PROGRESS REPORT II

Restoring prairies:
A synthesis of studies on vegetation and invasive species
in support of effective management
(Year two)

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Introduction

Willamette Valley wetland and upland prairies are some of the most endangered ecosystems in the United States, and provide habitat for many federally listed species. Because these are dynamic systems, subject to ecological succession and invasion by aggressive non-native species, prairies require active management to maintain management goals. This is true of all Willamette Valley prairies, whether intact, degraded, or reestablished. Yet restoration and conservation efforts often lack experimental validation. Our overall objective for this project is to synthesize the scientific information now available relevant to the prairie restoration efforts of the West Eugene Wetlands project and, where there is sufficient support, develop concrete and defensible management recommendations

Successful ecosystem restoration requires establishing and maintaining native plants. In turn, plant establishment hinges on having suitable environmental conditions, using species with adequate germination rates, and reducing competitive pressure from non-native plants. In *year one* of this project, we synthesized the wealth of data in the West Eugene Wetlands establishment data set on plant abundance after sowing native species during wetland restoration (Wilson 2004). In *year two*, we will build and expand on these results in several important ways:

- We are *generalizing* these results through the investigation of plant traits that consistently correspond to the patterns of establishment and vigor.
- We are systematically compiling the results from year one and year two of this project into a public *databas*e. We are adding to this database findings from similar ecosystems, both in the Willamette Valley and elsewhere.
- We are considering further the role of *microsite* variability on seedling establishment patterns.
- We are *synthesizing* these results into scientific conclusions.
- We are *integrating* these results, where there is sufficient support, into concrete and defensible management recommendations

Our goal is to develop an ability to predict key aspects of prairie restoration performance, such as establishment rates, based on knowing species traits, site conditions, and maintenance. These predictions can then be converted into management recommendations, such as which species to sow and which site preparation and maintenance regimes should be followed to maximize native plant abundance and minimize non-native plant abundance at a given site.

The two components of our project –plant traits and the database– are crucial to this goal.

- Without the generalization that traits allow, understanding of wetland restoration increases slowly and expensively, one case study at a time.
- The organization of the database will increase the power and efficiency of revealing the relationships between plant traits and plant performance. Perhaps even more important is the role of the database as a first step in developing a Web-based expert system for managers wishing to plan wetland restorations

Measurement of plant traits
This report describes our progress to date on the measurement of plant traits.

Many of the key plant traits are not yet available in the Willamette Valley Species Database or from the ecological literature. Thus, we started a series of studies to measure key plant traits based on our knowledge of the mechanisms influencing the seedling establishment process. The studies included measurements of plant growth under standardized growth chamber conditions, measurement of seed characteristics, and determination of other traits from direct observation or from published references. We conducted all three types of measurements for high priority species. Growth chamber procedures followed the general recommendations of Hendry and Grime (1993). Standardized conditions include specifications for germination media, transfer of germinants, pot size, growing media, nutrient solutions, growing illumination and temperatures, and dates of harvest. The use of standardized conditions allows us to integrate our results with those in the scientific literature. The Willamette Valley Species Database will house both sources of data. Thus, managers will have a larger pool of data–ours and the data from the literature–with which to make management and restoration decisions.

Appendix A: Key Willamette Valley prairie wetland species for which plant traits were measured.
Appendix B: List of plant traits to be measured in this study, their description, and the ecological functions to which they are associated. The information has been compiled from several sources principally, Weiher et al. 1999, Cornelissen et al. 2003 , Hendry and Grime 1993.
Appendix C: Protocol for measuring plant traits for seedlings under standardized conditions based on Hendry and Grime 1993.
Appendix D: Protocol for measuring seed mass.
Appendix E: Protocol for measuring seed dimensions.
Appendix F: Protocol for germinating seeds.
Appendix G: Seed germination requirements for target species.
Appendix H: Current plant trait data collected on target species.

Acknowledgments
We gratefully acknowledge the invaluable contributions of Rachael Roberts and Diana Wageman in the collection of the plant trait data. We also wish to acknowledge the contribution of seed from the Bureau of Land Management, Eugene District, in particular the assistance from Dharmika Henshel. Amy Bartow of the Plant Materials Center kindly shared her extensive knowledge of germination requirements of wetland species.

Citations

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Dias, S., Buchmann, N., Gurvich, D.E., Reich, P. B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G., and Poorter, H. 2003. A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51:335-380.

Hendry, G. A. F. and Grime, J. P. 1993. Methods in comparative plant ecology. Chapman & Hall, London.

US Fish and Wildlife Service. 1988. National list of vascular plant species that occur in wetlands. US Fish & Wildlife Service Biological Report 88.

Weiher, E. Ven der Weft, A., Thompson, K. Roderick, M., Garnier, E., and Eriksson, O. 1999. Challenging Theophrastus: A common core list of plant traits for functional ecology. Journal of Vegetation Science 10:609-620.

Wilson, M. V. 2004. Patterns of establishment success in West Eugene Wetlands Program restoration sites. Final report to the Bureau of Land Management, Eugene District. 42 pages.

APPENDICES

Appendix A. Key Willamette Valley prairie wetland species for which plant traits were measured.

Achillea millefolium
Agrostis exarata
Allium amplectans
Aster hallii
Beckmannia syzigachne
Camassia leichtlinii
Camassia quamash
Carex densa
Carex tumilicola
Carex unilateralis
Danthonia californica
Deschampsia caespitosa
Downingia elegans
Downingia yina
Epilobium densiflorum
Eriophyllum lanatum
Festuca roemeri
Glyceria occidentalis
Grindelia integrifolia
Hordeum brachyantherum
Juncus acuminatus
Juncus ensifolius
Juncus tenuis
Microseris laciniata
Panicum occidentale
Plagiobothrys figuratus
Prunella vulgaris
Ranunculus orthoryhnchus
Rumex salicifolius
Sidalcea campestris
Sidalcea cusickii var. purpurea
Veronica scutellata
Wyethia angustifolia
Zygadenus venenosus

Appendix B. List of plant traits to be measured in this study, their description, and the ecological functions to which they are associated. The information has been compiled from several sources principally, Hendry and Grime 1993, Weiher et al. 1999, and Cornelissen et al. 2003.

Plant trait	Trait description	Ecological function
Life history	Categorical trait: annual, biennial, or perennial, which may be further sub-divided into longevity categories.	Associated with plant longevity, space-holding ability, and disturbance tolerance.
Growth form	A classification of system of plant form defined a) by a single principal characteristic: the relation of the perennating tissue to the ground surface. Perennating tissue is the meristematic tissue that remains inactive during the winter or dry season and then resumes growth with return of a favorable season. b) or by canopy structure and canopy height.	Associated with plant strategy, climatic factors and responses to disturbance.
Clonality	Ability of a plant to reproduce itself vegetatively, thereby producing new ramets and expanding horizontally.	Associated with competitive vigour; the ability to exploit patches rich in key resources (e.g., nutrients, water, light) and space acquisition. Clonality can promote persistence after environmental disturbances. Clonality may also be an effective means of sort-distance migration in lieu of poor seed dispersal or seedling regeneration. Clonal organs, especially below round ones, may also serve as storage organs and the distinction between both functions is often unclear.

Height	Plant height measured near the end of the growing season, as the difference between the elevation of the highest photosynthetic tissue in the canopy and at the base of the plant.	Associated with competitive vigor, whole plant fecundity, and with the time intervals plant species are generally give to grow between disturbances. There are also important traded-offs between plant height and tolerance or avoidance of environmental (climatic, nutrient) stress. Height tends to correlate allometrically with other size traits in broad interspecific comparisons, e.g., aboveground biomass, rooting depth, lateral spread and leaf size.
Seed mass	Oven-dried mass of an average seed of a species.	Small seeds tend to be dispersed further away from adult plant. Related to dispersal distance, establishment success, and fecundity.
Seed shape	Dispersule shape is the variance of its three dimensions, i.e., the length, the width and the thickness of the dispersule, after each of these values has been divided by the largest of the three values.	Small dispersules with low shape values (relatively spherical) tend to be buried deeper in the soil and live longer in the seed bank. Associated with dispersal distance.
Dispersal mode	Categorical trait with 9 categories: unassisted, wind, internal animal transport, external animal transport, hoarding, ant, water, launching, bristle contraction	Mode of dispersal has consequences for the distances it can cover, the routes it can travel and the places it can up in. May be associated with seed longevity in soil seed bank.
Dormancy breaking	Three main types of dormancy breaking requirements: after-ripening; stratification, and scarification.	Associated with longevity in seed bank, season of germination, range of temperatures at which a specie's seed will germinate.

a) Relative growth rate ***b) Unit leaf rate*** ***c) Leaf area ratio*** ***d)Leaf weight ratio*** ***e) Specific leaf area***	a) The innate rate of increase in total dry weight per plant over a period 7-21 day after germination. b) The rate of dry matter production per unit leaf over the period 7-21 days after germination c) The allocation of leaf area to unit amounts of total dry weight over the periods 7-21 days after germination d) The allocation of leaf dry weight to unit amounts of total dry weight over the period 7-21 days after germination. e) The allocation of leaf area to unit amounts of leaf dry weight over the period 7-21 days after germination.	The innate differences between plant species in maximum rate of dry matter production are fundamentally important o plant strategy theory. Associated with competitive ability, seedling establishment and growth, plasticity, stress tolerance, evergreeness, leaf longevity. SLA is often a good positive correlate of potential relative growth rate or mass-based maximum photosynthetic rate. Lower values tend to correspond with relatively high investments in leaf 'defenses" particularly structural ones, and long leaf lifespan.
Aboveground and belowground biomass	Oven-dried aboveground and belowground biomass measured 7 and 21 days after germination	Correlated with competitive ability and fecundity.
Germination rates	The proportion of seed that germinate under suitable conditions.	Associated with seedling establishment
Wetland Indicator Status (functional group based on suite of traits, rather than a single trait).	Wetland indicator status for Region 9 (from US Fish and Wildlife Service, 1988), which indicate probability of occurring in different wetland types	

Appendix C. Protocol for measuring plant traits for seedlings under standardized conditions based on Hendry and Grime 1993.

West Eugene Wetlands Plant Traits Project
Protocol for Measuring Relative Growth Rate and Related Traits
Prepared by Deborah Clark
January 19, 2005

Equipment

germination chamber
growth chamber for seedlings
computer
scanner
leaf area software
balance for weighing
plant dryer
sink with soil trap
desiccator

Materials

General

- species list that includes complete name and the four letter acronym (make sure that there are not any duplicate acronyms)
- seeds of target species
- data sheets that include
    a. heading
        project name
        date of project
        project lead and contact information
    b. columns of the following variables:
        initials of person doing measurements
        species acronyms
        replicate number
        date germinant transferred
        data seedling harvested
        number of days growing (7 or 21)
        leaf area
        dry shoot mass
        dry root mass
- camera

Transferring germinants
forceps
magnifying glass
wash bottle (optional)
gloved fingers (optional)
fine spray of water (optional)

Growing seedlings
pots and undrained trays
sand
plastic funnel for pouring sand
net mesh for lining pot
scissors
nutrient solutions (Hoagland's solution, 1938)
beaker, graduated cylinder for mixing Hoagland's
pot labels
permanent marker for labeling
calibrated pipette for watering seedlings
a flat disc of black polyethylene

Harvesting, measuring and drying
fork or small spoon
paper towels
cut up sheets of overhead transparencies
envelopes
pens, pencils for labeling
small containers, such as Petri dishes for washing off roots
small artist brush (optional)
razor blades
very fine tip forceps for removing sand
Petri dish
Scotch tape
notepaper
clear 15 cm ruler

Data Collection (*Hendry and Grime 1993*)

The methods outlined are for measurements of growth parameters over the period 7 to 21 days from germination for one species only. Many species (>10) may be screened simultaneously using identical or overlapping harvesting schedules.

Germinating seedlings

- Use appropriate dormancy breaking strategies and optimal germination conditions to provide approximately 40 seedlings each species.

Methods for growth

Pots

- Use pots set in a trays.

Media

- Use water washed silica sand as the growing medium. Silica sand is water-washed to remove any fine clay fractions that may hold nutrients or pollutants.
- Green algae can colonize the sand surface if a full nutrient solution is applied, with the potential to outpace and thus interfere with the establishment of slow-growing species. To avoid this, place a flat disc of black polyethylene around the main stem of the plant to exclude light from the sand surface.
- Line plastic pots of the appropriate capacity with nylon mesh at the base to prevent loss of sand through the drainage holes. Fill pots with washed sand using a plastic funnel. Place in a tray and give a sufficient quantity of the desired nutrient solution to saturate the sand and also to cover the bottom of the tray. The amount of nutrient solution required for subsequent weekly applications can be calculated by weighing one pot-full of dry sand and multiplying the number of grams of sand by 0.25. The result gives the volume of solution required per pot per week, e.g., for 150 mm diameter pots: 2600 g dry sand x 0.25 = 650 ml nutrient solution per week. For our pots, we should use 5.0 ml per pot for each application.

Nutrient solution

- The basic composition of the nutrient solution used is given in Table 2.

Transfer of germinants to the growing environment

- Prior to transferring the seedling, water the pot filled with sand with 5 ml of nutrient solution.
- When radical of germinated seedling is 1 mm long, transfer the germinated seedling to prepared pots. Great care should be taken, as damage inflicted at this time generally results in permanently poor specimens thereafter. Remove seedling with soft-tipped forceps. If necessary, saturate sand medium in the germination boxes so seedlings are easily removed.
- While holding the seedling with the forceps, it may be helpful to dip the root in water to make the root heavy and allow it to drop into a depression made in the sand in the experimental pot. The root can then be surrounded with sand, either by flushing with water from a wash bottle or by carefully pressing the sand around the root with gloved fingers, eliminating any air pockets which could dry out the root tip. A fine spray of water applied at this stage can help remove any sand particles which may have adhered to the shoot.
- For particularly small, slow growing species, two seedlings may be planted per pot.

- Add a label to each pot with the following information:
    a) species 4-letter acronym
    b) planting date
    c) indicate whether 7 or 21 day harvest

Growing seedlings

- Water each pot with 5ml of Hoagland's solution on Mondays, Wednesdays, and Fridays.
- Set growing regime in the growth chamber (Table 1) using the standard regime of Hendry and Grime 1993 (Table 1). Monitor continuously using automatic monitors.

Harvesting and Measuring

Harvest of the entire plant

- At 7 days from planting out, harvest seedlings. Repeat at 21 days from planting out. Separate root and shoot, determine total leaf area per plant and dry weight of root, stem and leaf. Repeat at 21 days using the 8-pot subset.
- Carefully remove the sand and plant were from each pot, using the net lining and small spoon. Place in a small container of water, which is rinsed between each plant transfer.
- Carefully tease out any roots that have grown into the nylon mesh. Remove any sand remaining on the roots by rubbing gently under water.
- Place each plant between two small pieces of clear transparencies (used for overhead projectors) and place into an envelope labeled as follows:
    a) species 4-letter acronym
    b) date planted
    c) date harvested
    d) 7 day or 21 day harvest

Measuring leaf area: preparing leaf material

- To measure the leaf area, the root is separated from the shoot. Usually, there is clear morphological demarcation.
- Any remaining sand is removed from the leaf and root, by washing again in a shallow dish of water (e.g., Petri dish) and hand removing using a fine dissecting forceps under a dissecting scope.
- The root is placed into a an envelope, which is labeled
    a) species 4-letter acronym
    b) date planted
    c) date harvested
    d) 7 day or 21 day harvest
    e) belowground
- The shoot is placed on a large uncut transparency used for overhead projector, and then covered with a small piece of transparencies, which is taped to keep the leaf flat and to inhibit water evaporation.
- Sometimes rewetting the leaf while it is on the transparency helps smooth out any wrinkles.
- Leaves are cut apart only if they cover each other when lying on the transparency. Usually leaves of grasses were not cut apart.

- Stem measurements
- A label is placed below the image with the same information that is contained on the envelope.

Measuring leaf area: scanning

- The leaf should be scanned as soon as possible to prevent dehydration. If there is a delay, put the transparencies into a refrigerator.
- Scanning protocols
- After scanning, place the aboveground plant biomass (separated into stem and leaf components) into an envelope labeled as follows
    a) species 4-letter acronym
    b) date planted
    c) date harvested
    d) 7 day or 21 day harvest
    e) stem or leaf

Measuring leaf area: using Assess (software program for measuring leaf area)

- Assess protocol

Measuring dry weight

- Remove excess water is removed from the harvested plant using an absorbent paper towel, and plant of plant component is placed in a labeled paper towel or envelope.
- The aboveground biomass and belowground biomass is then placed in an oven at 80 degrees C for 2 days.
- Following drying, plant are removed from the oven and placed in a desiccator until cool and then weighed. Plants must not be left in the oven for long periods as material my degenerate into small fragments and dust. If weighing cannot be effected straight away, the plants must be stored and then re-dried for a short time at 80 degrees before weighing.
- Dried plants should be handled with forceps to prevent any moisture from the hands affecting the final recorded weight.
- Record data on data sheets.

Data Summary

1. Prepare data sheet in Excel. Include
    a. Heading
        Project name
        Date of project
        Name of project lead and contact information
        Name of person(s) entering data and date(s)
        Name of person(s) proof-reading data and date(s)
        Name of person(s) making any data corrections and date(s)
    b. Columns of the following variables:
    c. On separate worksheet define any variables and abbreviations. Include
        Project name
        Date of project
        Name of project lead and contact information
    2. Enter data
    3. Calculate

a. electronic
b. hard copies

Plant trait calculations

Mean relative growth rate (RGR), the innate rate of increase in total dry weight per plant over period 7-21 days after germination.
Mean unit leaf rate (ULR), the rate of dry matter production per unit leaf area over the period 7-21 days after germination.
Mean leaf area ratio (LAR) the allocation of leaf area to unit amount of total dry weight over the period 7-21 days after germination.
Mean leaf weight ratio (LWR) the allocation of leaf dry weight to unit amounts of total dry weight over the period 7-21 days after germination.
Mean specific area (SLA) the allocation of the leaf area to unit amounts of leaf dry weight over the period 7-21 days after germination.
Root/shoot allometric coefficient, the ratio of root mean relative growth rate to shoot mean relative growth rate over the period 7-21 days after germination.

Table 1. Growing Conditions Integrated Standard Regime (*Hendry and Grime 1993)*

Lamps	400 W metal halide and 100 W tungsten in the ratio of 2
Light irradiance	125 ± 10 µmol m^{-2} s^{-1} equivalent to 29 W m^{-2}
Red/far red ratio	1.4
*Daylength	14 h day, 10h night (the daily integral of PAR at 29 W m^{-2} over a 16 hour day is 1.46 MJ m^{-2}, corresponding approximately to the daily mean of short-wave radiation receipt on a winter's day at 53 ºN)
Temperature	20-22Cº day, 15-17Cº night
Humidity	not controlled
Sand	Water washed silica sand from Double Arches Pit No. 21, Joseph Arnold, Leighton Buzzard, Bedfordshire, UK
Compost	John Innes No. 1 (for germination) John Innes No. 3 and grit in the ratio 3:1
Watering	Deionized water added to saucers of pots as required
pH	4.5 ± 0.2
Nutrient addition	to sand: 0.40 ± 0.05 ml nutrients per cm^3 sand per week

Details light regime (Hendry and Grime 1993)
The metal halide lamps are 400 W "Kolorarc" units from Thorn Lighting plc. They are high-pressure mercury discharge lams with metallic additives. The discharge occurs in a cylindrical quartz tube which is contained within an elliptical glass envelope and operated in the horizontal position. The proprietary ballast used in conjunction with each lamp supplies approximately 3.5 A at 120 V. Two variants of lamp are in use: the MBI unit, which has a clear glass envelope, and the MBIF unit, which has a fluorescent phosphor coating on the interior of the glass envelope, claimed by the manufacturer to provide improved output, color and diffusion.

The tungsten-based lamps are of two kinds. The first is a domestic 100 W or 150 W tungsten-filament lamp with a "pear' coating and an Edison –screw cap. The second is a 300 W tungsten/halogen tube mounted horizontally in a commercial floodlight unit of 'open" design (Fitzgerald 'Light Ring.)

UCPE measurements showing typical spectral outputs for metal halide and tungsten/halogen units are given in Figure 2.4, together with a specimen comparison curve for direct solar irradiance on a horizontal surface. The two metal halide sources differ in the manner claimed by the manufacturer. The tungsten/halogen sources is like the tungsten filament bulb (not shown) in providing a rich source of red and far-red-illumination but, unlike the tungsten filament bulb, it also provides a useful supplement to PAR in the range of 400-700 nm

Mixtures of metal halide and tungsten-based units are used in appropriate combination at each of the f our locations at which UCPE maintains the standard ISP environments. For example, in the largest growing area there is a mixture of 24 MBI or MBIF metal halide lamps interspersed with 12 tungsten filament lamps, without a water filter, about a bench area of approximately 6 m^2. This combination provides 125± 15 umol $m^{-2} s^{-1}$ of PAR at a red/far-red quotient of 1.4. In the temperature gradient tunnel there is a mixture of 15 MBI or MBIF 400 W metal halide lamps interspersed with the 300 W tungsten/halogen lamps, all above a 50 mm water filter and a growing area of 2.5 m^2. This combination provides 125±5umol $m^{-2} s^{-1}$ of PAR at a re/far-red quotient of 1.2.

Table 2 Composition of the nutrient solution

Element	Sigma (Hoagland and Arnon 1938)	mg/L
Ca/N	calcium nitrate	656.4
Mg	magnesium sulfate	240.76
K/P	potassium nitrate	606.6
	ammonium phosphate	115.03
Fe	ferric tartrate	5.32
Mn	manganese chloride· 4H2O	1.81
B	boric acid	2.86
Mo	molybdenum trioxide	0.016
Zn	zinc sulfate• 7H2O	0.22
Cu	cupric sulfate• 5H2O	0.08

Appendix D. Protocol for plant trait of seed mass.

West Eugene Wetlands Plant Traits Project
Protocol for Weighing Seeds
Prepared by Deborah Clark
January 19, 2005

Introduction

"A basic conflict in resource allocation exists with regard to seed size and seed number. The production of few seeds with large reserves will increase the probability of seedling establishment under unfavorable environmental conditions or severe competition from other plants. By contrast, small seeds will tend to e associated with high fecundity and may allow for effective dispersal in time and possibly space. The importance of seed is further decribed elsewhere in relation ... to seed shape and persistence in the soil " (Hendry and Grime 1993).

Objective

To determine the average mass of 100 seeds of target wetland species of Willamette Valley wetlands.

Materials and Equipment

- Species list that includes complete name and the four letter acronym (make sure that there are not any duplicate acronyms)
- Seeds of target species
- Data sheets that include
    a. Heading
        -Project name
        -Date of project
        -Project lead and contact information
    b. Columns of the following variables
        -Initials of person doing weighing
        -Date of weighing
        -Species acronyms
        -Replicate number
        -Mass of 100 seeds (mg)
- Small 3-ring binder containing data sheets
- Balance
- Envelopes
- Drying oven
- Weigh paper
- Pencils and permanent marker
- Single edge razor blade or similar tool for counting seed
- Magnifying lamp
- Plastic storage container for seeds
- Desiccator

Protocol for data collection

1. Dry seeds at 80C for at least 48 hours (or 60C for 72 hours) in coin envelopes labeled with species name.
2. Be aware that, once taken from the oven, the samples will take up moisture from the air. If they cannot be weighed immediately after cooling down, put them in the desiccator until weighing, or else back in the oven to dry off again.
3. Count out five replicates of 100 seeds.
   There should be no chaff.
   Leave any attached appendages or structures to the seed.
4. Record the following on the data sheet
   a. name of person doing weighing
   b. date of weighing
   c. specie name
   c. replicate number
   d. mass (mg) of each replicate containing 100 seed
5. Put counted seeds in to coin envelopes labeled with the specie name and date.
6. Store envelopes in plastic storage container.
6. Each day make a copy of the raw data and give to Deborah.

Protocol for data summary

1. Prepare data sheet in Excel. Include
   a. Heading
      Project name
      Date of project
      Name of project lead and contact information
      Name of person(s) entering data and date(s)
      Name of person(s) proof-reading data and date(s)
      Name of person(s) making any data corrections and date(s)
   b. Columns of the following variables
      Species name
      Replicate number
      Mass of 100 seeds (mg)
      Average
   c. On separate worksheet define any variables and abbreviations. Include
      Project name
      Date of project
      Name of project lead and contact information
2. Enter data
3. Calculate average
4. Make backup copies
   a. electronic
   b. hard copies

Appendix E. Protocol for measuring seed dimensions.

West Eugene Plant Trait Project
Protocol for Measuring Seed Dimensions
Prepared by Deborah Clark
January 19, 2005

Introduction

"Persistent seeds in soil seed banks are strongly associated with small seed size, compact shape and inhibition of germination by darkness. The seed bank is a major determinant of the rate and direction of recovery of vegetation of disturbance, and is of vital importance to attempts to restore degraded or neglected vegetation" (Hendry and Grime 1993).

Objective

To determine seed shape for target Willamette wetland prairie species.

Materials and Equipment

- Species list that includes complete name and the four letter acronym (make sure that there are not any duplicate acronyms)
- Seeds of target species
- Data sheets that include
    a. Heading
        Project name
        Date of project
        Project lead and contact information
    b. Columns of the following variables:
        Initials of person doing measurements
        Species acronyms
        Fruit or seed
        Replicate number
        Length with any (mm)
        Length without awn (mm)
        Width (mm)
        Thickness (breadth) (mm)
- Small 3-ring binder containing data sheets
- Calipers or micrometer
- Dissecting scope
- Scanner with software for measuring perimeter
- Pencils
- Fine tip forceps
- Permanent marker
- Magnifying lamp

Protocol for data collection

1. "Of interest is the unit that is likely to enter the soil. Therefore, only parts that fall off easily (e.g., pappus) are removed, while wings and awns remain attached. The flesh of fleshy fruits is removed too, since the seeds are usually the units to get buried in this case. The seeds should be mature and alive. For naturally dry dispersules air-dry storage is also okay" (Cornelissen et al. 2003).
2. For 10 seeds of each species, use calipers or dissecting scope measure or scanner
    a) width
    b) length with awn
    c) length without awn
    d) thickness
3. Record the following on the data sheet
    a. name of person doing weighing
    b. date of weighing
    c. specie name
    c. replicate number
    d. mass (mg) of each replicate containing 100 seed.
4. Make back up copies and give to Deborah each time new data are entered.

Protocol for data summary

1. Prepare data sheet in Excel. Include
    a. Heading
        Project name
        Date of project
        Name of project lead and contact information
        Name of person(s) entering data and date(s)
        Name of person(s) proof-reading data and date(s)
        Name of person(s) making any data corrections and date(s)
    b. Columns of the following variables
        Initials of person doing measurements
        Species acronyms
        Fruit or seed
        Replicate number
        Length with any (mm)
        Length without awn (mm)
        Width (mm)
        Thickness (breadth) (mm)
    c. On separate worksheet define any variables and abbreviations. Include
        Project name
        Date of project
        Name of project lead and contact information
    2. Enter data
    3. Calculate average seed shape:
        a) Dispersule shape is the variance of its three dimensions, i.e., the length, the width and the thickness (breadth) of the dispersule, after each of these values has been divided by the largest of the three values (Thompson et al. 1993)

4. Make backup copies
    a. electronic
    b. hard copies

Protocol for data analysis

1. Plot seed/fruit mass against variance.
2. Broadly speaking, seeds that weigh less than 3 mg and have a variance of less than 0.14 are persistent in the soil seed bank. For fruits, the weight limit is the same, but there are persistent fruits (all grasses) with a rather higher variance.
3. Variances lie between 0 and 1 and are unitless. Small dispersules with low shape values (relatively spherical) tend to be buried deeper into the soil and live longer in the seed bank (Cornelissen et al 2003).

References

Thompson, K. Band, S.R., Hodgson, J.G. (1993) Seed size and shape predict seed persistence in the soil. Functional Ecology 7: 236-241.

Appendix F: Protocol for germinating seeds

Seed Germination Protocol
West Eugene Wetlands Plant Traits Project
Prepare by Deborah Clark
January 26, 2005

Objectives

- To germinate seeds in preparation for growing in growth chambers for seedling measurements in plant trait analyses
- To gather germination data, specifically temperature requirements and dormancy breaking requirements for use in plant trait analyses

Equipment and Materials

Provided by Seed Lab

- germination boxes
- labels
- filter paper for stratification
- germination chambers

Provided by grant

- species list that includes complete name and the four letter acronym (make sure that there are not any duplicate acronyms)
- seeds of target species
- data sheets
- small 3-ring binder containing data sheets
- sand
- sand paper for any scarification

Protocol for Germinating Seeds

Breaking dormancy

*Stratification (*Hendry and Grime 1993)

1. For each species, sow seeds into two germination boxes, which are first lined with thick germination paper and filled half-way with moistened sand. Label should contain:
   -species four letter acronym
   -replicate number
   -date seeds sowed
   -germination chamber number
   -initials of person sowing the seeds
2. Allow seeds to imbibe and check sand is still sufficiently damp. Do not allow seeds to stand in water.
3. Place in fridge at 2-5C. Chilling is effective in light or darkness but a clear germination box facilitates regular checks on the seeds.
4. A weekly check during the chilling period will avoid losses due to seed drying out or damage from fungal or bacterial attack.

5. The chilling requirement varies depending on the species and on the ripeness of the seed at the collection time.

Scarification

(Hendry and Grime 1993) Those seeds in which imbibition does not take place until the testa has been weakened or breached require scarification. In the Sheffield flora a requirement for scarification is virtually confined to the families Cistaceae, Geraniaceae and Leguminosae, and is strongly associated with the possession of a persistent seed bank.

1. Place a small quantity of seed on a sheet of paper on a firm surface, cover with medium sandpaper and gently but firmly rub seed in a circular motion.
2. When tiny chips of testa are viable on the paper scarifying is complete. Germination is more uniform when scarifying is done in this way.
3. Hand scarifying with a scalpel is both laborious and slow and germination intends to be more erratic.
4. As soon as the seeds are moistened, those which have been successfully scarified will visibly swell, providing an immediate visual check on the effectiveness of the treatment. 6. Germination of scarified seeds is often extremely rapid, commencing within 24 hours of imbibition.

Germination for non-dormant seeds

Time the germination the best that we can so that there will be room in the growth chamber and so that there is not a conflict with not being able to come in on weekends.

1. Prepare two germination boxes for each species.
2. Add label, which includes
   - -species acronym
   - -replicate number
   - -date seeds sowed
   - -germination chamber number
   - -initials of person sowing the seeds
3. Fill the germination boxes about ½ full with washed silica sand, which has been washed with tap water.
4. Saturate the sand with tap water.
5. Gently tap the germination box to level the sand surface.
6. Add 100 seeds using the vacuum seed dispenser to each of germination box, except for the largest seeds. For these, sow 50 seeds per germination box and sow a total of 4 boxes for each species.
7. Some species may require shading with muslin or nylon cloth to achieve optimal germination.
8. Place germination boxes in appropriate germination chamber.
9. Seeds must be checked on Fridays and Mondays as we do not have access to the seed lab during the weekends. Then check on Wednesday or Tuesday and Thursday. Water as necessary.
10. Record data as described below.
11. As seeds germinate remove and transfer to growth chamber using the protocols for growing in the growth chamber.

12. After sufficient seed have been removed to the growth chamber, keep track of germination of the remaining seeds until no germination has occurred for 7 days.

Protocol for Data Collection

1. Record the following on the data sheet
    a. Specie acronym
    b. Replicate number
    b. Date seeds sowed
    c. Date germinated seed removed from growth chamber
    d. Dates germination results recorded
    e. Number of germinants
    f. Initials of data collector
2. Each day of data collection make a copy of the raw data and give to Deborah.

Protocol for Data Summary

1. Prepare data sheet in Excel. Include
    a. Heading
        Project name
        Date of project
        Name of project lead and contact information
        Name of person(s) entering data and date(s)
        Name of person(s) proof-reading data and date(s)
        Name of person(s) making any data corrections and date(s)
    b. Columns of the following variables
        Specie acronym
        Replicate number
        Date seeds sowed
        Date germinated seed removed from growth chamber
        Dates germination results recorded
        Number of germinants
        Initials of data collector
    c. On separate worksheet define any variables and abbreviations. Include
            Project name
            Date of project
            Name of project lead and contact information
    2. Enter data
    3. Calculate germination averages
    4. Make backup copies
        a. electronic
        b. hard copies

Data Sheet for Seed Germination Trials
West Eugene Wetlands Plant Trait Project, Winter 2005
Deborah Clark, Project Lead
Department of Botany and Plant Pathology 737 -1734

Specie name _____
Specie acronym _____

Initials of data collector	replicate number	date seeds sowed	germination epigeal or hypogeal	date seed removed for growth chamber	# germinants removed for growth chamber	date remaining germination results	# germinates

comments

Appendix G: Seed germination requirements for target species.

Germination requirements for study species

CODE	SPECIES	PRIORITY	PREPLANTING TREATMENT	TEMP	HRS DARK:LIGHT	GERMINATION RATE (%)	GERM TIME	REFERENCE
ACMI	Achillea millefolium	1	none	20/30	8:16	84-100+	7 days	***
AGEX	Agrostis exarata	1	none					
ALAM	Allium amplectens	1	CS: 90 days					Amy
ASCU	Aster curtus	1	CS: 3-6º, 6 wks	9-18	winter photoperiod	9	13 days	Drake et al 1998
			CS: 4º, 8 wks	15/25	8/16	48		Kaye & Kuykendall 2001
			scarified	15/25	8/16	58		Kaye & Kuykendall 2001
ASHA	Aster hallii	1	none	15	8/16	100		kew (data for A. chilensis)
BESY	Beckmannia syzigachne	1	WC: growing conditions for 6 weeks, then alt. 8 hrs @ 5º & 16 hrs @ 10º	10/20	8/16	72		Guerrant & Raven 1995
CALE	Camassia leichtlinii	1	CS: 5º, 6 wks	10/20	8/16			
CAQU	Camassia quamash	1	CS: 5º, 6 wks	10/20	8/16	90		Guerrant & Raven 1995
CADE	Carex densa	1	CS: 5º, 6 wks	10/20	8/16	51		Guerrant & Raven 1995
CALE	Carex leporina (ovalis)	1	CS: 5º, 6 wks	10/20	8/16	98		Guerrant & Raven 1995
		1	none	19/33	12/12	100		kew
CATU	Carex tumilicola	1	CS: 5º, 6 wks	10/20	8/16	40		Guerrant & Raven 1995

CAUN	Carex unilateralis	1	CS: 5º, 6 wks	10/20	8/16	24		Guerrant & Raven 1995
DACA	Danthonia californica	1	CS: 5º, 6 wks	10/20	8/16	87		Guerrant & Raven 1995
DACAR	Daucus carota	1	CS optional	9/23	12/12	100		kew
DECA	Deschampsia caespitosa	1	CS or WC	10/20	8/16	85		Guerrant & Raven 1995
DOEL	Downingia elegans	1	none	15	8/16			kew (based on other Downingia sp.)
EPDE	Epilobium densiflorum	1	none	21	12/12			kew (based on other Epilobium sp.)
ERLA	Eriophyllum lanatum	1	CS: 3-6º, 6 wks	9-18	winter photoperiod	31	6	Drake et al 1998
			none	20/20	8/16	86	7 days	***
FERO	Festuca roemeri	1	none	5	24/0	90	21 days	***
GLOC	Glyceria occidentalis	1	WC: growing conditions for 6 weeks, then alt. 8 hrs @ 5º & 16 hrs @ 10º	10/20	8/16	90		Guerrant & Raven 1995
FERO	Festuca roemeri	1	none	5	24/0	90	21 days	Guerrant & Raven 1995
HOLA	Holcus lanatus	1	none					
HOBR	Hordeum brachyantherum	1	CS: 5º, 6 wks	10/20	8/16	98		Guerrant & Raven 95
HOCO	Horkelia congesta	1	CS: 4º, 12 wks (8 wks gives 20% germ)	15/25	8/16	60		Kaye & Kuykendall 2001
JUAC	Juncus acuminatus	1	none	21	12/12			kew (based on other Juncus sp.)

LOBR	Lomatium bradshawii	1	CS: 16 wks WC: growing conditions for 6 weeks, then alt.	20		100		BBG(mobot)
MILA	Microseris laciniata	1	8 hrs @ 5º & 16 hrs @ 10º	10/20	8/16	66		Guerrant & Raven 1995
PACA	Panicum capillare	1	none	19/33	12/12			kew (based on other Panicum sp.)
PLFI	Plagiobothrys figuratus	1	none	10	8/16	88		kew
POGR	Potentilla gracilis	1	CS: 5º, 6 wks	10/20	8/16	42		Guerrant & Raven 1995
			none	20/30	8/16	14		***
PRVU	Prunella vulgaris	1	WC: growing conditions for 6 weeks, then alt. 8 hrs @ 5º & 16 hrs @ 10º	10/20	8/16	100		Guerrant & Raven 1995
			none	20/20	8/16	70		***
RUAC	Rumex acetosella	1	scarified (chipped w/ scalpel)	9/23	12/12	86		kew
SICA	Sidalcea campestris	1	none	20/20	8/16	33		***
SICU	Sidalcea cusickii var. purpurea	1	CS: 5º, 6 wks	10/20	8/16	23		Guerrant & Raven 1995
SIHI	Sisyrinchium hitchcockii	1	CS: 5º, 6 wks	10/20	8/16	6		Guerrant & Raven 1995
VESC	Veronica scutellata	1	CS: 6º, 4 wks	9/23	12/12	60		kew
WYAN	Wyethia angustifolia	1	seeds subjected to ambient outdoor conditions			42		Guerrant & Raven 1995
ZIVE	Zigadenus venenosus	1	CS: 3-6º, 6 wks	9-18	winter photoperiod	72	29	Drake et al 1998

DOYI	Downingia yina	2						
RAOC	Ranunculus occidentalis	2	CS or WC	10/20	8/16	87		Guerrant & Raven 1995
RAOR	Ranunculus orthorhynchus	2	seeds subjected to ambient outdoor conditions			34		Guerrant & Raven 1995
RUSA	Rumex salicifolius	2						
ALPL	Alisma plantago-aquatica	3	CS	20				
JUEN	Juncus ensifolius	3						
JUTE	Juncus tenuis	3	WC: growing conditions for 6 weeks, then alt. 8 hrs @ 5º & 16 hrs @ 10º	10/20	8/16	79		Guerrant & Raven 1995
PAOC	Panicum occidentale	3						
AGGR	Agoseris grandiflora	4	none	5	24:0	43		***
	Agoseris grandiflora	4	none				5-7 days	Native Plant Network
BADE	Balsamorrhiza deltoidea	4						
BRCA	Bromus carinatus	4	none	15/25	8/16	94		***
CATO	Calochortus tolmiei	4						
CLQU	Clarkia quadrivulnera	4						
ELAC	Eleocharis acicularis	4						
ELOV	Eleocharis ovata	4						
ELPA	Eleocharis palustris	4	CS: 5º, 6 wks	10/20	8/16	3		Guerrant & Raven 1995

ELGL	Elymus glaucus	4	none	20/20	8/16		95	***
ERPE	Eryngium petiolatum	4						
FERU	Festuca rubra	4						
GNPA	Gnaphalium palustre	4						
GREB	Gratiola ebracteata	4						
JUOX	Juncus oxymeris	4						
LOUN	Lotus unifoliolatus	4						
MAGL	Madia glomerata	4						
MASA	Madia sativa	4	CS: 5º, 6 wks	10/20	8/16	95		Guerrant & Raven 1995
			CS: 3-6º, 6 wks	9-18	winter photoperiod	20	18	Drake et al 1998

Resources for germination requirements of target wetland prairies species.

1. Willamette Valley Database from Mark Wilson
2. Propagation Protocol Database www.nativeplantnetwork.org
3. Information compiled by Dan Irvine for the Butterfly Meadows and Finley Fender's blue restoration project.
4. Dale Brown (Seed Lab)
5. Barbara Wilson may have information on Carex species
6. Berry Botanical Garden study (Deborah has copy)
7. Published paper on Puget Sound species (Deborah has a copy)
8. Ecological Database of the British Isles
   http://www,york.ac.uk/res/ecoflora/cfm/ecofl/index.cfm
9. Kew Gardens Seed Information Database Tweddle, J.C., Turner, R.M., and Dickie, J.B. 2003. Seed Information Database http://www.rbgkew.org.uk/data/sid
10. Graham Simpson's Bibliography of References Related to Seed Dormancy and/or Germination in Higher Plants (there is a link on the Kew Gardens database page)
11. Lisa Lantz's thesis
12. Plant Materials Center: Amy Bartow

References cited by Dan Irvine

1. Native Plants Network. <http://www.nativeplantnetwork.org>
2. Tweddle, J.C., Turner, R.M., and Dickie, J.B. 2003. Royal Botanic Gardens, Kew. Seed Information Database. <http://www.kew.org/data/sid/>
3. Brown, Dale. Personal interview. 27, Jan. 2004.
4. Biological Services Program, FWS /OBS –82/29. *Growing Colorado Plants from Seed: A State of the Art. Volume II: Grasses and Grasslike Plants*. August 1982.
5. <http://www.anet-chi.com/~manytimes/page43.htm>
6. Taylor, Jeanie. Seattle Department of Parks and Recreation, Citywide Horticulture. *Propogation Successes, Failures and Lessons Learned.*
7. Holloway, P., and Matheke, G. "Seed Germination of Burnet, *Sanguisorba spp*." *Native Plants.* Fall 2003: 95-99.
8. Biological Services Program, FWS. *Growing Colorado Plants from Seed: A State of the Art. Volume III: Forbs.*

Appendix H: Current plant trait data collected on target species.