Development of High Yielding Turf-type Kentucky Bluegrass Varieties for Non-burn Seed Production

Submitted to: Agricultural Burning Practices and Research Task Force

Funding: $14,000 (July 1, 2005 to June 1, 2007)

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Major Participants:
Dr. Richard C. Johnson
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Pullman, WA 99164-6402
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Grower Cooperators - Washington State grass seed producers to be identified.
University Cooperators – To be identified for turfgrass quality trials.
Development of High Yielding Turf-type Kentucky Bluegrass Varieties for Non-burn Seed Production

PROPOSAL SUMMARY

In Washington State, grass seed production traditionally included open-field burning after harvest to remove residue and stimulate seed production the following year. A ban on field 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 residue was removed, similar to baling, yield was reduced 27%. Therefore, bailing alone will not attain desirable seed yields. Although variety development is a long-term process, excellent progress has been made on this project toward the goal of developing high yielding turf-type bluegrass that does not require field burning (initiation of project funded 2003-2005 by ABPRTF).

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 (completed). The selected material will then be planted into seed increase nurseries (Johnston et al., 2005) to obtain sufficient seed to carry out Objective 2 (currently ongoing).

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 WA over several years. In addition, selections will be evaluated for turf quality to insure this factor is not lost in the selection of germplasm for high seed yield.

In Objective 1, based on past research, 10 entries; 8 PI accessions and 2 check cultivars (‘Kenblue’ and ‘Midnight’) were evaluated in field plots at Pullman, WA, 2002-2003. Each plot, replicated 3 times, consisted of 28 plants spaced 100 cm apart within a row of seven plants, four rows wide. Seed production from individual plants was used to select individual plants within each accession. Selected material (5000 plants) was transplanted at the USDA seed increase nursery at Central Ferry, WA, fall 2004, to obtain sufficient seed to carry out Objective 2.

In Objective 2, seed production plots will be established on grower fields at diverse locations in 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 40 selections, 10 additional entries of remnant seed from the original populations, and 2 to 3 check cultivars. Seed yield will be the primary measurement. The experiment will continue for three seasons (harvests) past the establishment year. If selection was effective, high seed yield selections should have higher 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 high seed yield affected turfgrass quality and how this interacts with the individual entries.

For 2005-2007, we are asking for funding to complete Objective 1 (harvest bluegrass seed increase plots) and to initiate Objective 2. In due course, requests for funding and detailed procedures will be presented to complete the project. Findings will be made available to the ABPRTF and grass seed producers by presentations at meetings and publication in scientific journals. Our goal is to release new, high yielding Kentucky bluegrass varieties with good turfgrass quality for seed production in Washington State without burning.
PROJECT NARRATIVE

1. Background:
   Open-field burning of Kentucky bluegrass (*Poa pratensis* L.) seed fields in the fall normally maximizes 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-burn residue management does exist. To sustain bluegrass seed production at economically viable levels, new germplasm that enhances yield in non-burn management systems needs to be identified, selections made, germplasm enhancement carried out, field testing conducted, and ultimately high yielding turf-type bluegrasses be made available to Washington growers.

2. Related and Current Work:
   Traditionally in WA, grass seed production practices have included open-field burning after harvest to remove residue and stimulate seed production the following year. A ban on grass seed field 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 from the Western Regional Plant Introduction Station at Pullman, WA and 17 commercial cultivars 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 total genetic diversity within the entire USDA Kentucky bluegrass collection. An additional 16 PI accessions with high yield and high turf quality 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-burn residue management. This set of material was also established in turfgrass 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). In Idaho, Lamb and Murray (1999) found seed yield response to residue management was cultivar dependent, as we did for PI accessions.

   The reduction in seed yield 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 Kentucky 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 commercial check cultivars under non-burn management systems. Sufficient genetic variability appears available to permit development of germplasm for non-burn 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 commercial check cultivars. What is needed now is to determine if, and to what extent, variation within accessions is available for yield selection and if turfgrass quality is changed though 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 news 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 the individual plant from each accessions with the highest panicles per m², seed per panicle, seed weight, and overall seed yield; plus plants from the original PI collection. Establish and harvest bluegrass seed increase plots.

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 WA environments and over years. In addition, at the same time, test the selections for turfgrass quality at diverse USA locations.

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-burn 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 long-term nature of the project there are clear benefits to pursuing these objectives: 1) there is the potential for developing enhanced bluegrass germplasm with improved seed yield without buming that will benefit 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 turfgrass quality will be better understood and can be used to direct future research.

4. Approach: Material and Methods:

Objective 1. Assess the within and among variation in selected Kentucky bluegrass accessions utilizing agronomic and molecular characterization. Select the individual plant from each accessions with the highest panicles per m², seed per panicle, seed weight, and overall seed yield; plus plants from the original PI collection. Establish and harvest bluegrass seed increase plots.

The experiment will consist of 10 entries; eight are PI accessions from the USDA collection and two are commercial 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 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, seed 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 for highest panicles per m², seed per panicle, seed weight, and overall seed yield. Individual plant harvesting (280 plants) and eventual plant selection will be done for two harvests. As a major project cooperator, Dr. Richard Johnson’s USDA/ARS laboratory at WSU will perform the DNA analyses. Dr. Johnson’s personnel will also assist in seed harvesting of field plots at Pullman.

Selection is necessary to understand how seed yield selection parameters affects turfgrass quality in subsequent tests. The seed from the selected material will be cleaned, weighed, and yield components determined. In addition, plants from the original PI collection for each accession will be used to represent the original population. The seed will be germinated in vermiculite and individual plants will be established in flats. Individual plants (5000) will be transplanted into a seed increase nursery (USDA/ARS nursery at Central Ferry, WA) to obtain sufficient seed to carry out Objective 2. Dr. Richard Johnson’s personnel will oversee the management of the nursery at Central Ferry and assist WSU personnel in harvesting.

**Objective 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 WA environments and over years. In addition, at the same time, test the selections for turfgrass quality at diverse USA locations.

Seed production plots will be established at diverse locations in WA on grower fields. The seed production plots (experimental units) will consist of seven, 7-ft rows spaced 7 to 14 in. apart, and a 2 ft border area between each plot. Soil fertility, irrigation for establishment (if warranted), 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. There will be a total of 50 entries. Forty resulting from the divergent seed yield component selection from the 10 accessions and 10 entries of remnant seed from the original population. Also, planted will be two to three additional commercial 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 approximately 53 entries at two, or more, locations. The experiment will continue for three harvest seasons past the establishment year. The results will be analyzed using analysis of variation. The factors examined will include effects associated with year, location, accession, and selection. Interactions with environment and location will be important in assessing the yield selection. If selection was effective, high seed yield selections should have higher yield than their parent populations.

Turf plots of the same set of entries will be established at the same time with university cooperators to be determined. 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 seed 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 (collect field data, harvest the bluegrass seed increase plots, clean seed, analyze data) and begin Objective 2. In due course, requests for funding 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:
Fall 2004: Establish bluegrass seed increase plots at Central Ferry, WA.
June – July 2005: Collect field data, harvest bluegrass seed increase plots.
August – December 2005: Clean seed and analyze data.
June – July 2006: Collect field data, 2$^{nd}$ harvest of bluegrass seed increase plots.
2006: Establish seed production trials and turfgrass trial plots.
2007: Harvest seed production trials on grower fields and evaluate university turf plots.
Future years:
2008: Harvest seed production trials on grower fields and evaluate university turf plots.
2009: Enter superior germplasm in the National Turfgrass Evaluation Program for turfgrass evaluation across the U.S. (approximately 25 locations). This may occur as early as 2008.

Give grower presentations and publish results as warranted.

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.

DOE ABRPTF High Yielding KBG PROPOSAL 2005v2
### UNITED STATES DEPARTMENT OF AGRICULTURE
### COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE
### BUDGET

**ORGANIZATION AND ADDRESS**
Washington State University, 423 Neill Hall, PO Box 643140, Pullman, WA 99164-3140

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**PROJECT DIRECTOR(S)**
William J. Johnston

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**A. Salaries and Wages**

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### B. Fringe Benefits (If charged as Direct Costs)

1826

### C. Total Salaries, Wages, and Fringe Benefits (A plus B)

11457

### D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)

506

### E. Materials and Supplies

1000

### G. Publication Costs/Page Charges

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**H. Computer (ADPE) Costs**

---

**I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)**

---

**J. All Other Direct Costs (In budget narrative, list items and dollar amounts, and provide supporting data for each item.)**

---

**K. Total Direct Costs (C through J)**

12963

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**L. F&A/Indirect Costs (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)**

1037

**M. Total Direct and F&A/Indirect Costs (K plus L)**

14000

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**N. Other**

---

**O. Total Amount of This Request**

14000

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**P. Carryover -- (If Applicable) Federal Funds: $**

---

**Q. Cost-Sharing/Matching (Breakdown of total amounts shown on line O)**

Cash (both Applicant and Third Party)

- Non Cash Contributions (both Applicant and Third Party)

---

**AME AND TITLE (Type or print)**

Project Director: William J. Johnston

Authorized Organizational Representative

---

**SIGNATURE (required for revised budget only)**

WJ

**DATE**

5/21/05

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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-2604 (12/2000)
Budget Justification
W. J. Johnston
Washington State University

“Development of High Yielding Turf-type Kentucky Bluegrass Varieties for Non-burn Management”

Salaries/Wages ($9631)

Graduate student (PhD): $3,921. Summer time-slip. Assist in layout of research field plots, data collection, and data analysis. Assist in preparation of research reports, presentations, posters, and manuscripts. Give presentations of research results at meetings as warranted.

Pre-baccalaureate student: $2,112. Assist in plot maintenance, seed harvest, and seed cleaning.

Benefits. $1,826
Golob @34%, $1,223
Graduate student @ 10%, 392
Pre-baccalaureate students @10%, $211

Materials and Supplies ($506)
Harvest supplies and equipment maintenance (bags, machine parts, gas, oil, etc.), film and processing, misc. office supplies. Laboratory supplies for seed cleaning.

Travel ($1,000)
Travel to research sites in eastern Washington ($1,000).

Total direct costs: $12,963
Overhead @ 8% TDC: $1,037 (8% overhead limit on grass seed research set by RCW 70.94.656)

TOTAL COST: $14,000

T4/WDOE High Yielding KBG JUSTIFICATION 2005
### 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
2. All current research to which principal investigator(s) and other senior personnel have committed a portion of their time must be
3. Provide analogous information of all proposed research which is being considered by, or which will be submitted in the near

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<th>Supporting Agency &amp; Agency Number</th>
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**Pending:**

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Form CSRS-663 (9/92)
William J. Johnston  Grass Seed - Vitae  May 2005

Professor - Agronomist
Department of Crop and Soil Sciences
Washington State University
Pullman, WA  99164-6420
Phone: (509)335-3620  FAX: (509)335-8674  e-mail: wjohnston@wsu.edu

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 evaluation of Kentucky bluegrass residue for paper making and by-product use as fertilizer and mulching material, the use fungicides for disease control in grass seed production fields, the development of turf-type Kentucky bluegrasses for seed projection without burning.

Professional Organizations and Societies:

Current Grass Seed Research Publications:
RICHARD C. JOHNSON          GRASS VITA          17 May 2005

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: rcjohnson@wsu.edu

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.

Title: Photosynthesis, transpiration, and light penetration in contrasting
wheat canopies.


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):


Appendix A

CERTIFICATIONS AND ASSURANCES

I/we make the following certifications and assurances as a required element of the bid or proposal to which it is attached. I/we understand that the truthfulness of the facts affirmed here and the continuing compliance with these requirements are conditions precedent to the award or continuation of the related contract(s):

1. The prices and/or cost data have been determined independently, without consultation, communication or agreement with others for the purpose of restricting competition. However, I/we may freely join with other persons or organizations for the purpose of presenting a single proposal or bid.

2. The attached proposal or bid is a firm offer for a period of 60 days following receipt, and it may be accepted by the Department of Ecology without further negotiation (except where obviously required by lack of certainty in key terms) at any time within the 60-day period.

3. In preparing this proposal or bid, I/we have not been assisted by any current or former employee of the State of Washington whose duties relate (or did relate) to this proposal, bid or prospective contract, and who was assisting in other than his or her official, public capacity. Neither does such a person nor any member of his or her immediate family have any financial interest in the outcome of this proposal or bid. (Any exceptions to these assurance-- are described in full detail on a separate page and attached to this document.)

4. I/we understand that the Department of Ecology will not reimburse me/us for any costs incurred in the preparation of this proposal or bid. All proposals or bids become the property of the Department, and I/we claim no proprietary right to the ideas, writings, items, or samples.

5. I/we understand that any contracts awarded as a result of this RFP will contain terms and conditions substantially similar to those attached as Appendix B. I/we certify that I/we will comply with these or substantially similar Terms and Conditions if selected as a contractor.

Signature

Dan Nordquist
Director, Authorized Inst. Official
Office of Grant and Research Development

Title

5/31/2005

4/18/2005

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