed production from accessions identified in previous work. Establish, and harvest, bluegrass seed increase plots in central and eastern WA.

The experiment will consist of 10 entries; eight are PI accessions and two are check cultivars (‘Kenblue’ and ‘Midnight’). Kenblue is a common type with generally high yield and Midnight had the highest turf quality of the nine checks in the 1998-1999 study (Johnson et al., 2003). Each plot (experimental unit) will consist of 28 plants spaced 100 cm apart within a row of seven plants, four rows wide. The experimental design will be a randomized complete-block with three replications. Research plots will be located at Pullman, WA.

The selected bluegrass accessions represent a range of responses to residue treatments. For example, PI 230132 had a yield averaging nearly 1500 lbs per acre when burned, and more than 1000 lbs per acre when residue was mechanically removed. Its turf quality was as high as that of Kenblue, the highest yielding check cultivar, with 900 lbs per acre when burned and 580 lbs per acre when residue was removed. PI 349188 had essentially the same yield as Kenblue, but with significantly higher turf quality. PI 371768 and PI 574523 had high turf quality but were low yielding. Four of the eight PIs had seed yields that did not differ statistically between the burned and residue removed treatments.

For plot establishment, seeds of each accession will be germinated in water-saturated vermiculite in a growth chamber at 25°C. Seedlings will be transplanted into styrofoam flats consisting of 96 cells filled with potting soil. Plants will be grown under greenhouse conditions and transplanted to the field. Heading date, anthesis date, physiological maturity date, seed yield, vegetative spread, plant height, leaf length, and leaf width will be measured on individual plants. In addition, ratings of leaf habit, abundance and color will be made on a 1 to 9 scale with 9 the most upright, most leafy, and darkest green. Leaf tissue will be gathered and DNA extracted from one replication of each entry. This will total 280 total extractions. The DNA will be used in RAPD analysis as described by Johnson et al. (2002) for Kentucky bluegrass. Thus, the agronomic and molecular variation within and among entries will be assessed along with seed production. Agronomic factors such as irrigation, soil fertility, weed and disease control will be optimized. Seed production from individual plants will be used to select individual plants from each accession and within each block for high and low yield. The divergent selection is necessary to understand how seed yield selection affects turfgrass quality in subsequent tests. The selected material will be increased, germinated in vermiculite, and individual plants established in flats. These will be transplanted into seed increase nurseries to obtain sufficient seed to carry out Objective 2.

**Objective 2.** Determine the selection response for yield by testing the resulting selections in Objective 1 for seed production under a residue removed (baled) management system in diverse environments and over years. In addition, at the same time in turf plots, test the selections for turfgrass quality.

Seed production plots will be established at diverse locations (e.g., central WA and eastern WA). The seed production plots (experimental units) will consist of seven, 7-ft rows spaced 7 in. apart, and a 2 ft of border area between each plot. Soil fertility, irrigation for establishment, weed control, and other agronomic factors will be those used by the growers. The experimental design will be a randomized complete-block with three replications. The entries will include a total of 20 entries resulting from the divergent selection; that is, 10 high and 10 low yielding plants from each of
the entries. In addition, 10 entries of remnant seed from the original population will be planted plus three additional check cultivars. Seed yield will be the primary measurement, but additional data on crop development and seed yield components will also be collected. This will result in an experiment with 33 entries at two locations. The experiment will continue for three seasons past the establishment year. The results will be analyzed using analysis of variation. The factors examined will include effects associated with year, location, selection group, and entry. Interactions with environment and location will be important in assessing the yield selection. If selection was effective, then high and low seed yield selections should have higher and lower seed yield than their parent populations.

Turf plots of the same set of entries will be established at the same time and near the same locations as above. Plots will be evaluated by criteria established by the National Turfgrass Evaluation Program (NTEP). Plots will be 4 x 5 ft and seeded at a rate of 11 g m$^2$ in a randomized complete-block experimental design with three replications. Of most interest will be to determine if, and to what extent, the selection for high or low yield affected turf quality and how this interacts with the individual entries.

5. Anticipated Schedule for Achieving Objectives:

It will take several years to complete both objectives. At this point we are asking only for funding to complete Objective 1 and to establish and harvest the bluegrass seed increase plots. In due course, requests for funding and detailed procedures will be presented to complete Objective 2 and to carry the project forward to the goal of releasing new, high yielding bluegrass varieties with good turf quality for seed production in Washington State without burning.

Current proposal time-line:
November 2003 – April 2004: Finish Objective 1 data collection, select plants for genotype by environment studies of selection response.
April – May 2004: Establish bluegrass seed increase plots in central and eastern WA.
June 2005: Harvest bluegrass seed increase plots. Publish results.

Future years:
Year 2006: Establish seed production trials and turf plots.
Year 2007: Harvest seed production trials and evaluate turf plots.
Year 2008: Harvest seed production trials and evaluate turf plots.
Year 2009: Harvest seed production trials and evaluate turf plots. Publish results.
Enter superior germplasm in the National Turfgrass Evaluation Program for turfgrass evaluation across the U.S. (approximately 25 locations). Potentially this may occur as early as July 2007.

Based on NTEP testing, release new high yielding Kentucky bluegrass for seed production in Washington State without burning.

6. Evaluation: The ultimate project goal is to release new, high yielding Kentucky bluegrass germplasm and/or varieties with good turf quality for bluegrass seed production in Washington State without burning. Project success will be measured by meeting the objectives according to the timeline outlined above. Meeting this goal will enhance the economic stability of a valuable agricultural industry in Washington State.
Budget Page: Attached.

Justification: Attached.

References:


Current and Pending Support: Attached.

Vitae: Attached.

T4/DOE HIGH YIELDING KBG PROPOSAL 2003v4
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According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Form CSREES-2004 (12/2000).
Budget Justification
W. J. Johnston
Washington State University

“Development of High Yielding Turf-type Kentucky Bluegrass Varieties for Non-thermal Management in Washington State”

Salaries/Wages ($8,598)
Technician – Technician duties: Golob 8%/month for 12 months, $3,598 (budgeted as one month, July 2004). Assist in layout of research field plots and field plot maintenance. Assist in maintaining equipment during project duration. Assist in data collection. Prepare spread sheets for data analysis. Assist in preparation of research reports, presentations, posters, and manuscripts.

Pre-baccalaureate students – duties: $5,000. Assist in plot establishment, maintenance, seed harvest, and seed cleaning.

Benefits. $1,047
Golob @27%, $972
Pre-baccalaureate students @1.5%, $75

Materials and Supplies ($1,500)
Planting and harvest supplies and equipment maintenance (bags, machine parts, gas, oil, etc.), film and processing, misc. office supplies. Laboratory supplies for DNA extraction and RAPD analysis, i.e., taq polymerase, buffers, nucleotides, and primer.

Travel ($1,818)
Travel to research sites in eastern and central and eastern Washington ($1,818).

Total direct costs: $12,963
Overhead @ 8% TDC: $1,037

TOTAL COST: $14,000

TIA/WDOE High Yielding KBG Justification 2003
UNITED STATES DEPARTMENT OF AGRICULTURE  
Cooperative State Research, Education, and Extension Service  

**Current & Pending Support for WILLIAM J JOHNSTON**

**Instructions:**
1. Record information for active and pending projects. (Concurrent submission of a proposal to other organizations will not prejudice its review by CSREES).
2. All current research to which principal investigator(s) and other senior personnel have committed a portion of their time must be listed, whether or not salary for the person involved is included in the budgets of the various projects.
3. Provide analogous information of all proposed research which is being considered by, or which will be submitted in the near future to, other possible sponsors including other USDA programs.

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Form CSRS-663 (9/92)
William J. Johnston  Grass Seed - Vitae  August 2003

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Education:
B.S. Geology  1965  Pennsylvania State University
M.S. Agronomy  1974  Auburn University
Ph.D. Agronomy  1980  Auburn University

Grass Seed Research:
Current emphasis is on the characterization of diseases and insects in grass cropping systems, the evaluation of Kentucky bluegrass residue for paper making, the use fungicides for disease control in perennial ryegrass seed production fields, the evaluation of Kentucky bluegrass germplasm under alternative management regimes, and the characterization of emissions from post-harvest residue burning.

Professional Organizations and Societies:

Current Grass Seed Research Publications:


WJJ Short Grass Seed Viace 8-2003
RICHARD C. JOHNSON

GRASS VITA

29 August 2003

Regional Plant Introduction Station Rm. 59, Johnson Hall, Washington State University, Pullman, WA 99164-6402, Phone: (509) 335-3771, FAX: (509) 335-6654
E-mail: rjohnson@wsu.edu

BORN: 12 July 1951, Tonasket, WA. USA

AREA OF SPECIALIZATION: Conservation, regeneration, and enhancement of plant genetic resources; molecular and agronomic assessment of genetic diversity; plant response to environmental stress.

EDUCATION:

Ph.D. - Evapotranspiration Laboratory, Kansas State University, Major: Agronomy, 1981. Thesis Title: Crop development and yield in winter wheat at elevated temperatures and under water stress.


PROFESSIONAL EXPERIENCE:

1987-Present USDA-ARS Research Agronomist (GS-12, 1987-90; GS-13, 1990-94; and GS-14, 1994 to present) Western Regional Plant Introduction Station, Pullman WA. Acquisition conservation, and management of plant genetic resources including major collections of forage legumes, grasses, and safflower. Research involving diversity, regeneration, characterization, and enhancement of grass germplasm using molecular and agronomic techniques.

1981-87 Assistant (1981-84) and Associate Professor (1984-87) of Agronomy, Oklahoma State University. Research on physiological and genetic mechanisms of biotic and abiotic stress resistance in crops.

SELECTED PROFESSIONAL ACTIVITIES

American Society of Agronomy
Crop Science Society of America
Associate Editor, Agronomy Journal, 1987-89
Adjunct Agronomist and Professor, Washington State University (1988-present)
Member Graduate Faculty, Washington State University (1988-present)
Ex-officio member, Forage and Turf Crop Germplasm Committee (1988-present)
Member, Technical Advisory Committee for the Grass Seed Cropping Systems for a Sustainable Agriculture Special Grant Program (1994-present)
Associate Editor, Crop Science (1995-1997)
Chair, Crop Science Subcommittee C-852.12, Crop Registration for Sunflower, Safflower, and other oilseeds, 1995-2000
Chair-elect (1996), Chair (1997), Past-chair (1998), Plant Genetic Resources Division (C-8) Crop Science of America
Board of Directors, Crop Science Society of America, (1996-98)
Ex-officio member, Committee on the Frank N. Meyer Medal for Plant Genetic Resources (1997)
Chair, Nominations Committee for Plant Genetic Resources Division (C-8), Crop Science Society of America (1998)
Secretary and Host, Grass Breeders Work Planning Conference, 2001-2002, host for 2002 meeting, 14-16 May

SELECTED AWARDS
James Whatley Award for Excellence in Agricultural Research, Oklahoma State University, Division of Agriculture (1987)
Outstanding Paper in the 1998 Plant Genetic Resources Section of Crop Science, Crop Science Society of America (1999)
Elected Fellow, Crop Science Society of America (2001)
Elected Fellow, Agronomy Society of America (2001)
Letter of Commendation for superior grass germplasm preservation and evaluation by the Grass Breeders Work Planning Conference (2002)
Award as a “Model for Conservation of Genetic Resources” presented by the International Scientific Committee for the Second International Conference on Sustainable Agriculture for Food, Energy and Industry, Beijing, China (2002)

GRASS RESEARCH PUBLICATIONS FOR THE LAST FIVE YEARS (excluding abstracts):


STATEMENT OF POLICY - Institutions receiving CSREES funding for research are responsible for protecting human subjects, providing humane treatment of animals, and monitoring use of recombinant DNA. To provide for the adequate discharge of this responsibility, CSREES policy requires an assurance by the institution's Authorized Organizational Representative (AOR) that appropriate committees in each institution have carried out the initial reviews of protocol and will conduct continuing reviews of supported projects. CSREES also requires AOR certification by citing a timely date that an appropriate committee issued an approval or exemption.

NOTE: Check appropriate statements, supplying additional information when necessary.

1. INSTITUTION
   Department of Crop and Soil Sciences
   201 Johnson Hall
   Washington State University
   Pullman, WA 99164-6420

2. CSREES PROJECT NUMBER OR AWARD NUMBER (if known)

3. PROJECT DIRECTOR(S) W. J. Johnston

A. BIOSAFETY OF RECOMBINANT DNA
   X Project does not involve recombinant DNA.
   Project involves recombinant DNA and was either approved ( ) or determined to be exempt ( ) from the NIH Guidelines by an Institutional Biosafety Committee (IBC) on ___________ (Date).

This performing organization agrees to assume primary responsibility for complying with both the intent and procedures of the National Institutes of Health (NIH), DHHS Guidelines for Research Involving Recombinant DNA Molecules, as revised.

B. CARE AND USE OF ANIMALS
   X Project does not involve vertebrate animals.
   ☐ Project involves vertebrate animals and was approved by the Institutional Animal Care and Use Committee (IACUC) on ___________ (Date).

This performing organization agrees to assume primary responsibility for complying with the Animal Welfare Act (7 USC, 2131-2156), Public Law 89-544, 1966, as amended, and the regulations promulgated thereunder by the Secretary of Agriculture in 9 CFR Parts 1, 2, 3, and 4. In the case of domesticated farm animals housed under farm conditions, the institution shall adhere to the principles stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Federation of Animal Science Societies, 1999.

C. PROTECTION OF HUMAN SUBJECTS
   X Project does not involve human subjects.
   ☐ Project involves human subjects and
   ☐ Was approved by the Institutional Review Board (IRB) on ___________ (Date). Performing Institution holds a Federally wide assurance number ___________; if not, a Single Project Assurance is required.
   Is exempt based on exemption number ___________.

Specific plans involving human subjects depend upon completion of survey instruments, prior animal studies, or development of material or procedures. No human subjects will be involved in research until approved by the IRB and a revised Form CSREES-2008 is submitted.

This performing organization agrees to assume primary responsibility for complying with the Federal Policy for Protection of Human Subjects as set forth in 45 CFR Part 46, 1991, as amended, and USDA regulations set forth in 7 CFR 1c, 1992. All nonexempt research involving human subjects must be approved and under continuing review by an IRB. If the performing organization submits a Single Project Assurance, supplemental information describing procedures to protect subjects from risks is required.

SIGNATURE OF AUTHORIZED ORGANIZATIONAL REPRESENTATIVE

Director, Office Grants and Research Development

DATE 9/2/03

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average .50 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

CSREES-2008 (12/02/00)