ANNUAL REPORT

FOR THE

WESTERN REGIONAL PLANT INTRODUCTION STATION

FOR

CALENDAR YEAR 2000
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1. Note: changes in personnel due to retirements are listed in the Personnel Activities section on pages 6-7.
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EXECUTIVE SUMMARY

Most of agriculture in the United States is based upon crop plants which are native to other parts of the world. Recently, there is an increasing sensitivity to environmental issues, especially regarding pesticides. The research of this unit is consistent with the mission of the collection, preservation, evaluation, and use of plant germplasm. We utilize current technologies and information to best achieve germplasm conservation goals. The group is comprised of scientists in disciplines such as agronomy, horticulture, entomology, plant pathology, genetics, botany and natural resource management. Acquisition technology has been enhanced recently by use of geographic information systems (GIS) and global positioning system devices when planning and carrying out collecting trips. Genetic monitoring of regeneration protocols is being enhanced by use of DNA techniques such as RAPDS, RFLPS, and SSRs, especially when used in conjunction with morphologic genetic markers. Seed storage technology has progressed and facilities are in place to allow long-term storage of original and regeneration seed lots at -18 C. Regeneration population size has been increased from 20 to 60 plants for grass species to ensure freedom from genetic drift and loss of minor alleles. Native insect pollinators are being evaluated and utilized in caged field increases. Accessions that are not adapted to the Washington locations are increased at Parlier, CA if long seasons and milder weather is need, or in Palmer, AK if arctic conditions are needed. In the entomology program, we tested 3 species of Lesquerella to determine that house flies are an effective pollinator for quality seed increase under caged conditions. A project was developed to characterize fungal endophyte diversity in Mediterranean tall fescues to learn more about the complexity of grass-endophyte-insect relationships by studying different tall fescue-endophyte associations on aphid resistance. The entomology program participated in a multi-disciplinary team researching the sustainability of spring wheat production in the arid and semiarid wheat-fallow region of central WA. Agronomy research included; comparison of agronomic and biochemical traits in balanced and bulk populations of annual ryegrass; a test for the effective population size of heterogenetic grass accessions in perennial ryegrass, meadow fescue, and bluebunch wheatgrass; the evaluation of diverse Kentucky Bluegrass germplasm for seed production in alternative residue management systems. Horticultural research included; a collaborative project comparing polymorphism between 45 accessions of garlic selected from diverse morphological types; finalization of experiments to test for survival of A. sativum meristems in liquid nitrogen (LN). We will establish a collaborative program with NSSL for long term LN storage. We evaluated safflower germplasm for ornamental potential. Also evaluated accessions of other agronomic and horticultural genera for ornamental potential. Significant data input into GRIN has occurred. Examples include field data on 1385 accessions of lentil, passport data on four large Phaseolus collections, evaluation data on field observations of the wild Allium collection. We established an excellent working relationship with the international Beta group for exchanging and increasing Beta germplasm. Approximately 200 PI accessions are being included in the IDBB Beta core collection. We have received 16 of these that were on our increase priority list. Continued to evaluate and develop core collections in lettuce, chickpea, and pea as well.
OVERALL STATION ACTIVITIES and ACTIVITIES FOR RESEARCH LEADER
Richard M. Hannan

Plant Material Activity

At year’s end W-6 was responsible for 2,572 taxa in 361 genera (23% of the NPGS) with a total of 70,582 accessions. In 2000, we received 1,212 new accessions from collections, plant explorations and germplasm exchanges. We also received 274 accessions from the National Seed Storage Laboratory, Ft. Collins, CO for inclusion into our Pullman collections and 10 accessions were transferred from other sites. Therefore, we had a total of 1,486 accessions in 57 genera added to the W-6 collections.

During the year, 14,800 accessions were distributed in 24,694 packets. There were 598 separate orders filled, and of these, 456 (76%) were domestic and 142 (24%) were sent to foreign scientists. Within the 110 genera distributed there were 770 taxa represented in the 24,694 packets sent. Within the Western Region alone, there were 761,482 accessions received by users of germplasm. Of this 14% was provided by the Western Regional Plant Introduction Station.

By the end of CY2000 the number of PI accessions (50,783) backed up at NSSL increased to 72% of our collection. During the year a total of 197 accessions were sent to NSSL for back-up. The goal continued to be getting back-up samples of all of the accessions which are so difficult to propagate. As yet, we have not had the resources to implement an ongoing tissue culture/cryogenic program at the Pullman location, but will continue trying to coordinate with the cryogenic laboratory at NSSL.

Personnel Activities

Andrew Bell began working for USDA, ARS in March, 2000, as a Biological Technician at the Prosser, Washington, site. Drew previously was employed by Washington State University in the same capacity.

Bill Luna came to us in March as a transfer from Riverside, California. Bill has been with the USDA for six years and is working as the greenhouse technician under Wayne Olson.

Kevin Miller resigned his position as Plant Technician II in April.

Merle Peters resigned his position as a Biological Technician in Parlier, California, in April.

Jacob McNally was hired as a Biological Technician in the Parlier, California, unit in April.

Nancy Robertson recruited Clare Stockert to fill the vacancy created by the departure of Denice Knight-Slater as the plant pathology technician in May.
Dan Cervantes was hired on W6 Regional Research Funds in July, as a Plant Technician II and will be working in Wayne Olson’s program.

Michael Cashman was hired in August to fill the two-year term position in Clare Coyne’s Cool Season Food Legume program.

Ted Kisha was hired in August to serve as geneticist and lab 35 manager for the unit.

Jerry Serimian accepted the appointment to Biological Technician in the Parlier Unit in August.

Russell Staska who worked for Barbara Hellier’s program as a temporary employee was hired to a full-time, permanent position on W6 Regional Research Funds in September, 2000, as a Plant Technician II for Washington State University and is continuing in Barbara’s program.

On October 6, Dave Stout retired from WSU after serving 30 years, six months and three days for WRPIS. His knowledge and skill are not gone. Dave accepted a two-year term appointment from the USDA, ARS and will continue on in basically the same capacity as he was prior to retiring from WSU.

Julie Thayer was hired in December on W6 Regional Research Funds to fill the position of Plant Technician II and will be working with Molly Welsh in her greenhouse Phaseolus increase program.

**STATION VISITORS for 2000**

Jan. 18 Randall Ireson of the American Friends Service Committee visited the station. He is the DPRK Development Assistance Coordinator and is developing crop improvement programs in North Korea.

Feb. 25 Dr. Lothar Frese, German Federal Centre for Breeding Research on Cultivated Plants (BAZ) Gene Bank visited the Beta program at WRPIS.

Mar. 4 Aynalem Haiki, The Volcan Inst. Bet-Dagan, Israeli Gene Bank, Israel

Mar. 16 Dan Skinner came as visiting scientist working on molecular characterization of germplasm.

Apr. 20 Orlin Reinbold, Landmark Seed Company.

Apr. 20 Steve Stilson and Bill Rucheret, Dye Seed Company.

Aug. 21 Evert Byington, National Program Leader-Range came for a site visit.

Nov. 5 Dr. Gowsela Sivam and four students, Bastyr University, Bothell, Washington, for Plant Introduction Station tour.

Nov. 6 Dr. Hohd Hashim Tajudin, Director and Aslan Hy. Ismail, Sr. Research Officer, R&D, Tolden Hope Plantations Berhad, Malaysia.

Nov. 16 Dr. Ram Nath Arora and Dr. Jai Vir Singh, Forage Section, Department of Plant Breeding, C.C.S. Haryana Agricultural University, HISAR-125004 INDIA.
Administrative, Germplasm and Research Activities

After the final tally for the FY2000 period, the total budget for our combined program was nearly $3.3 million when Regional Research Funds and ‘soft’ monies were included in the total. The activity of managing these funds was quite complex. At the end of the fiscal year, we came to within 0.04% of a balance budget. At Palmer, the program is more than sufficiently funded. The National Arctic Plant Genetic Resources Unit (NAPGRU) is now housed at two sites. The germplasm maintenance activities are located at the State of Alaska, Plant Materials Center, and the Plant Pathology Research program is located at the University of Alaska Research Station in Palmer. At Parlier we have been constantly trying to balance between spending on the operation of the germplasm conservation effort, and getting a decent place for our employees to work. Right now the germplasm related operations are being run well, but the housing for office and laboratory activities is inadequate. Hopefully, we will remedy this problem in 2001.

At Pullman, this was a banner year for upgrades and innovative advances. We finally have installed a drip irrigation system to serve the 65 acre farm at Central Ferry. The advantages to this system are 1. Less water use, therefore, less negative impact on the water table, 2. By not watering between the rows, less herbicide use, 3. A potentially safer delivery system (chemigation) for insecticide applications. 4. Less physical impact on the farm crew by not moving sprinkler pipes every time we cultivate. Another big item was the conversion of a hand labor transplanting operation to a mechanical transplanter. This will save a lot of backs in our aging staff. Our Safety Officer oversaw all aspects of our Pullman program as well as provided technical guidance on all computer related issues. Space is always a problem, but this year we acquired WSU office space for two of our scientists. Lastly, we constructed a building to centralize the greenhouse operations and equipment in order to more efficiently operate the 30,000 sq. ft. of greenhouse space on campus.

Research projects were primarily continuations or culminations of existing projects. All of my research involved collaboration with scientists here at WSU and other institutions around the US and abroad. One project involved collaboration with scientists from the Vavilov Institute in St. Petersburg, Russia, as well as one scientist from Kazakhstan. The project included the use of GIS technology to determine appropriate collection strategies to fill genetic gaps in germplasm collections. A three-week germplasm collection trip was taken in August/September. Publications have primarily been as abstracts and less rigorous journals, with one co-authorship in a peer reviewed journal. See attached publication list. International germplasm related collections and/or scientific and information exchange included Bolivia (self funded), Kazakhstan (funded by NPGS, PEO), China (funded by PRC) and Armenia (funded by CSREES/IP).
Talks and Presentations for R. Hannan (unless otherwise indicated)

Feb. 7 Melissa McClendon’s seminar entitled, “Fusarium Wilt on Pea: Locating a Novel DNA Marker for Resistance” to Department of Crops and Soils, WSU.
Mar. 6 Vicki Newman seminar entitled, “Culinary Herbs,” to Department of Horticulture and Landscape Architecture, WSU.
Mar. 20 Marie Laurie Pilet seminar entitled, “Quantitative Trait Loci for Resistance to Blackleg in Canola.”
Mar. 24 Dan Skinner seminar entitled, “Experiences with Molecular Markers.”
May 11 Lecture to Pullman Kiwanis Club entitled, “Conservation of Plant Diversity and the Affect on American Agriculture.”
Jun. 1 Ted Kisha seminar to crops and soils entitled, “The Relationship Between Genetic Diversity and Variation in Soybean.”
Oct. 23 Presentation to Chinese Extension Professionals, in Guizhou Dushan, China, regarding and overview of the operations of the Western Regional Plant Introduction Station.

Travel, Invitations, Special Awards and/or Assignments for Hannan or as indicated.

Jan. 24-30. To Tucson, Arizona to meet with the Desert Legume Project, and serve on the Advisory Board.
Feb. 10. To Prosser, Washington to review and work with the National Forage Legume Genetic Resources Unit.
Mar. 30. Met with Laurie Sundraman of the Spokesman Review for an interview on the role of WRPIS in the NPGS.
Apr. 3-5. To Atlanta, Georgia, to attend and participate in the Plant, Microbial, and Insect Genetic Resources, Genomics and Genetic Improvement National Program (301) Workshop.
Apr. 6-19. To Bolivia, South America to visit Dr. Walter Kaiser and establish germplasm exchange relationship with Bolivia.
May 24-26. To Parlier, California to visit and work with Dr. Jenderek of the National Arid Land Plant Genetic Resources Unit.
Jun. 25-28. To Palmer, Alaska to attend the Annual W6 TAC Meeting which was hosted by the National Arctic Genetic Resources Unit.
Jul. 12. To Prosser, Washington to review and work with the National Forage Legume Genetic Resources Unit.
Jul. 17. Served as the WSU, Graduate School Representative in a PhD defense.
Jul. 18-22. To Beltsville, Maryland to attend the CGC/PGOC Meetings.
Aug. 23- Sep. 13. Traveled with Stephanie Greene to Kazakhstan on a plant exploration/collection trip.
Oct. 22-27. Paula Rose traveled to Baltimore, Maryland, to attend the USDA-GRIN meetings.
Oct. 22-27. David Stout traveled to Baltimore, Maryland, to attend the USDA-GRIN meetings.

Nov. 27- Dec. 5 Traveled to Armenia at the invitation of USAID-MAP to evaluate the development of an Armenian plant germplasm conservation system.

Committees, Other Assignments, Activities, and News

WSU, Graduate School Representative for Ph.D. and MS defenses
WSU, Adjunct Faculty, Department of Horticulture and Landscape Architecture
Serve as Chair of Committee for Master’s Degree Candidate, Barbara Hellier
Serve as Chair of Committee for Master’s Degree Candidate, Vicki Newman
Serve on Committee for Doctoral Candidate, Khalid Al-Saad
Serve on Committee for Doctoral Candidate, Mark Minton
Bean Improvement Cooperative (BIC)
PGOC Movement of Germplasm Subcommittee (Chair)
Washington State University Plant Growth Facilities Committee
Sigma Xi

RESEARCH ENTOMOLOGIST
Stephen L. Clement

Research Activities

Preserving Neotyphodium Endophyte in Stored Seed. We completed an 8-year study investigating the survival of endophyte in wild barley, perennial ryegrass and tall fescue seed stored under different temperatures to determine if W6 seed storage conditions are optimal to maintain this microbial germplasm. In short, our 4°C conditions seem adequate to preserve endophyte. Experiments showed that endophyte viability and seed germination were maintained after 8 years of storage at -196°C, -10°C and 4°C. However, endophyte viability and seed germination was adversely affected by storage at 20°C.

Endophyte Diversity in Tall Fescue Accessions and Aphid Resistance. We completed a series of greenhouse experiments to examine extent of bird cherry-oat aphid (BCOA) resistance in endophyte-infected accessions of tall fescue from Tunisia. While some infected accessions were resistant to BCOA, several infected accessions supported aphid development and reproduction. It was expected that most, if not all, infected accessions would be resistant to BCOA. Variation in the type and levels of alkaloids produced by infected accessions likely mediated the differential survival of BCOA on some test accessions.

Endophyte Diversity in Wild Barley Accessions and Aphid Resistance. We completed a series of greenhouse experiments to quantify resistance of endophyte-infected and uninfected Hordeum accessions to bird cherry-oat aphid (BCOA) and rose grass aphid (RGA). Results showed that
infected wild barley is susceptible to BCOA; however, some infected accessions exhibited resistance to RGA development and reproduction. Thus, the expressions of aphid resistance is dependent upon the aphid species, *Hordeum* genotype, and *Neotyphodium* strain involved in the aphid-plant-endophyte interaction. A field plot to investigate the same relationships was established at Central Ferry with four *Hordeum* PI lines; however, aphid populations failed to materialize so this phase of the study was not completed in 2000.

**Pollination of Caged Allium Accessions.** A pilot study to examine the effectiveness of house flies to pollinate caged *Allium* for seed regeneration was initiated with Barbara Hellier, W6 Curator. Initial results indicate the feasibility of using these insects. The study will be expanded in 2001.

**Cereal Cropping Systems and Insect Pests.** We completed a 5-year study to quantify populations of aphid pests and Hessian fly in different cereal cropping systems established in large replicated plots near Ralston, Washington. This research was supported with extramural grant funds. Year-to-year variability in Russian wheat aphid and English grain aphid densities were recorded, along with variability in ladybird beetle populations. While this result was expected, based on previously published research, we did not anticipate finding large Hessian fly populations. Hessian fly densities increased every year, especially in the continuous no-till spring wheat plots. Producing spring wheat under no-till conditions, and continuously on the same piece of ground, will likely exacerbate problems with Hessian fly. The solution to the problem is to plant Hessian fly resistant wheats from the breeding program at Washington State University.

**Talks and Presentations**

- **Jun. 7** Reviewed aphid and Hessian fly results from cropping systems study, Ralston, Washington, at field day.
- **Aug. 22** Enhancing insect resistance in grasses through deployment of fungal endophytes. Foz do Iguassu, Brazil, International Congress of Entomology. Invited symposium presentation.
- **Sep. 28** Overview of *Neotyphodium* incidence in pastures and seed collections worldwide. Soest, Germany, International *Neotyphodium/Grass Interactions* Symposium. Invited address.
- **Sep. 27** The relationship between storage temperature, grass endophyte viability, and seed germination. Soest, Germany, International *Neotyphodium/Grass Interactions* Symposium. Poster presentation.
- **Sep. 27** Endophyte diversity in tall fescue and the issue of aphid resistance. Soest, Germany, International *Neotyphodium/Grass Interactions* Symposium. Poster presentation.
- **Dec. 6** Hessian fly: population densities in no-till cropping systems. Montreal, Canada, annual meeting of Entomological Society of America. Poster presentation.
Travel, Invitations, Special Awards and/or Assignments

Jun. 25-28 Leslie Elberson traveled to Costa Mesa, California, to attend the Pacific Branch Meeting of the Entomological Society of America on behalf of Dr. Clement to present his paper.

Aug. 18-28 Traveled to Foz do Iguassu, Brazil, to attend the XXI International Congress of Entomology and give two invited talks.

Sep. 24-30 Traveled to Soest, Germany to chair an invited talk, chair a paper session and display two posters, 4th International Neotyphodium/Grass Interactions Symposium.

Nov. 30- Dec. 8 Traveled to Montreal, Canada to attend national meeting of Entomological Society of America, present a poster, and serve as Chair of Arrangements Committee.

Dec. 6 Received plaque from Entomological Society of America for exceptional service as Arrangements Committee Chair for annual meeting, Montreal, Canada.

Committees, Training, Other Assignments, Activities and News

Chair, Arrangements Committee for 2000 meeting in Montreal, Canada, Entomological Society of America
Member, Graduate Faculties, Entomology, University of Idaho and Washington State University. Committee member for two M.S. graduate students
Faculty Advisor, Alpha Zeta, Washington State University
Reviewed 6 manuscripts for various editors and authors and one grant proposal for U.S. State Department
Received approximately 20 calls of a ‘cooperative extension’ nature on a variety of subjects.
Acting Research Leader on multiple occasions in absence of Dr. Hannan.
Wrote letter supporting tenure candidacy of assistant professor at a major university in southeastern United States
Organized and moderated a session of presentations on Neotyphodium diversity, International Neotyphodium/Grass Interactions Symposium, Soest, Germany.

Plans for 2001

Will complete last portion of a multi-year project to determine incidence of Neotyphodium endophytes in W6 grass accessions collected in North Africa in 1994. This work will focus on accessions of Lolium perenne accessions.

Will cooperate with W6 Allium curator in controlled pollination experiment involving caged accessions and fly pollinators. Will be responsible for the entomological aspects of this study.
Will expand upon research started in 2000 on tall fescue-endophyte-aphid interactions by conducting more glasshouse experiments to ascertain levels of bird cherry-oat aphid resistance and susceptibility in endophyte-infected accessions of tall fescue from Tunisia.

Will participate in service work by working with greenhouse managers and curators to refine decision making in use of pesticides to control insects and mites in W6 greenhouses devoted to seed increase activities.

RESEARCH AGRONOMIST
Richard C. Johnson

Activities

With funding provided under the Agricultural Genome Project in FY 2000, Dr. Ted Kisha was hired as Geneticist-Lab Manager to lead research to characterize genetic resources using molecular markers. Ted received degrees from Hiram College (Chemistry), Montana State (Agronomy) and Michigan State (Plant Breeding and Genetics). He has extensive experience in molecular markers associated with genetic diversity in germplasm.

Registration of mapping populations is now possible in CROP SCIENCE. R.C Johnson served on a special committee that outlined and published procedures in CROP SCIENCE.

R.C. Johnson compiled a summary of Crop Science Society of America, Division C-8 history. This will be posted at the C-8 web site.

Mandatory training on “Diversity in the workplace” and “Violence in the workplace was completed.

Johnson worked to help agency recruiting and visibility through Washington State University (2/15/00) and University of Idaho (2/16/00) career days (assisted Pam Dean, Location AO), and by developing a program for “Join your parents at work day”through ARS-Pullman (4/47/00).

As part of an effort to strengthen safflower utilization, a safflower web site was developed (http://safflower.wsu.edu/) (Funded by IPGRI for $2,000), and an information site at the FAO Ecoport System was also developed by Peter Griffée, FAO.

Johnson consulted with the International Plant Genetic Resources Institute (IPGRI) office in Beijing and Institute of Industrial Crops, Xinjiang Academy of Agricultural Sciences, Urumqi, PR China. As part of this trip 28 accessions of safflower were collected from China. This included eleven from the Xinjiang Province in extreme Northwest China from Professor Chen Yuehua, where the majority of the Safflower in China is grown. The additional 17 accessions were acquired from Professor Li Dajue and developed for improved cold resistance (7/17/00-7/29/00).
Johnson served as the lead source for a newspaper story on our Station written by Mike Lee of the Tri-City Herald. The story was also published in the Lewiston Morning Tribune (11/00).


Working with the grass curator, Vicki Bradley, improved standards for regeneration and maintenance of outcrossing grasses are being developed. This includes increasing plant populations from 60 to 120 plants, increasing isolation distance form 25 m to 50 m, and developing sampling methods to reduce genetic drift.

Through a cooperative program with José Fernández-Martínez, oilcrops geneticist and breeder of the Instituto de Agricultura Sostenible CSIC, Córdoba, Spain, numerous accessions of safflower relatives have been regenerated for the Pullman Station that otherwise would have been lost.

Dr. Dan Skinner, USDA-ARS, Kansas State University, Manhattan KS, visited Johnson and helped initiate work on the use of chloroplast primers for identifying species and distinguishing accessions (from 3/3 to 3/24, 2000).

Participated in the Workshop on Bioinformatics, Minneapolis MN (11/5/00)

Johnson was ask by the Grass Breeders Work Planning Conference to host the 2002 meeting at Pullman. As part of this he will hold the positions of Secretary, Vice-chair and Chair of the Grass Breeders Work Planning Conference for 2000, 2001, and 2002, respectively.

Suichang Sun, Simplot Turf and Horticulture, Post Falls Idaho, visited Johnson concerning the utilization of molecular and agronomic core collections of Kentucky bluegrass and molecular techniques for fingerprinting grasses (11/20/00)

Johnson wrote and received funding from the ARS-Plant Exploration Office for collections trips to Argentina. The proposal title is “EXPLORATION FOR COOL-SEASON GRASSES IN ARGENTINA.” Trips are scheduled for November 2001 and January 2002.

Research Activities

1. **Effective Population Size of Heterogenetic Grass Accessions.** Cooperative with Vickie Bradley.

   **Background.** Normally, in a population of plants, not all are effectively randomly mating, so the effective population size ($N_e$) is usually less than the census population ($N_c$)(Frankel et al., 1995). High variation in the number of progeny per plant in a population is a major factor leading to lower $N_e$ and a higher potential for random genetic drift. As outlined below, Heywood (1986) provided equations for estimating how much variation in progeny numbers per plant affects $N_e$. The proportional reduction in effective population size associated with variation in potential fecundity is:
1) \[ N_e/N_c = 1/ [(1 + F) (\sigma^2/\mu^2) + 1)], \]

where F is the fixation index, and \( \sigma \) and \( \mu \) the standard deviation and mean of family size respectively. Thus when each parent contributes equally to the gamete pool, \( \sigma^2 \) is zero and \( N_e = N_c \). Under these conditions genetic drift is equal to the traditional Wright-Fisher binomial sampling model.

When the variance and mean number of seeds sampled per plant is known then an estimate of \( \sigma^2/\mu^2 \) can be obtained as:

2) \[ \sigma^2/\mu^2 = s^2/z^2 - 1/z, \]

where \( s^2 \) is the variance of seeds per plant in a given population and \( z \) is the mean seeds per plant. Seed production of most plants sampled is usually high enough that the correction term, \( 1/z \), has little effect. Thus, \( N_e \) in a population approaches \( N_c \) as the seeds per plant variance approaches zero.

Using Heywood’s equations, we found that variation in seeds per plant reduced \( N_e/N_c \) to an average of 0.73 for three annual ryegrass accessions. Thus, in a population of 100 plants only 73 are on average randomly mating. But this information is needed on a broader set of accessions and species to gain insight into how sampling will affect genetic drift in our outcrossing accessions.

Objectives:

1. Determine the reduction in effective population size, \( N_e \), associated with variation in seed production per plant in three heterogenetic grass species.

2. Determine the utility of inflorescence sampling procedures for a wide range of species and accessions.

3. Compare cutting, rubbing, and inflorescence sampling methods in three grass species to determine effective population size differences.

Objective 1. Determine the reduction in effective population size, \( N_e \), associated with variation in seed production per plant in heterogenetic grass species.

Progress:

The effective population size (\( N_e \)) is the key parameter for predicting genetic drift associated with germplasm regeneration. The objective of this study was to estimate \( N_e/N_c \) resulting from variation in seed production in three model wind pollinated grass species, and to recommend cost effective sampling methodology to maximize \( N_e/N_c \) during seed regeneration.
In 1997, three accessions of three perennial grass species were established at two locations within current regeneration plots at the Western Regional Plant Introduction Station. The species were diploid accessions of perennial ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*), and bluebunch wheatgrass (*Pseudoroegneria spicata*). For each of the nine entries there were two replications of 30 plants each at both locations spaced 0.5 m apart and isolated by at least 50 m distance. In 1998, harvest of seeds from individual plants was completed. Estimates of $N_e$ were made as outlined by Heywood (1986). This requires data on the variance in seed production per plant within each population.

There were significant differences among species, and accessions within species, for mean seeds per whole plant, standard deviations, and $N_e/N_c$. The interactions with location, however, were not significant ($P>0.05$). For whole plant samples, the average $N_e/N_c$ values for each species averaged over locations differed with values of 0.42, 0.51, and 0.63 for *Lolium perenne*, *Festuca pratensis*, and *Pseudoroegneria spicata*, respectively. This means that on average only half the plants were effectively randomly mating. The $N_e/N_c$ for seeds per plant from inflorescence samples, consisting of two spikes or panicles per plant, was calculated for one accession of each species. The $N_e/N_c$ for inflorescence samples averaged 0.69, 0.88, and 0.86 for *L. perenne*, *F. pratensis*, and *P. spicata*, respectively. Thus, compared to whole plant sampling, the maternal contribution to variation in fecundity was reduced with inflorescence sampling. This resulted from the elimination of the variation of spikes or panicles per plant from the total seeds per whole plant variance. The results showed that variation in seeds per whole plant resulted in $N_e$ values that were on average only about half of $N_c$. A combination of increased plant populations during regeneration and harvesting a constant number of inflorescences per plant appeared to be a cost effective way to increase $N_e$ and reduce genetic drift in future regenerations.

This work has been included in the manuscript “Reductions in effective populations size during grass regeneration and improvements with sampling,” which has been accepted with revisions by CROP SCIENCE.

**Objective 2**: Determine the utility of inflorescence sampling procedures for a wide range of species and accessions.

The above work on effective population size shows the need for more plants per regeneration and the potential utility of inflorescence sampling as a cost effective way to minimize genetic drift in regeneration samples. Inflorescence sampling appears to be a way to control the variance in fecundity associated with maternal gamete production. Additional information is needed on the extent to which inflorescence sampling will help maintain higher $N_e$ in a wide range of species and accessions.

**Progress**: In 2000, inflorescence samples were taken from regeneration plots on 6 grass species with 4 accessions per species at Pullman and at Central Ferry. Two inflorescence samples were taken per plant and are now being cleaned to determine seeds per inflorescence and associated variance. From this, $N_e/N_c$ for inflorescence sampling will be calculated. This will be repeated in 2001. Information concerning how many inflorescences to take per plant and species, and the
extent to which this will improve effective population size in a wide range of species and accessions, will be determined.

**Objective 3**: Compare cutting, rubbing, and inflorescence sampling methods in three grass species to determine effective population size differences.

There has never been a comparison of how different harvest methods effect effective population size in grass accessions. This will be examined in field plots at Central Ferry and Pullman with accessions on *Lolium perenne*, *Festuca pratensis*, and *Pseudoroegneria spicata*.

Cut method: Each plant will be cut with a sickle and placed in a separate bag when the maximum number of seeds are mature.

Rub method: Each plant will be rubbed one or more times as needed and seeds from each plant kept separate.

Inflorescence method: Inflorescences from each plant will be harvested and kept separate. At least two will be obtained from each plant.

There will be four replications at each location. Each plot will consist of 30 plants which will be divided into three treatments of 10 plants each. The experimental design is a split block with species as main plots, and harvest method as the subplots. Calculation of \( \frac{N_s}{N_i} \) for each method will be made. The experiment will be completed at Central Ferry and Pullman research sites and analyzed over locations.

**Progress**: Plots for this study were established in 2000 and sampling will be completed in the summer of 2001. Samples will be processed that winter and treatment comparisons completed.

2. **Evaluation of Safflower Core Collection for Agronomic Descriptors**.

In 1993 a safflower core collection representing about 10% of the total accessions was developed from the entire collection of more than 2,000 accessions based on country of origin and morphological data. However, the core has not been characterized for many basic crop descriptors.

**Objective**: Evaluate the diversity of USDA safflower core collection for numerous agronomic descriptors and determine if regional differences in agronomic factors could be distinguished.

**Progress**:

The USDA safflower core collection, consisting of 207 accessions, was grown at the Central Ferry Research Farm. On 8 April 1996, accessions were planted in randomized complete blocks with two replications. Seeds were drilled approximately 2 cm deep and planted about 5 cm apart
in rows 3-m long and on 1.5 m centers. Prior to anthesis, screen mesh was placed over the plants to prevent insect cross-pollination. Irrigation from sprinklers was applied as needed.

Twenty-eight descriptors were evaluated for both replications. The descriptors were those outlined by the International Plant Genetic Resources Institute (IPGRI) (Safflower descriptors, 1983). Of these 28 descriptors, seven quantitative factors were evaluated. These were outer involucral bract (OIB) width, OIB length, primary head diameter, date of 50% flowering, plant height, weight per seed, and yield per plant. For these quantitative factors, analyses of variance was conducted for each factor using SAS general linear models (GLM) with treatment differences declared at P<0.05. In addition, the accessions were divided into ten geographic regions based on their country of origin and analyses of variance conducted for each factor evaluated to determine differences among regions. The regions were: the Americas (17 accessions), Australia (6), China (12), East Africa (Kenya, Ethiopia, and Sudan)(14), Europe (excluding countries bordering the Mediterranean Sea) (30), Japan (3), the Mediterranean (countries bordering the Mediterranean Sea) (61), South-Central Asia (Bangladesh, India, and Pakistan) (28), South-West Asia (Afghanistan, Iran, Iraq, Kazakhstan, Kuwait, Tajikistan, and Uzbekistan) (34), and Thailand (2). In addition to univariate analyses, data for regions were standardized to have a mean of zero and a variance of one, and multivariate analysis was completed using canonical discriminant functions (CANDISC, SAS Institute).

Accessions were highly variable for all factors measured indicating considerable diversity within the core collection. Correlation analysis showed the strongest associations were between plant height and flowering date r = 0.62), and between OIB width and OIB length r = 0.54) (P<0.05). To test regional differences, accessions in the core collection were partitioned into the ten major regions described above. Significant differences were found among regions for all factors except OIB length and yield per plant. Canonical discriminant functions showed that the first and second functions explained 83% of the total variation. Among the regions, accessions from SW Asia and S. Central Asia were the most distant from other regions. The results showed that the core collection was a highly diverse germplasm source, and that agronomic attributes could distinguish regional variation among accessions.

This work has been summarized in a manuscript “Evaluation of the USDA Core Safflower Collection,” and submitted to the Proceedings of the Fifth International Safflower Conference.

3. Evaluation of diverse Kentucky Bluegrass (*Poa pratensis* L.) germplasm for seed production in alternative residue management systems (Cooperative with Bill Johnston, WSU turf program)

**Background.** This project “Evaluation of diverse Kentucky Bluegrass germplasm for seed production in alternative residue management systems” has been funded through a USDA Special Grant program for Grass Seed Cropping Systems For A Sustainable Agriculture. Since 1994 we have received a total of $112,473 for this project. We also received $4,300 from the Forage and Turf CGC for turf evaluation of the core subset and promising turf types identified in our collection. The goal is to use molecular and agronomic evaluations to characterize the
USDA Kentucky Bluegrass collection and identify accessions and/or characteristics that give good turf quality and improved seed production under no-burn residue management systems. The first step was to set up replicated evaluation plots representing the Kentucky bluegrass collection as a whole represented by 228 accessions. Both agronomic and molecular characterization was completed using these plots. Then a core collection and selections showing agronomic potential were grown in larger plots under different residue management systems to determine the genetic potential for maintaining high seed production on non-thermal management systems.

Objectives.

1. Characterize the USDA *P. pratensis* collection and compare diversity based on RAPDs with diversity based on agronomic evaluations. The extent of duplication, and associations between germplasm origins and diversity patterns in the collection, will also be assessed along with recommendations for germplasm acquisition.

2. Using morphological and RAPD data, test methods of core collection development in Kentucky bluegrass.

3. Determine seed production potential of diverse Kentucky bluegrass germplasm in burn and alternative residue management systems while evaluating for turf quality.

Progress:

**Objective 1.** Using morphological and RAPD data, characterize the USDA *P. pratensis* collection and compare diversity based on RAPDs with diversity based on agronomic evaluations.

Characterization of germplasm collections is critical to assess collection diversity and enhance utilization. A *Poa pratensis* L. germplasm collection of 228 accessions representing 26 countries, along with 17 commercial check cultivars, was characterized using 86 random amplified polymorphic DNA (RAPD) markers and 17 agronomic descriptors. The Dice similarity coefficient used for RAPD data ranged from 0.56 to 0.95 and average Euclidean distance used for agronomic data ranged from 0.28 to 2.52. No two accessions had a similarity of one or a distance of zero, showing there were no duplicate entries. Cluster analysis of RAPD data with the unweighted pair-group method using arithmetic averages (UPGMA) revealed 11 accessions with particularly low similarity values. These were subsequently found to be misidentified *Poa* species (one each of *P. aplina*, *P. compressa* *P. glauca*, *P. urssulensis*, and seven *P. trivialis*). For RAPD data, 62% of the entries were in one large cluster with 46 additional clusters containing one to 13 accessions. For agronomic data, 89% of the entries were in four main clusters. This clustering pattern for RAPD and agronomic data suggested unique genotypes were generally under represented in the collection. The agronomic-based clusters showed some broad separation by accession origin, but in general, origin did not correspond closely with the clustering pattern. The correlation between the RAPD and agronomic-based
distance matrices, excluding misidentified accessions, was highly significant (P<0.01) (n=234, r = -0.14). However, the correlation represented a relatively small fraction of the total variation, indicating that both molecular and agronomic characterizations were needed to assess overall diversity.

This work has been completed, written-up, peer reviewed, and submitted for publication in the journal GENETIC RESOURCES AND CROP EVOLUTION. The title of the paper is “Characterization of the USDA Poa pratensis Collection Using RAPD Markers and Agronomic Descriptors.”

Objective 2. Use agronomic and RAPD data to study core subset development methods in Kentucky bluegrass.

Core collections offer a way to improve access to germplasm collections by providing a highly diverse, representative subsample of the total collection. The future of the Poa pratensis L. (Kentucky bluegrass) seed production industry in the U.S. depends on the development of new germplasm with both high turf quality and seed production potential. Evaluation of the entire USDA-ARS collection for these attributes would, however, be prohibitively expensive and impractical. Our objectives were to (1) utilize a core collection to identify germplasm with high turf and seed production, and (2) compare several methods of developing core collections using agronomic and molecular data. From 228 total accessions, cores representing 10% of the collection were developed using random sampling, hierarchal cluster analysis (Ward’s or UPGMA), and stratification by broad geographic regions using both agronomic and RAPD data. Cores developed from cluster analysis provided more uniform sampling of accessions from the total collection resulting in cores with increased variances and ranges of agronomic attributes compared to cores developed without cluster analysis. Stratification over broad geographic areas without clustering produced cores that were similar to a core selected at random. In field evaluations, a core developed using Ward’s clustering of agronomic data showed a wide range of variability in turf quality and seed production, and several accessions were identified with values of turf quality and seed production greater than the mean values of the check cultivars. Cluster analysis appeared to be essential for producing the most highly diverse core collections in Poa pratensis.

This work has been published in the book “Core Collections for Today and Tomorrow” published by the International Plant Genetic Resources Institute (IPGRI) under the title Core Utilization and Development: An Example with Poa pratensis L”.

Objective 3. Determine seed production potential of diverse germplasm in burn and alternative residue management systems while evaluating for turf quality.

From the initial evaluations and core development described above, a set of material for more in-depth evaluation of seed production and turf potential were selected. This included the core subset developed from morphological data plus accessions that showed a combination of high turf potential and high seed yield in previous tests. The resulting 38 accessions derived from the
collection of over 200 have been evaluated under different residue management systems. Nine commercial cultivars from the National Turfgrass Evaluation Program (NTEP) were included among the entries to represent the current genetic base and provide checks. The seed production plots were established in the fall of 1996 and each plot consisted of five, 1.8 m rows spaced 0.18 m apart, and there was 0.6 m of border area between each plot. The first year seed production data was in 1997 and thus there was no residue that year. After harvest in 1997, three residue treatments were established: open-field burning, mechanical residue removal (bale), and no residue removal. The experiment was randomized in complete blocks with a strip-plot arrangement with three replications. The main plots are the residue treatments, arranged in strips, and the subplots are the 38 accessions.

Of particular interest will be the accession by residue environment interaction. If significant, it will indicate that individual accessions differed in their response to residue environment. The next question will be why did some accessions yield more under residue than others. In order to examine this question, we will monitor crop development, yield and yield components and correlate these factors with yield in the different residue treatments.

Turf plots using the same entries as the seed production plots were established in the spring of 1997 in 1 m² plots randomized in complete blocks with three replications. On these plots, turf quality factors are being evaluated (leaf texture, leaf color, spring green-up, and overall quality assessment).

**Progress:** The data from 1997,1998, 1999 has been collected and analyzed. A wide range of variation in turf quality and seed yield was found within the USDA Kentucky bluegrass germplasm collection. Entries varied in their ability to yield under different residue management systems. Some accessions yielded almost as well under the “bale” treatment as under burning and may be useful for developing improved cultivars for “no-burn” management systems. As open-field burning becomes more restricted, the greater genetic potential of some genotypes to yield under non-thermal residue management, such as baling, needs to be exploited. The plan is to write-up aspects of this work in 2001 for peer review and submission for publication.

**4. Genotyping germplasm collections for duplication and diversity analysis.** Cooperative with Ted Kisha and Stephanie Greene.

**Background:** Use of molecular markers will have a large impact on germplasm management. Molecular markers can be used to identify duplicates, develop and test special groups of collections (such as core collections), estimate and compare diversity among countries or regions, and identify acquisition needs. Within the general area molecular marker use for germplasm management, there are three areas of investigation planned. First, there will be a study to develop the techniques needed for sampling and tests for duplication; second, testing and validation of core collections; and third, if possible, genotyping entire collections. Initially this work will be completed in a heterogenetic species (alfalfa). Normally molecular variation within heterogenetic species is high making distinguishing accessions more difficult. But since so many of our species are heterogenetic, effective genotyping methods to efficiently distinguish
accessions are critically needed. With such methods, duplication can be assessed, genetic variation between geographic regions compared, core collections developed and refined, and gaps in collections determined.

**Objectives:**

1. Determine methods for genotyping accessions in heterogenetic species (alfalfa).

2. Testing diversity of core collections.

3. Characterize the diversity of entire collections of heterogenetic species such as alfalfa using molecular markers.

Of the above objectives, we are currently working on objective 1, which needs to be completed before we can proceed to the other objectives.

**Objective 1.** Determine methods for genotyping accessions in heterogenetic species (alfalfa)

Often in heterogenetic accessions from outcrossing plant species, such as alfalfa, the variance within a population tends to be high relative to the variance among accessions so that separation between accessions is difficult. In addition, if accession genotyping is to be possible on a large scale, it will probably need to be done on bulked leaf tissue rather than populations of individual plants. In other words, separate extractions of perhaps 24 individual plants per accession times perhaps 4,500 accessions as in the case of alfalfa would be 108,000 extractions. Even if this was possible, scoring and data management for up to 100 makers, times 108,000 DNA extractions, quickly becomes unrealistic. Thus we need to develop methods for genotyping heterogenetic accessions from bulk tissue and applying data analysis systems to distinguish accessions.

Stephanie Greene has picked out a set of alfalfa accessions that will allow us to complete the first objective. We are working with three alfalfa cultivars (Aragon, Hunter River, and Yonca) each with a set of at least three different PI numbers or duplicates. In one case for each cultivar we will also be comparing original and increased seed.

There will be different sampling methods and marker systems used to determine the most effective sampling/marker system combination for genotyping alfalfa. This will be done on the three increased seed populations for each cultivar to work out the best procedures and then applied to the sets of duplicate accessions to determine their genetic distance. The basic population number per accessions will be 48 plants. There will be extractions of individual plants in each population plus bulk leaf extraction of 6, 12, 24, plants. In addition there will be bulks of DNA made from individual plant extractions from 6, 12, 24, and 48 plants. There will also be different marker systems applied to the different sampling methods. The marker systems will be
1. Chloroplast primers. There are two primer pairs developed from the alfalfa chloroplast genome by Dan Skinner (ARS, Kansas State) that show considerable variation in alfalfa populations. When Dan visited last spring we worked with these and they may be useful to distinguish accessions and duplications.

2. AFLP (Amplified fragment length polymorphisms). This marker system that is now being widely used for genotyping work. It has the advantage of being highly reproducible and usually provides numerous markers for each primer pair. The disadvantage is the markers are generally dominant and therefore calculation of factors related to heterozygosity are not possible. But they will provide good genetic distance information. Since these are random markers they do not require the development inputs needed for some other marker types.

3. SSR’s (Simple sequence repeats). These have the reliability and high number of markers per primer pair that AFLP’s provide, and they have the advantage of being co-dominant. In some ways these may be the best marker type for germplasm work to date. They require significant development inputs beyond what we plan to do, so we are hoping to use primers already developed by others in the alfalfa research community.

4. RAPD’s (Random amplified polymorphic DNA). These are relatively easy and inexpensive but are not as reproducible as the other markers listed above. We used these successfully for work with the Kentucky bluegrass collection, but they require a lot of care in data scoring and in replication of samples to ensure reproducibility.

Genetic distances will be calculated for each sampling and marker system using a distance (or similarity) coefficient. Statistical procedures will be developed to use in comparing samples. Comparisons will be made among accessions and sampling method to determine if statistical separation is possible, and if so, at what P value.

Progress:

All populations are planted and growing in the greenhouse. DNA has been extracted from the increase populations of each cultivar. We have completed the initial analysis on the three increase populations using chloroplast primers. There appears to be a difference between ‘Yonca’ and the other cultivars based on preliminary information. We have started the procedures needed to complete the AFLP analysis (DNA digestion and ligation), and this, along with completing the chloroplast primer work, will be the emphasis for now.

Talks and Presentations

Jul. 26  “Safflower genetic resources” (Invited, expenses paid), Institute of Industrial Crops, Xinjiang Academy of Agricultural Sciences, Urumqi, PR China, (Oral).

Nov. 2  “Characterizing the USDA bluegrass collection with RAPD and chloroplast primers” (Invited, expenses paid), workshop speaker at “PCR-based Approaches to Identify and Quantitate Botanicals in Dietary Supplements.” Invited by Dr. Michael McClure, National Institute of Health, (Oral).

Nov. 7  “Characterization of the USDA *Poa pratensis* collection using RAPD markers,” Crop Science Society of America, Minneapolis MN, (Poster).

**Travel, Invitations, Special Awards and/or Assignments**

Jan. 8-13  Traveled to San Diego, California, to present a paper at the Annual Meeting of the Plant and Animal Genome VIII Conference.

May 21- Jun. 8  Traveled to Ardmore, Oklahoma, to attend the Grass Breeders Conference.

Jul. 17-29  Traveled to Beijing, China, as an invited guest of the Chinese Institute of Industrial Crops to share information on safflower.

Nov. 1-3  Traveled to Triangle, North Carolina, as an invited guest to present a paper at the NIH Workshop.

Nov. 4-9  Traveled to Minneapolis, Minnesota, to attend the ASA-CSSA-SSSA annual meeting.

**Committees, Training, Other Assignments, Activities and News**

Ex-officio member, Alfalfa Crop Germplasm Committee.

Ex-officio member, Forage and Turf Grass Crop Germplasm Committee.

Chair, C852.12 Crop Registration Subcommittee for Sunflower, Safflower, Rapeseed, and other misc. oilseeds, 1995-2000. Responsible for reviews, revisions, and decisions on publications of approximately 12 registration manuscripts per year.

Member, C111.08 nominations committee for CSSA Division Plant Genetic Resources (1998-2000)

Appointed member of Ad-hoc committee for 1999-2000 on “Registration of mapping populations in Crop Science,” by CSSA Committee C852, Crop Registration. A document outlining procedures was developed and approved the CSSA Board. Guidelines have been published in CROP SCIENCE.

Member of Graduate Faculty, Washington State University.

Appointed to the Organizing Committee, Vth International Safflower Conference, to be held at Williston, North Dakota, June 2001.

Member of the Technical Advisory Committee for the Grass Seed Cropping Systems for a sustainable Agriculture Special Grant Program.

Cooperator with Bill Johnston and Jerry Sitton on project entitled "Disease control in bluegrass cropping systems without open-filed burning." Funded for 1999 at $26,786.


Served on Graduate Committees for Grant Poole and Pamela Scheinost, Department of Crop and
Soil Sciences, Washington State University
Peer Reviews for journals (7) for ARS (4).
Chair, committee to recommend changes in the Note category of Publication for CROP
   SCIENe to include germplasm evaluations and other germplasm work currently difficult to
   publish.

Plans for 2001

Continue research on:
a. Determining the reduction in effective population size, N_e, associated with variation in
   seed production per plant in heterogenetic grass species and develop improved sampling
   techniques.
b. Seed production potential of diverse germplasm in burn and alternative residue
   management systems while evaluating for turf quality.
c. The use molecular markers to genotype accessions, identify duplicates, and characterize
   germplasm collections, initially in alfalfa.

Obtain acceptance for publication of the following manuscripts:
a. “Reductions in effective populations size during grass regeneration”
b. “Evaluation of the USDA Core Safflower Collection,”
c. “Characterization of the USDA Poa pratensis Collection Using RAPD Markers and
   Agronomic Descriptors,”

Write and have approved for submitting for publication the manuscripts:
a. “Evaluation of diverse germplasm for seed production under contrasting residue
   management.”
b. “Comparison of agronomic and biochemical traits in balanced and bulked populations of
   annual ryegrass population after three regeneration cycles.”

Other goals:
a. Continue supervision of agronomy research program and genetics lab. The goal is to
   develop a genetics lab to serve as a station resource for germplasm characterization.
b. Work with curators as requested to advise on program needs, regeneration procedures and
   evaluation.
c. Collect forage germplasm in Argentina as part of a proposal funded through the ARS
   Plant Exploration Office. Continue working toward germplasm exchange and evaluation
   of South American grasses with G. Becker in Patagonia, Argentina
d. Continue as Chair, International Safflower Germplasm Committee (1997-2001). Organize
   meeting; review crop descriptors, safflower web page, current research priorities;
   recommend needs concerning germplasm conservation, collection, distribution,
   utilization and storage; identify training needs for international safflower workers and
   how can they be funded
e. Participate in the Vth International Safflower Conference, Williston ND, present paper.
f. Organize entry of evaluation data for safflower not currently in GRIN.
g. Continue to serve as Ex-officio member: Alfalfa CGC and Forage and Turf Grass CGC.
h. Participant in the Crop Science Society of America National Meetings, present paper.
i. Continue cooperative project to regenerate accessions of safflower relatives with José Fernández-Martínez, oilcrops geneticist and breeder of the Instituto de Agricultura Sostenible CSIC, Córdoba, Spain.

**RESEARCH PLANT PATHOLOGIST**

Frank M. Dugan

**Activities**

The pathology lab isolated *Sclerotinia* from sclerotia on PI 380712 *Astragalus ovinus*. It seems to be *S. sclerotiorum* because the sclerotia occur in a ring around the margin of the plate, and are greater than 2 mm in length. Technician Shari Lupien was given task of producing apothecia for confirmation of id. for Barbara Hellier.

The lab undertook isolation of fungi & bacteria from lupine accessions grown in Parlier for Clare Coyne and Maria Jenderek. The only consistently isolated microorganism was a *Pythium*, which proved non-pathogenic when inoculated to the host. The damage was likely from misapplication of herbicide.

The pathologist provided consultations with Vicki Bradley and Wayne Olson on identification and management of *Puccinia* species causing rust on grass accessions. Advice: non-systemic fungicide if *Neotyphodium* endophytes are to be preserved. Otherwise, systemics per PNW Plant Disease Management Handbook.

The pathologist detected ergot sclerotia in PI 578671 *Dactylis glomerata*, and PI 611150 *Pseudoroegneria spicata* and relayed the information to Dave Stout.

The lab isolated *Fusarium* from roots of beans for Molly Welsh; the problem was over-watering.

The pathologist provided documentation on host and geographic range of *Puccinia carthami* and *Fusarium oxysporum* f.sp. *carthami* in Washington State, for Vicki Bradley.

The pathologist provided host-pathogen indices for *Papaver* spp. to Barbara Hellier.

The pathologist and technician demonstrated for Bill Luna techniques for monitoring pasteurized soil for microorganisms.

The pathologist assisted local growers/field staff in disease diagnosis.
Provision/curation of microbial strains:

We accessioned strains chickpea pathogens from Walt Kaiser (in progress).

We distributed *Fusarium oxysporum* f.sp. *ciceris* strains to Fred Muelhbauer.

We distributed strains of *Ascochyta rabei* to Tobin Peever.

Visitors:

ARS National Program Leader Evert Byington (Aug. 21); Crops and Soils Tour of WRPIS for Prof. David Bezdichek and faculty (Aug. 22); Dr. Jim Fortune of Grains Research and Development Corporation, Kingston, Australia (Nov. 27).

Research Activities

1. Fungi quiescent in grasses and grass seeds

Rationale: An abundant literature exists on the fungal flora of stored cereals, but information of corresponding depth is lacking with regard to stored seed of important forage and weedy grasses. This is especially true regarding those fungi which may reside quiescently or endophytically in such materials. The Western Regional Plant Introduction Station (WRPIS) conserves germ plasm of 16,350 accessions (over 800 species) of grasses. Because of the increasing attention paid to quiescent and endophytic infections of plants, and because fungi may be distributed along with the seeds of the host, we have undertaken a floristic survey of such fungi in grass seeds. We have also examined vegetative tissues of the same hosts. We focus on important forage and weedy grasses endemic (but not necessarily native) to the Pacific Northwest. In all samples, we screened to eliminate materials bearing external signs of infection.

Summary: For each of two growing seasons (1999, 2000) we have plated 100 seeds and 50 culm nodes onto agar for each 15 species (*Aegilops cylindrica, Agropyron cristatum, Agropyron repens (Elytrigia repens), Agropyron spicatum (Pseudoroegneria spicata), Arrhenatherum elatius, Avena fatua, Bromus inermis, Bromus tectorum, Elymus cinereus (Lymus cinereus), Festuca idahoensis, Dactylis glomerata, Melica subulata, Phalaris arundinacea, Phleum pratense, and "feral" Triticum aestivum). For all taxa but *B. inermis, Melica* and *Triticum* we have subjected 100 seeds of PI accessions to the same procedures. Approximately 700 isolates have been recovered for the entire project. Most commonly isolated is *Alternaria*, a genus in strong need of taxonomic revision. Other frequently isolated genera included *Cladosporium* spp. (agents of sooty molds) and *Selenophoma* (*Pseudoseptoria*) spp. (agents of halo spot). Significantly, *Fusarium, Aspergillus* and *Penicillium* (the classic mycotoxin producers) have been completely absent to rare in isolations from non-symptomatic seeds. Miscellaneous taxa of ascomycetes, hyphomycetes and coelomycetes have been isolated but identification is not complete.
2. Production of keys and/or descriptions for important fungal taxa recovered from non-symptomatic grass nodes and seeds:

Rationale (*Alternaria*): In our studies, *Alternaria* was the most commonly isolated fungus from seeds and culm nodes of grasses. Other than *Fusarium*, *Alternaria* is the genus with the most entries in standard texts of seed pathology (Agarwal and Sinclair, 1997, Principals of Seed Pathology, 2nd ed., CRC; Maude, 1996, Seedborne Diseases and their Control, CAB). *Alternaria* species occur endophytically in other plants with effects on vertebrate herbivores (Braun and Liddell, 1998, Phytopathology 88:S10) and are known producers of mycotoxins in Gramineae and other plants (Chelkowski and Grabarkiewicz-Szczesna, 1991, *Alternaria* and their metabolites in cereal grain, in: Cereal Grain Mycotoxins, Fungi and Quality in Drying and Storage, Elsevier; Chelkowski and Visconti (eds), 1992, *Alternaria* Biology, Plant Diseases and Metabolites, Elsevier). *Alternaria* species are agents of black point, black head & other diseases of graminicolous plants. Available taxonomic treatments of the genus *Alternaria* are inadequate for identification and keying of graminicolous species of *Alternaria*. (The limitations of each of the previously published keys and sets of descriptions are specifically addressed in my progress report for the previous year.)

Summary (*Alternaria*): Using type, authentic and other well-documented materials, we have now produced photographs and a morphotaxonomic key to the following graminicolous species: *Alternaria alternata, A. cetera, A. infectoria, A. longissima, A. metachromatica, A. oregonensis, A. padwickii, A. saparva, A. tenuissima, A. triticicola, A. triticimaculans and A. triticina*. In the later part of year 2000 and in 2001, we will collaborate with Tobin Peever (Department of Plant Pathology, WSU) on molecular analysis of these same species.

Rationale (*Selenophoma*): Other than the agents of black point (*Alternaria and Cladosporium*), *Selenophoma* (Pseudoseptoria) was the most commonly isolated pathogen in our studies of fungi quiescent in grass tissues. *Selenophoma* (Pseudoseptoria) spp. are widely distributed, seed-borne pathogens causing halo spot on grasses and cereals. Damage is usually described as minor, but instances of severe damage are periodically recorded, especially from areas with cool, moist climates (Allison, Phytopathology 35:233-240, 1944; Cooke and Brokenshire, Trans. Br. myc. Soc. 64:153-156, 1975; Holton and Purdy, Pl. Dis. Rept. 46:728, 1962; Pluck et al., Trans. Br. myc. Soc. 77:509-518, 1981; Polley et al., Ann. appl. Biol. 123:287-307, 1993). Varietal resistance and fungicides have been utilized for experimental control (Griffin and Lessiter, Tests of Agrochem. & Cult. 10:30-31, 1989; Jenkins et al., Plant Pathol. 21:49-58, 1972; Pluck et al. ibid; Slopek and Labun, Can. Pl. Dis. Survey 72:5-7, 1992). Published sources for identification (Punithalingam and Waller, CMI Descriptions No. 400, 1973; Smiley et al., Compendium of Turf Grass Diseases, 1992; Sprague, Diseases of Grasses and Cereals, 1950; Sprague and Johnson, Species of Selenophoma on North American Grasses, 1950; Sutton, The Coelomycetes, 1980) describe pycnidia and pycnidiospores produced on host plants. However, strains isolated into pure culture, including strains from non-symptomatic host tissues, often fail to produce diagnostic characters matching published descriptions. Brokenshire and Cooke (1975) noted that cultures on agar media often produce conidia without the production of pycnidia, but cultural characters have not been adequately described. The coelomycetous state was absent or
inconsistently produced in a high proportion of strains. This study has the objective of describing and illustrating diagnostic, morphological characters of the hyphomycetous and yeast-like states which often dominate in agar cultures. Results are discussed with regard to the segregation of graminicolous Selenophoma spp. into Pseudoseptoria by Sutton (1980).

**Summary (Selenophoma):** Strains of *Selenophoma (Pseudoseptoria)* spp. were isolated from asymptomatic, surface-disinfested seeds and/or culms of *Arrhenatherium*, *Bromus*, *Dactylis*, *Phalaris*, and *Pseudoroegneria* species. Strains were grown on malt extract or half strength V8 agar and examined at 40-1000X with differential interference contrast. Cultures produced hyphomycetous synanamorphs commonly exhibiting synchronous conidia production typical for members of the *Aureobasidium-Kabatiella* complex. Elongated, micronematous conidiophores bore intercalary and terminal cells with 1-5 conidiogenous loci. Some isolates also produced conidiogenous cells in palisades on a stroma, and a minority of strains readily produced pycnidia similar to those described from the host. Yeast-like polar budding of 1-celled conidia, secondary conidia formation from multiple loci on septate conidia, and catenate conidia were common in most strains. Several strains produced two distinctive conidial morphologies: typical selenophomoid and irregularly vermiform. No annellations could be discerned on conidiogenous loci. Descriptions and illustration are provided for identification of *Selenophoma* in culture.  

**Manuscript sent for RMIS pre-submission review (Aug '00); submitted to Can. J. Phytopathol. (Sept. '00): F.M. Dugan. Cultural characters of coelomycetous, hyphomycetous, and yeast-like states of *Selenophoma* strains from Poaceae.**

3. **Location of *Neotyphodium* in tissues of wild barley:**

Rationale: Wild *Hordeum* species have been shown to contain *Neotyphodium* endophytes with effects on plant-insect interactions (Clement et. al. 1997, Entomologia Experimentalis et Applicata 82:275-282). The precise location of such endophytes within host tissues is therefore of interest. Summary: Histological techniques enabled visualization of fungal hyphae of *Neotyphodium* sp. within coleoptile leaf tissues of seedlings of wild barley, *Hordeum brevisubulatum* subsp. *violaceum*. Hyphae were predominantly intercellular and commonly distributed at low density within mesophyll tissues; hyphae were also immediately adjacent to or just inside sheath cells of the vascular bundle, or in contact with the inner walls of epidermal cells. Intracellular hyphae were extremely rare; extensive colonization of vascular tissue was lacking. With N. Youssef (University of Idaho).

4. **Fungi endophytic in asymptomatic grape berries and dormant buds**

**Rationale:** Endophytic and/or quiescent fungi have been demonstrated in several horticultural crops, including grapes, in which *Botrytis* is of special interest (Coley-Smith, Verhoeff and Jarvis 1980, The Biology of *Botrytis*; Nair et al. 1995, Australian J. Exp. Agri. 35:1177). *Botrytis* is a primary target of standard management practices in central Washington and the Pacific Northwest (Pscheidt, 1997, An on-line guide to plant disease control,
www.orst.edu/dept/botany/epp/guide; Grove, 1998, Bunch rot of grape, http://disease.tfrec.wsu.edu). Our objective is to ascertain the degree to which Botrytis or other fungi might persist as quiescent infections in non-symptomatic grape tissues in central Washington. Taxa recovered should be tested with Koch's postulates to see which are pathogenic to ripe fruit.

**Summary as of Aug 2000:** We have detected presence of *Botrytis cinerea* at low frequency in dormant buds (approx 1.5%) and in young grape berries (approx. 0.05%). *Alternaria, Cladosporium, Ulocladium* and *Penicillium* appear to predominate; *Aureobasidium*-like strains and lesser numbers of ascomycetes and coelomycetes were recovered. Note: Pre-existing fungicide trials, planned and executed by WSU's G.G. Grove near Prosser, WA., presented the possibility of cooperative research; no labor, monies or materials are expended by WRPIS for establishing plots or conduct of the trials, but sampling is available to W6 in the control replications for recovery of endophytic fungi. This effort is projected at approximately one quarter of the effort devoted to the floristic survey of grasses.

**5. Identification of fungus responsible for production of a bioactive compound**

**Rationale and summary:** I have for several years identified fungi in exchange for authorship for persons engaged in characterization of natural products originating from fungi. In this instance, Horace Cutler of Mercer University (and formerly of USDA-ARS, Athens, GA) contacted me regarding identification of a fungus producing a compound with bioactivity (inhibitory of growth of wheat coleoptiles) and possessing a novel spiroditerpenoid skeleton. The fungus was identified as *Penicillium brevicompactum*.

**Talks and Presentations**

Poster: Synanamorphs of *Selenophoma* species in culture. Frank M. Dugan. Annual Meeting of the Mycological Society of America, July 30-Aug 3, Burlington VT.

Poster: Fungi quiescent in field and storage samples of grass seeds. Frank M. Dugan and Shari L. Lupien. Annual Meeting of the American Phytopathological Society, Aug 12-16, New Orleans LA.

**Travel, Invitations, Special Awards and/or Assignments**

Feb. 14-17 Traveled to Albany, California, to attend the Pacific West Area new scientist orientation.

Mar. 1-3 Traveled to Beltsville, Maryland to attend APHIS Safeguarding initiative meetings, at request of Dr. M. Palm, leader Science and Technology Committee..

Mar. 26-31 Traveled to Washington DC to attend APHIS Safeguarding initiative meetings, at request of Dr. M. Palm, leader Science and Technology Committee.

Jul. 29- Traveled to Burlington, Vermont to attend the Mycological Society of America Annual Meeting and present a poster.
Aug. 4-17 Traveled to New Orleans, Louisiana to attend the American Phytopathological Society meeting and present a poster.

Committees, Training, Other Assignments, Activities, and News

Bibliography of Kaiser/Hannan Reprint Library. Citations are made available on searchable diskettes or hard copy which have so far been distributed to interested parties in ARS, WSU and internationally. The bibliography covers citations for pathology and resistance in beans, chickpeas, lentils and pea up until approximately 1996. The library has about 3,000 citations, each corresponding to a reprint. Reprints can be readily accessed, copied, distributed and re-filed.

Chair, Mycology Committee of the American Phytopathological Society.

Member-at-Large, Executive Board, U.S. Federation for Culture Collections (rotated off in 2000).
Member, Science and Technology Committee, APHIS implementation working group for National Plant Board Review: Safeguarding American Plant Resources.

President: Palouse Mycological Association (mushroom club - community-based activities), a member and affiliate of the North American Mycological Association.

Manuscripts reviewed:


States, J.S., and M. Christensen. Fungi associated with biological soil crusts in desert grasslands of Utah and Wyoming. For Mycologia (James Correll, ed.)

Pre-submission reviews for W6 and WSU Colleagues:

Clement, Stephen L. Overview of *Neotyphodium* incidence in seed bank collections and plants in managed and unmanaged habitats. For IOBC wprs Bulletin.

Coyne, C., M. Pilet and M. McClendon. Selected AFLP primer pairs for fine mapping in cultivated pea. For Pisum Genetics.

Coyne et al. Comparison of two methods to evaluate quantitative resistance to eastern filbert blight in European hazelnut.

Douhan, L. and D. Johnson. First report of *Colletotrichum coccodes* associated with Mentha. For Plant Disease Notes.


Kerrigan, J., M.T. Smith and J.D. Rogers. *Ascobotryozyma americana* gen. nov. et sp. nov., an unusual yeast from the surface of nematodes. For Antonie van Leeuwenhoek.

Rogers, J.D., and Y.-M. Ju. *Ascovirgaria occulta* gen. et. sp. nov., Jumillera hawaiensis sp. nov., and *Lopadostoma hawaiianum* sp. nov. from Hawaii. For Mycological Research.


**Plans for year 2001**

Produce and submit manuscript for above project Fungi quiescent in grasses and grass seeds.

Finish late winter and early spring samples for grape tissues; do pathogenicity tests; produce initial draft manuscript for Fungi endophytic in asymptomatic grape berries and dormant buds.

Refine existing morphotaxonomic key for graminicolous *Alternaria* species, and conduct molecular studies on these same species in cooperation with Tobin Peever. Submission is projected as two "back-to-back" morphotaxonomy/molecular analysis articles sometime later in year 2001.

**AGRONOMY CURATOR**
**Vicki L. Bradley**

**Activities**

The Agronomy regeneration program personnel are responsible for maintaining the grass and the *Carthamus* collections.

The *Carthamus* collection at the WRPIS consists of 2,401 accessions, 2,288 of which are cultivated safflower. Eighty accessions of safflower were planted for regeneration at Central Ferry, Washington in the spring. A drip irrigation system was installed in the nursery, and the
accessions were caged using a new caging technique that allows more room and better air circulation than did our old bagging system. Visually, the quality of the seed appeared to be high, however, germination tests indicated that there was mechanical damage from threshing. This problem will be addressed in 2001.

The temperate grass collection consists of 17,092 accessions and represents approximately one quarter of the WRPIS holdings. There are 97 genera and 967 taxa. We planted 550 grass accessions for increase in the 2000 nursery. We harvested approximately 600 grasses in the second year, 1999, nurseries as well as 200 first year grasses.

Changing the Grass Regeneration

With the help and cooperation of the farm managers and crew, we initiated several changes in the 2000 grass nurseries. Accessions were transplanted into the field using a mechanical transplanter. In the Central Ferry nursery, drip irrigation lines were laid under a layer of plastic mulch, and the plants were planted through the mulch. We hope these improvements will help lower both the incidence of fungal diseases associated with sprinkler irrigation and the need for hand removal of weeds and volunteer grass plants. The isolation distance between the 10 planting strips at Central Ferry was increased from 25 to 38 meters. The isolation distance between cross-pollinating accessions within each planting strip was increased from 25 meters to 50 meters. We also changed the labeling system in the nurseries. We used plastic stakes which hold cards made of vinyl siding. Label information was imported from the nursery data file into a label file and clear plastic labels were printed with a laser printer and placed on the cards. Prior to this, the information on each card was manually typed on a card printing machine. We also began placing small labels into each harvest bag. This helped to reduced recording errors due to hand-written bag labels.

In 2001 nurseries we will use a type of planting flat that is more compatible for use with the tranplanter than the flats used in the past. These flats also hold more plants, so we will increase the plant population of grass regeneration plots from 60 to 100. We will also increase the isolation distance in all nurseries to 50 meters in all directions. We feel that this level of isolation and increased plant population are a critical need, although the number of accessions regenerated per year will decrease.

Prior to these changes we regenerated about 625 accessions per year at 60 plants per accession. With the new protocol we will be able to regenerate only 525 of the 100-plant accessions. Nevertheless, the number of plants we will plant, maintain, and harvest will increase by approximately 15,000 per year. We will continue to explore ways to increase the number of accessions grown.

A major reason for reducing the number of accessions grown per year is lack of land at our Central Ferry research farm. In order to isolate properly and continue to maintain our present levels of crop rotation, fallowing, weed control and soil fertility we need more than the 60 acres currently utilized. We have estimated the cost of preparing an additional 40 acres at Central

-33-
Ferry for planting to be $133,500 (Table 1). In addition, another full-time position would be needed for proper maintenance of the farm, and we would need to hire more seasonal help for planting, maintaining and harvesting the additional plants. The work load could also increase for the seed-cleaning personnel. Harvest techniques to properly sample all plants, harvest adequate seed, and also keep the amount of plant material harvested to a minimum are being investigated. In most years we have regenerated enough accessions to keep up with the influx of new ones (Table 2). However, newly acquired accessions are only a part of the accessions needing regeneration. We estimate that it will take twelve years to successfully regenerate the grass accessions that currently need to be grown (approximately 6,000). This estimate was made for a static collection. A more realistic estimate for a working collection, taking into account decreased viability and/or depleted seed quantity in some accessions, as well as newly acquired accessions, is 40 years.

Table 1. Costs related to the new regeneration protocol.

<table>
<thead>
<tr>
<th>Item</th>
<th>Estimated one-time cost</th>
<th>Estimated recurring cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation main line(materials only)</td>
<td>$37,000</td>
<td></td>
</tr>
<tr>
<td>Road-rock</td>
<td>$10,000</td>
<td></td>
</tr>
<tr>
<td>Fencing</td>
<td>$10,000</td>
<td></td>
</tr>
<tr>
<td>Lathhouse at Central Ferry</td>
<td>$6,500</td>
<td></td>
</tr>
<tr>
<td>Bubblehouse &amp; Lathhouse construction at Pullman</td>
<td>$70,000</td>
<td></td>
</tr>
<tr>
<td>Bubblehouse maintenance per season</td>
<td>$2,000</td>
<td></td>
</tr>
<tr>
<td>Salary- 2 seasonal employees</td>
<td>$7,500</td>
<td></td>
</tr>
<tr>
<td>Salary &amp; benefits -1 full-time employee</td>
<td>$50,000</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>$133,500</td>
<td>$59,500</td>
</tr>
</tbody>
</table>

Table 2. Newly acquired accessions compared to accessions regenerated, 1995 - 1999.

<table>
<thead>
<tr>
<th>Calendar Year</th>
<th>New Accessions</th>
<th>Regenerated Accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>1,077*</td>
<td>640</td>
</tr>
<tr>
<td>1996</td>
<td>348</td>
<td>614</td>
</tr>
<tr>
<td>Year</td>
<td>Accessions of Phleum</td>
<td>Accessions of Arrhenatherum</td>
</tr>
<tr>
<td>------</td>
<td>----------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>1997</td>
<td>377</td>
<td>642</td>
</tr>
<tr>
<td>1998</td>
<td>339</td>
<td>650</td>
</tr>
<tr>
<td>1999</td>
<td>518</td>
<td>600</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2,659</td>
<td>3,146</td>
</tr>
</tbody>
</table>

*Includes 574 accessions of *Phleum* and 155 accessions *Arrhenatherum* transferred from NE9 in 1995.

**Research Activities**

In a cooperative effort with the Research Agronomist, we planted an experiment to continue the analysis of harvest methods in grass regeneration plots. We also planted 16 accessions of *Achnatherum splendens* in an initial evaluation of this species for use as a soil stabilizer as Vetivier grass is used in warm climates.

Sixty safflower accessions were planted in Pullman, WA, in late September to be evaluated for winter hardiness in the Palouse region. D. L. Auld, S.E. Ullrich, and B.L. Bettis reported in a 1980 University of Idaho Agricultural Experiment Station study that fifty of these accessions exhibited some degree of winter hardiness when planted at Pullman, WA, and Moscow, ID.

**Talks and Presentations**

Nov. 5 Presented annual summary of the grass regeneration program at the Forage and Turfgrass CGC meeting in Minneapolis, MN.

**Travel, Invitations, Special Awards and/or Assignments**

Mar. 3-6 Traveled to Eugene, Oregon, for the Farmers Cooperative Genome Project meeting.

Oct. 15-28 Traveled with Rich Hannan to Beijing, China, as an invited guest to exchange germplasm information.

Nov. 4-9 Traveled to Minneapolis, Minnesota, to attend the ASA-CSSA-SSSA annual meeting.

**Committees, Other Assignments, Activities and News**

Ex-officio member of the Forage and Turf Grass Crop Germplasm Committee.
Ex-officio member of the New Crops Crop Germplasm Committee.
Member of the Association for the Advancement of Industrial Crops.
Member of the Turfgrass Breeders Association.
Member of the Crop Science Society of America.
Member of the Western Society of Crop Science.
Prepared crop curator statements for one plant exploration proposal, and reviewed five PI determination forms.
Trained 6 employees on Worker's Protection Standards.
COOL SEASON FOOD LEGUMES CURATOR
Clarice J. Coyne

Activities

Cool Season Food Legume germplasm increases were carried out in greenhouses, screen houses, and farms in Pullman, WA, Central Ferry, WA, Prosser, WA and Parlier, CA. We expanded the greenhouse increases over twelve months in 2000 by utilizing three bays in current facility and by utilizing excess greenhouse capacity in other programs during the summer months. We are experimenting with planting dates to maximize seed production by pre-germinating *Pisum* and *Vicia* accessions to fill both screen houses in one day to ensure two cycles per year. I focused
greenhouse seed increase *Cicer, Pisum, Vicia,* and *Lens* on accessions with low seed quantity i.e. accessions without regeneration seed numbers for normal increase in the field, or screen house in the case of *Pisum.* Greenhouse increases included 30 *Cicer, 45 Pisum, 20 Vicia* and *20 Lens* accessions. The Vicia increases included accessions without species identification so progress was made in completing the passport information for GRIN. We converted the annual species of *Lupinus* direct seed increase program to a transplant program to ensure an effective population size is reached for this genera at Parlier, CA in collaboration with Maria Jenderek USDA-ARS, Parlier, CA. Field increases in 2000 included 60 *Cicer, 140 Pisum sativum* lines for 2001 international genotype x environment disease study (U.S., France, and New Zealand), and 505 *Cicer* core collection at Central Ferry for increase and descriptor data; 60 *Lupinus albus* at Parlier for increase; maintained and harvested perennial accessions at Pullman; *Vicia faba* increases at Prosser (48 accessions) and 9 in cages at Pullman, along with 20 *Lens, 20 Vicia, 30 Lathyrus* in the field, along with two cycles of *Pisum* (approximately 180 accessions) increase in screen house facilities. All *Pisum* accessions were virus indexed twice using ELISA to ensure PSbMV-tested-free seed for distribution and backup at the National Seed Storage Laboratory. Germplasm research projects: (1) Comparison of seed yield and quality of *Vicia faba* with and without pollinators; and irrigated vs.dryland seed production in collaboration with Stephanie Greene, USDA-ARS Prosser, WA. (2) Optimization of planting density and potting medium for legume germplasm increases in greenhouse and screen house environments (replicated greenhouse and screen house using one wild and one domesticated *Pisum sativum* accessions) to maximize yields of high quality seed. (3) Chemical control of pod dehiscence (replicated two year field experiment using *Lens culinaris* in 2000 and 2001. (4) Molecular and morphological characterization of *Cicer arietinum* core collection. We collected additional field data in 2000 and are currently isolating DNA from the core accessions for analysis of genetic diversity using microsatellites on our automated genotyping system.

**Research Activities**

The demands placed on U.S. agriculture can only be meet by easily accessible, healthy, and well-characterized genetic resources. My research program objectives are to 1) expand genetic marker and comparative genomic characterizations of cool season food legumes and transfer this information to the scientific community and 2) develop, maintain, and enhance genetic marker and genomic data management and bioinformatic capabilities associated with my crops. We have made good progress on the marker development and development of genomic tools in 2000. Our most significant contribution of genomic tools was the completion of one-haploid genome equivalent large-insert library of pea, published in September 2000 and completion of 3.8 haploid genome equivalent library of chickpea which we reported at the Plant and Animal Genome meeting. Our marker work focuses on molecular genetics of disease resistance in *Pisum sativum* germplasm, including QTL analysis of Aphanomyces root rot resistance in field and greenhouse studies of germplasm in collaboration with Kevin McPhee USDA-ARS Pullman, WA; pea seedborne mosaic virus in greenhouse studies of germplasm in collaboration with Robert Martin and Karen Keller, USDA-ARS Corvallis, OR, and Fusarium wilt race 1, 2, and 5 resistance in germplasm in collaboration with Debra Inglis, WSU Mount Vernon and Kevin McPhee, USDA-ARS Pullman, WA. Our most significant contribution to markers for analysis and
characterization of germplasm was identifying the first flankers markers for three major QTL conferring complete field resistance to Aphanomyces root rot in pea. Post-doctoral research scientist, Marie-Laure Pilet-Nayel, completed a linkage map and QTL analysis of Aphanomyces root rot resistance and reported these results in June 2000 at the Molecular Genetics of Model Legumes meeting held in Norwich, UK. In addition, we are preparing four manuscripts for publication in 2001 to report (1) the QTLs for use in marker assisted selection and germplasm characterization, (2) to report the results of surveying germplasm with aphanomyces isolates from four countries, (3) report differences in QTL between French and American isolates, and (4) report the comparison of field versus greenhouse screening for characterizing germplasm for reaction to this devastating disease. Visiting scientist Karen Keller, USDA-ARS Horticulture Crops Unit from Corvallis, OR began the genotyping germplasm using our automated genotyping equipment and software to identify a closely linked marker to pea seed-borne mosaic virus, and phenotyping recombinant inbred lines to verify the markers for use in germplasm characterization of the NPGS Pisum collection, and we are preparing a competitive grant to fund this project. We published our latest results on a marker for Fusarium wilt race 5 in September, and applied for funding to locate a marker for race 2, an emerging disease in pea production areas in the U.S. Graduate student (M.S.) Melissa McClendon identified a closely-linked marker to Fusarium wilt race 1, the closest marker identified to date for disease resistance loci in any grain legume crop, and is preparing a manuscript for publication in Theoretical and Applied Genetics. In the bioinformatics area, we upgraded our LI-COR automated genotyping system and software to speed the collection of the marker data.

Grants Awarded

2000 USDA-ARS-CRSEES Cool Season Food Legume grant: Inglis, Coyne, and McPhee. Post-doctoral salary (full-time, 6 months) from UNIP, Paris, France (in kind) for French visiting scientist.

Talks and Presentations

Jan. 28 Invited seminar presentation, “Chickpea Germplasm Resources in the United States” The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India

Travel, Invitations, Special Awards and/or Assignments

Jan. 20- Feb. 1 Traveled to Hyderabad, India, for the international exchange of *Cicer* germplasm between NPGS and ICRISAT.
Feb. 24-17 Traveled to Albany, California, to attend the Pacific West Area new scientist orientation.
Apr. 10-12
May 9-10
Jun. 24-28 Traveled to the John Innes Centre, Norwich, UK for Molecular Genetics of Model Legumes conference and visited Mike Ambrose, John Innes Pisum collection curator.
Nov. 4-9 Annual meeting of the Crop Science Society of America, Cool Season Food Legume CGC meeting.

Committees, Other Assignments, Activities and News

Appointed, Washington State University, Graduate School Faculty. Member, Cool Season Food Legume Crop Germplasm Committee. Member, Pisum Crop Germplasm Committee. Member, American Association for the Advancement of Science Member, Crop Science Society of America Member, North American Pulse Improvement Association

Graduate Student Advisory Committees
- Major Advisor, Melissa McClendon. Master of Science, Department of Horticulture, WSU.
- Co-advisor, Makito Mimura, Master of Science, Department of Crop and Soil Science, WSU.
- Jeff Shultz, Master of Science, Department of Crop and Soil Science, WSU
- Jena Lewinsohn, Master of Science, Department of Botany, WSU
- Kyung-Mi Yun, Master of Science, Department of Horticulture, WSU News

Michael Cashman joined WRPIS as the Cool Season Food Legume technician August, 2000. Dr. Marie-Laure Pilet-Nayel, a post-doctoral researcher with the CSFL program 1999-2000 accepted a research scientist position with Unité Mixte de Recherches INRA/ENSAR Amélioration des plantes et biotechnologies végétales at Rennes, France. Dr. Eric Storlie, a very short term post-doctoral researcher with the CSFL program accepted an appointment working on a wheat collaboration between Australia and China at Toowomba University, Queensland, Australia. Melissa McClendon was awarded 3rd place in the Graduate Student Research presentation competition at the Western Society of Crop Science Annual meeting.

Plans for 2001
Develop a transplant program for *Vicia*, *Lathyrus*, *Trigonella* and *Lupinus* species to increase effective population size during regeneration cycles.

Prepare screen houses for larger plant populations for each accession increased in 2001 to insure distribution quantities produced, seed available to ship to NSSL backup storage, and lengthen the time-frame for next regeneration cycle.

Develop *in vitro* germination procedure for difficult-to-germinate food legume species and write report.

Complete the field experiment of chemical control of pod shatter on lentil and write report.

Write report on 1999-2000 field experiments comparing *Vicia faba* regeneration with and without pollinators.

Submit several USDA competitive grants to fund the molecular genetics of pea germplasm disease resistance in germplasm resources research.

Automate the collection of descriptor data to speed the entry of the information into GRIN.

Attend North American Pulse Improvement Association (formerly National Pea Improvement Association), Pisum Crop Germplasm Committee, Fargo, North Dakota.

Attend Crop Science Society meeting, Food Legume Germplasm Committee, Charlotte, North Carolina

Invited talk 2001, AEP (European Grain Legume) conference, Cracow, Poland

Invited talk 2001, Department of Horticulture, Oregon State University, Corvallis, OR

Invited talk 2001, Crop Improvement and Protection Research Unit, USDA-ARS, Salinas, CA

Organize technology transfer workshop for pea researchers on disease screening and application of molecular markers in screening germplasm, October 2001, Pullman, WA (subject to grant award).

**HORTICULTURE CROPS CURATOR**
**Barbara Hellier**

**Activities**
The Western Regional Plant Introduction Station Horticulture Crops program has curatorial responsibility for 211 genera, 58% of the generic diversity held at the station. The major collections within this program are *Lactuca* and *Lactuca* relatives (1388 accessions), *Allium* (969 accessions), *Astragalus* (752 accessions), *Onobrychus* (604 accessions), *Papaver* (556 accessions), *Sanguisorba* (126 accessions), *Hedysarum* (110 accessions), *Scorpiurus* (80 accessions), *Anthyllus* (66 accessions), *Plantago* (60 accessions) and *Salvia* (48 accessions). The remaining 200 genera are represented by 40 or fewer accessions each. The total number of accessions maintained by the Horticulture Crops program is 5774.

Increase activities were the major focus in 2000 for the Horticulture Crops program. 567 increase plots were established/maintained/harvested during the year for a selection of genera within the program. Many of the species in the program require 2-4 years of growing time before producing seed. Consequently, our increase plots are long term projects. Also, the majority of the species within the program need multiple harvests per year and can only be harvested by hand. Both of these factors limit the number of accessions which can be grown each year.

One of the major activities of the Horticulture Crops program is maintaining and distributing the *Allium sativum* collection; the whole collection requires yearly increase. Also, the Horticulture Crops program staff package all seed orders for *Allium sativum*. For many years garlic has been one of the most requested collections. This year was no exception. There were 26 requests with 778 packets distributed (the 8th most requested species of the Station collections).

In addition to increase activities, progress was made in upgrading the information in the Germplasm Resource Information Network (GRIN) available for the program crops. In late fall of 1999 and early winter of 2000, digital images of bulbs of the *Allium sativum* collection were taken by Russ Staska and Vicki Newman, temporary employees. During the year these images were downloaded by Gwen Penticost, the Station computer specialist, into GRIN making them accessible to the public via the world wide web.

Two major improvements were made in the operations of the Horticulture Crops program in 2000. The most important and influential was the program was given a full time, permanent technician position. In July, Russell Staska was hired to fill this position. This is a boon to the program as only temporary help was previously available. The second improvement was the purchase of a small seed germination chamber placed in the main greenhouse space used by the program. It will be used to start seed which require specific germination temperatures and to perform germination tests to help augment the Station germination program.

**Talks and Presentations**

Jul. 5  
Presented “Genetic, morphologic, and habitat diversity of two species of *Allium* native to the Pacific Northwest, USA and their implications for the in situ seed collection for the National Plant Germplasm System”, Masters Defense, Washington State University, Pullman, Washington.

Jul. 11  
Presented a program summary to the Herbaceous Ornamental Crop Germplasm
Committee, Columbus, Ohio.

Jul. 24  Presented a program summary to the Root and Bulb Crop Germplasm Committee, Orlando, Florida.

Sep. 28  Presented a program summary to the Leafy Vegetable Crop Germplasm Committee, Monterey, California.

Oct. 4   Gave tour of garlic storage and drying facilities to Dr. Manford Morris of Little Rock, Arkansas.

Oct. 12  Presented “Introduction to the NPGS Garlic collection and the GRIN Database”. Garlic is Life Symposium and Festival, Tulsa, Oklahoma.

Oct. 15  Presented a program summary to the New Crops Crop Germplasm Committee, St. Louis, Missouri.

Oct. 30  Presented “Morphological data collected from proposed National Plant Germplasm System in situ seed collection sites of two *Allium* species native to the Pacific Northwest” (poster). The Third International Symposium on Edible Alliaceae, Athens, Georgia.

Travel, Invitations, Special Awards and/or Assignments

Mar. 3-6  Traveled to Eugene, Oregon, to attend the Farmer’s Cooperative Genome Project Advisory Counsel and Curators Meeting.


Jul. 8-11 Traveled to Columbus, Ohio, to attend the Herbaceous Ornamental Crop Germplasm Committee meeting and the Ohio Florist’s Association Short Course and Trade Show.

Jul. 22-27 Traveled to Orlando, Florida, to attend the Root and Bulb Crop Germplasm Committee meeting and the 97th Annual International Conference of the American Society for Horticultural Science.

Sep. 24-28 Traveled to Monterey, California, to attend the Leafy Vegetable Crop Germplasm Committee meeting and the International Lettuce and Leafy Vegetable Conference.

Oct. 11-13 Traveled to Tulsa, Oklahoma, to attend the Garlic is Life Symposium and Festival.

Oct. 14-16 Traveled to St. Louis, Missouri, to attend the New Crops Crop Germplasm Committee meeting.

Oct. 29-  Traveled to Athens, Georgia, to attend the Third International Symposium on Edible Alliaceae.

Nov. 4

Committees, Training, Other Assignments, Activities and News

Ex-officio member, Root and Bulb Crop Germplasm Committee.

Ex-officio member, Leafy Vegetable Crop Germplasm Committee.

Ex-officio member, Herbaceous Ornamental Crop Germplasm Committee.

Ex-officio member, New Crops Crop Germplasm Committee.
Ex-officio member, Clover and Special Purpose Legume Crop Germplasm Committee.

In conjunction with other members of the Root and Bulb Crop Germplasm Committee, prepared priority statements for *Allium sativum* and the Miscellaneous *Alliums*.

Apr. 3       USDA Ethics training.
Aug. 7       USDA Work Place Violence training.
Sep. 8       USDA Credit Card use training.

**Plans for 2001**

Field and greenhouse increases: Most of the genera in the program have accession which are unavailable or are not backed-up at the National Seed Storage Laboratory. We will continue to increase accession with low quantity, germination or are not backed-up. Accessions of *Astragalus* (8 species), *Onobrychis* (3 species), *Papaver* (3 species), *Anthyllis*, *Asclepias* (2 species), *Callistephus*, *Echium*, *Hedysarum* (2 species), *Lavandula*, *Marrubium*, *Mercurialis*, *Nepeta*, *Plantago*, *Polygonum* (2 species), *Ratibida*, *Sanguisorba*, *Satureja*, *Scabiosa*, *Scorpiurus*, *Teucrium*, *Tragopogon*, *Krascheninnikovia*, and *Camphorosma* are targeted for starting in 2001.

*Allium ampeloprasum* pollinator density trial: In cooperation with Dr. Steve Clement and the Station entomology program we will conduct a replicated trail to determine optimal fly densities needed for pollination of *Allium ampeloprasum* within our controlled pollination units.

Information gathering: Many of the species represented within the collections maintained by the Horticulture Crops program are wild relatives of cultivated species. Little to no cultivation and pollination biology information is easily available for these species. A major task for the Horticulture Crops program is finding this type of information on the program species. Information gathering is planned for 2001 with an emphasis on the genera/species in the major collections in the program and *Artemisia*.

**BETA CURATOR**
Alan Hodgdon

**Activities**

Fifty-four accessions were increased at W-6 in 2000. Of these thirty-three were done in the greenhouse system. Six of these increases will have to be redone; all of these were done in the field. The increases were given a combined rating for seed number and quality. Of the greenhouse increases nineteen were good, ten were fair, and four were poor. Of twenty-one field increases four were good, six were fair, and eleven were poor. Thirty-seven accessions were
started in 2000; three of these had no germination. The Beta increase program has a carryover of fifty-five accessions largely due lack of complete flowering induction. Flowering deinduction seems to occur when growth conditions, especially night temperatures, are too warm. This problem has been solved in some of the greenhouses where we can control the temperatures well. Deinduction is a problem in field increases of wild Beta accessions. We will continue to work on the deinduction problem.

Seventy-three accessions of Beta were germination tested in 2000. Forty-two of the tested lines were new (1999) seed. Only one of these had less than 50% viability; fourteen of these had greater than 50% dormancy. There is a large backlog of Beta accessions that need germination testing which will be reduced in the next few years.

A total of 420 accessions were distributed in 2000 in twenty-nine seed orders. We acquired 100 new accessions. One accession was backed up at NSSL.

In 2000 I was a member of the WSU Growth Facilities Advisory Committee and the W-6 Greenhouse Committee. Also I am making a spreadsheet of Beta descriptors from notes taken from seed increases since 1994.

Committees, Other Assignments, Activities and News

WSU Growth Facilities Advisory Committee

Plans for 2001

I plan to attempt to solve the deinduction problem in wild accessions of *Beta vulgaris*. I will get descriptor data organized from seed increase notes. I will work on a *Beta* Procedures Manual.

*PHASEOLUS CURATOR*
Molly Welsh

Activities

During the year 2000 approximately 446 accessions from the collection were increased. All accessions in the increase cycle were tested for the presence of BCMV and presently 15.8% of the collection is labeled 'tested virus-free'. During the year 10 accessions, testing positive, were run through the virus cleanup program and are now labeled 'tested virus-free'. Seventeen accessions were increased in sufficient quantity to send as backup to the NSSL.

The BCMV files continued to be updated as data obtained during the increase cycles was collected. The protocol manual of the current lab procedures for the BCMV testing continued to
be upgraded as needed. The manual is available upon request. The 'BCMV clean-up' program has been modified and the following guidelines applied: 1) if infection is <10% the accession is rogued of infected seedlings, 2) if infection is >10% and <20% the accession is separated into "clean" (labeled PBF) and "dirty" sections (moved to another area until the remainder of the clean-up process is finished), and 3) if infection is >20% the accession is labeled non-BF and is entered into the list of accessions to be cleaned-up. The program to clean-up the labeled "non-BF" accessions was continued, with an envisioned goal of 2 non-BF lines planted and entered per month into the program.

Two accessions within the collection not previously identified as to species were identified. Additionally, 5 accessions with incorrect identification have been re-identified as to species. A uniform system of recording increase information, including plant character data, BCMV testing results and harvest results is used in the Phaseolus germplasm increase program, and collection and recording of this information was done on a regular basis.

Information contained in the GRIN web-page descriptor site has been updated and continues to be monitored and changed as additional data is obtained. Emphasis is placed on updating the information to keep it a current resource. A program to update and clarify the information in the GRIN passport data has been initiated.

The program for bio-control of insect pests GH 44 and GH 109 was considered successful during the year. A combination of constant monitoring and the use of newly identified, as well as the previously effective, predators contributed to the success of the program.

Aphid proof netting has been installed in GH CF. The field planting experiment at Central Ferry was a complete failure. Although the beans themselves grew well, shortly after aphids appeared in the field, BCMV was evident in the ELISA test results. A pattern of spread outward from the originally infected plants was seen over time. All the plants were removed and no seed was harvested. Outdoor plantings will not be repeated.

**Research Activities**

My work in embryo rescue and tissue culture program included practice with, and refining of technique. Accessions that had been in storage for more than 20 years without being regenerated were used for the practice sessions.

Seed was harvested from plants (produced via the embryo rescue procedure) of 3 such accessions.

I contacted Dr. Daniel DeBouck of CIAT to initiate a program to increase the number of wild Phaseolus accessions in the NPGS collection. In discussions with Dr. DeBouck concerning which accessions we might obtain from CIAT we have developed a plan whereby we could determine the accessions present in the CIA collection that we did not have in the NPGS collection. We then plan to begin the transfer of such material to the NPGS collection.
Travel, Invitations, Special Awards and/or Assignments

Feb. 14-17  Traveled to Albany, California, to attend the Pacific West Area new scientist orientation.

Mar. 3-6   Traveled to Eugene, Oregon, to attend the Farmer’s Cooperative Genome Project and meetings.

Aug. 2-4   Traveled to Twin Falls, Idaho, to attend the Bean Improvement Conference meetings.

Committees, Other Assignments, Activities and News

Member, Phaseolus Crop Germplasm Committee
Member,  Bean Improvement Conference Genetics Committee
Member,  W150 Regional Project
Member,  Seed Savers Exchange

Plans for 2001

During the year 2001 my plans include: I will continue to increase the scope of the "BCMV clean-up" program by adding non-BF accessions to the schedule for "clean-up". There will be further study in the area of Phaseolus systematics and botany to identify previously unidentified and/or mis-identified species within the collection.

The GRIN (Germplasm Resource Information Network) resource will be kept current and will be maintained as "user-friendly" as is possible.

I will work with clients to obtain data from their research with the requested seed, and will continue to enter the data into the GRIN database.

I plan to pursue my informal study of the Spanish language.
I will improve my skills in the utilization of the GRIN database.

The collection will be further organized, making better accessibility for interested parties to accessions and the related data.

I plan to improve my experience with the control of greenhouse pests and the nutritional requirements of beans.

All of the regular increase, germplasm acquisition and distribution, and data collection programs are in the 2001 plans.

I intend to pursue my work in the program to "save" endangered accessions through the embryo rescue work.
I will continue to update and clarify the information in the GRIN passport data.

I want to attend classes to learn the protocol, etc. for handling the information in the GRIN database.

I will review "odd" material stored at the W6 site, considering it to be added to the collection. The number of wild Phaseolus accessions being grown will be increased; we plan to learn more about how to get better results from such increase attempts.

We plan to increase the NPGS collection by obtaining new accessions from CIAT, particularly wild accessions.

**SEED STORAGE/GERMINATION PROGRAM**  
David Stout, Seed Manager

**Talks and Presentations**

Oct. 24 Gave a presentation at the GRIN meeting regarding how SQL is used at the Western Regional Plant Introduction Station.

**Travel, Invitations, Special Awards and/or Assignments**

Oct. 22-27 David Stout traveled to Baltimore, Maryland, to attend the USDA-GRIN meetings.

**Plans for 2001**

Continue packaging seed into sealed waterproof packets for -10C storage.

Start germinating genera never tested before like, Astragalus, Sanquisorba and others.

Monitor the time it takes to package, plant and count emergence of several genera.

Continue reorganizing the cold storage room in alpha-numeric order under each curator’s crops.

**INFORMATION TECHNOLOGY/SAFETY OFFICER**  
Gwen Pentecost

**Activities**
Computer Resources

This appears to have been a fairly typical year. My tasks included the expected range of hardware support, software support, system purchases and installation, troubleshooting, instructional assistance, security issues, website development and GRIN data entry. In 1999, we focused on upgrading most of our older PCs. Therefore, in 2000 we were able to turn our attention to redesigning the WRPIS website and fine-tuning computer operations within the group.

Computer-related expenses were significantly lower this year than in 1999. Primary expenses were fairly evenly divided between computers for new employees, PC systems for group, and hardware and software upgrades. Minor expenses included typical accessories, data storage media, reference material and repairs.

Through the purchase of additional hardware and software, we enhanced the group’s technical capabilities. An imaging system PC was assembled to provide a central location for tasks such as scanning slides, negatives, photographs and documents; preparing images for papers, posters and web pages; and backing up data with a CD-RW.

We noticed gaps in access to computers by staff outside the office. To solve this problem, we purchased two notebook PCs. Their main functions are to provide computer access while on collecting trips, at meetings, and in the lab; enable staff to meet deadlines and work at home; and for temporary use by employees who do not have their own computer.

I also redesigned and updated the Pullman USDA-ARS Plant Introduction website. The website included auxiliary sites such as a Personnel page, web pages for individuals, an overview of WRPIS facilities, and web pages for four years of annual reports and TAC meetings. The web page that generated the most interest displays comprehensive current weather data at our Central Ferry facility, collected with a Davis Weather station and downloaded to the WSU server. In addition, I made minor revisions to the Safflower website.

Computer security required almost constant attention throughout the year. The following list details efforts that appeared to prevent some problems: stressing the importance of increased security threats to computers, distributing prompt warnings about particularly dangerous new viruses, providing guidelines for effective, basic techniques to prevent damage to hardware and data, periodically making sure that every PC had the latest anti-virus software and special updates, distributing step-by-step instructions on how to perform weekly anti-virus software updates, installing a simple icon on every PC that launches an extra-thorough virus detection scan, and making damage control my top priority when necessary. As always in a rapidly changing technical field, information resources are extremely valuable. I constantly increased my technical knowledge by searching trade magazines, the web, and manuals. This enabled me to identify solutions to technical problems, locate troubleshooting procedures, and study software and hardware issues. I also collected reference sources, read product evaluations, and gathered
information about situations that were likely to occur. To keep all of this information accessible, I am compiling a growing index of information about computer resources.

Safety Awareness

The concepts behind a safety program are knowing what you have and being prepared. With those ideas in mind, I concentrated on two areas: training and inventories. I also tried to identify the most important safety issues and expedite their implementation.

I encouraged as many people as possible to take First Aid/CPR training and Hands-on Fire Extinguisher training, and coordinated the classes. Mandatory training that I also helped coordinate included WSDA Licensed Pesticide Applicator Recertification, and USDA Respirator Fit-Testing and Training.

With help from Plant Introduction lab and farm managers, we have a thorough inventory of our chemicals and pesticides. The data will be updated annually. Plant Introduction personnel also helped me inventory facility safety equipment such as eyewashes and safety showers. The Area Office is now working with WSU Facilities Management on upgrading some of the eyewashes. I was also able to confirm that the vermiculite that we use is not contaminated by asbestos.

To increase my own safety awareness, I attended two local workshops. The first was a Lab Safety seminar, presented by national safety consultant, Jim Kaufman. The second was a class in WSU Chemical Disposal Procedures, presented by WSU Environmental Health and Safety (EHS).

Talks and Presentations

I gave brief presentations on the most important safety and computer-related issues at our Plant Introduction group meetings.

Travel, Invitations, Special Awards and/or Assignments

May 9-12 Traveled to Spokane, Washington, to attend a course presented by CompuMaster entitled “Advanced Troubleshooting, Maintaining & Upgrading PCs.”

Aug. 4-11 Traveled to Portland, Oregon, to attend the “Collateral Duty Course for Other Federal Agencies” workshop presented by OSHA representatives.

Oct.20-28 Traveled to Baltimore, Maryland, with Dave Stout and Paula Rose to attend GRIN annual meeting.

Committees, Training, Other Assignments, Activities and News

Washington State University CAHE Safety Committee.
ARS Pullman Location Safety Committee.
Attended ARS training on Ethics, Diversity, Workplace Violence, and Sexual Harassment.

**Plans 2001**

**Website**
- Continue to develop websites for our stations in Parlier, Palmer and Prosser.
- Refine the Pullman website.

**Imaging PC**
- Add and update the peripherals when necessary.
- Demonstrate its capabilities to the group.
- Increase its use for data backups using the CD-RW.

Attend additional training in the areas of computer support and safety awareness.

Continue emphasizing good PC security practices within the group.

**FARM AND FACILITIES MANAGER, PULLMAN**
Wayne L. Olson

**Seed Conditioning**

Seed cleaning has changed dramatically in 2000 here at the Pullman Plant Introduction Station. Until this year, every machine was dedicated to a specific crop. The beet machine was used for beets, bean machine for beans, and the hammer mill for grasses. A Westrup LAH Brush Machine was added to the cleaning equipment lineup in late 1999. This piece of equipment which turned out to be the backbone of the cleaning facility in 2000.

Dan Cervantes joined our staff in July of 2000 and with his vision and ingenuity procedures have changed dramatically. Efficiency has increased as well. Today, the brush machine threshes 65 percent of our crops, which includes most of our grasses, and odd crops such as *Lesquerella*, and *Limnanthes*. The belt thresher (previous beet machine) is used on 15 percent of our crops, which includes numerous grasses, *Guizotia*, *Iris*, and poppy. The hammer mill is still used for some of our larger seeded grasses. The bean machine has been modified to thresh beans, peas, and lentils.

In-house, we have been working with Brad Baugh who oversees our Environmental and Occupational Health and Safety here in the Northern Cluster, Pacific West Area. Together we have been monitoring the particulate air matter in our seed threshing and cleaning facilities to develop a base line for new air filtration systems we have planned for in the near future.
Scott McGee developed an opportunity to work with Gary Miller of Alf Christensen Seed on new methods of cleaning small lots of clover, alfalfa, and other similar seed types. Working together has benefited both our seed cleaning unit and the knowledge base for the folks of Alf Christensen Seed.

**Field Activities**

Early in the fall of 1999 our Beet Curator, Alan Hodgdon, came up with an idea of a unit to prepare a planting bed for his beets. Throughout the winter Wayne Olson and Scott McGee developed a design for this bed preparation unit and took it on into construction. On April 25th the unit was finished and was used to plant beets on April 27th. Other than a small design calculation flaw in material seam strength that was quickly modified, the bed preparation unit worked very well.

Kurt Tetrick of the Central Ferry Farm and Wayne Olson of the Pullman Farm purchased a Mechanical Transplanter from RDO Inc. (John Deere Dealership) in Pasco, Washington. This unit can plant any band, plug, or cone from the greenhouse which is 1.75 inches square in size or smaller either straight into the soil or through plastic as fast as you can feed plants into the transplanter machine. Kurt worked tirelessly in the spring of 2000 to design, develop, and construct a drip irrigation system for the Central Ferry Plant Introduction Farm which until this time has all been hand-line irrigated. Kurt finished roughing in the drip irrigation system the first week in May, and in the second week of May Kurt, Wayne, and Scott were getting their first taste of working with the Mechanical Transplanter with curator Vicki Bradley's grasses. After a couple of day's worth of frustration and heading back to the shop once or twice for modifications to the equipment, things started to come together and ended in a the clean, swift, and very efficient, planting system that will be used for all of Pullman's grasses, lettuce, and other numerous crops here in 2001. Throughout the fall of 2000 Kurt has perfected the use of a commercial one-pass plastic mulch layer which mounts on the rear 3-point of one of his tractors. On the front of the same tractor he has mounted a drip tube layer that can place the drip tube at any desired depth under the plastic. Throughout the summer of 2000 desires of less water usage, cleaner accessions, fewer weeds, and less disease within the crops all came into reality for the Central Ferry Farm.

**Potting Soil Tests**

The Pullman Plant Introduction Station has an interesting situation just handling the logistics of soil requirements with multiple tons of potting soil used on an annual basis. If all our greenhouse and screenhouse area is evaluated together, we operate very close to an acre under glass within the Pullman unit. As an economic and ecology measure, years back the Pullman Station started recycling the potting soils through a Siebring Steam Trailer system, with both end dump and side load trailers. Problems have developed over time that came to a point of investigation because of impact on seed production in the winter of 2000. Today, with the help of Dr. Frank Dugan, our Plant Pathologist, we have a system in place to plate out soils and monitor for optimum sterility of our soils coming off the Siebring Trailer System. Periodically we soil test the recycled soils looking at nutrient buildup and imbalance of our recycling system.
In cooperation with Dr. Clare Coyne, our Cold Season Legume Curator, we have been soil testing and growing out test plants in potting soils of different origins and manufacturers throughout the year. Dr. Coyne has been documenting this data in yield count, soil test parameters, and visually through photography of the growth habits and production of the plants in these different soils. Results and documentation has revealed a lot to our unit from this study and helps us today decide on which manufacturer's potting soils to purchase and why. In one case, Dr. Coyne's documentation was used to return 105 cubic feet of soil to the manufacturer for a full rebate because it did not meet our unit's growing requirements and needs.

**Spodnam Trial for Seed Retention**

Throughout the summer of 2000 curators Dr. Clare Coyne, curator for Cold Season Legumes, Barbara Hellier, Horticulturist Curator, and Vicki Bradley, Agronomy Curator worked with Wayne Olson and Scott McGee of the Pullman Station's farm in observation and documentation of a product marketed as SPODNAM.

SPODNAM, marketed by Miller Chemical, is a spray application product claiming retention of potential seed yield in all pod bearing and grass seed crops. This is done through controlling pod splitting (shatter); eliminating swathing, pulling, and windrowing; maintaining seed quality and germination by excluding water contact with the seed in the field; and generally controlling seed loss in grass seed crops.

Results were mixed. Barbara Hellier's work and data with lettuce showed no difference between treated and untreated areas. Vicki Bradley's work with a split block trial on four different grass accessions showed no visual signs of seed retention. Clare Coyne's work with *Pisum*, *Vicia*, *Lens*, and *Lupinus* in a randomized complete block design showed some statistical advantage with the use of SPODNAM, enough of an advantage with these accessions that we plan to replications the study again in 2001 for further observation and data.

**2000 in overview**

The year of 2000 has been a great year for reproduction of accessions here on the Pullman Station farm.

New ideas and personal ingenuity resulting in fabrication of new equipment and development of new planting and harvesting methods have yielded better quality and quantity of seed this year as well as more efficient methods of working with those accessions. Ideas and ingenuity we see as an ongoing strength of the Pullman Station farm and facilities unit as we continually strive to educate ourselves as often as possible by going to outside meetings and seminars and bringing back information to be shared by all.

**Travel, Invitations, Special Awards and/or Assignments**
Jan. 5  Wayne Olson and Kurt Tetrick attended the Central Basin Ag Equipment Show at “The Track” in Pasco, WA.
Jan. 25-26  Wayne Olson and Kurt Tetrick attended Eastern Washington Ag Recertification Courses at the University Inn/Best Western in Moscow, Idaho
Jan. 24-26  Scott McGee attended Eastern Washington Ag Education & Testing at the University Inn/Best Western in Moscow, Idaho. Scott passed and received his Washington State Pesticide License.
Jan. 27-28  Wayne Olson traveled to Caldwell, Idaho, to pick up soil bins for a new soil treatment system put in place in Feb 2000.
Apr. 18  Wayne Olson and Scott McGee attended respirator training and fit test required by USDA.
Jul. 18  Wayne Olson and Scott McGee attended forklift training and testing required by Washington State University.
Nov. 13-14  Scott McGee and Kurt Tetrick traveled to Pasco, Washington, to attend the Pacific Northwest Vegetable Association Annual Conference and Trade Show.
Dec. 8  Wayne Olson and Dan Cervantes attended the Annual Dry Pea & Lentil Association meetings at the University Inn/Best Western in Moscow, Idaho.
Dec. 12-14  Wayne Olson traveled to Spokane Washington for the Far West Ag Chemical Association meetings covering crop fertility, pesticide use, and new Ag Chemical Regulations for 2001.

Committees, Training, Other Assignments, Activities and News

Sep. 12- Dec. 19  Wayne Olson attended the Administrative Professional Leadership Training series sponsored by Washington State University for their administrative professional staff. Forty (40) hour course.

Plans for 2001

Expanded use of the mechanical transplanter for Barbara Hellier in the lettuce program.

Research new uses of the mechanical transplanter for Clare Coyne in the Cold Season Legume program.

Expanded use of the mechanical transplanter for Vicki Bradley in the grass transplant operation.

Finish modification and start use of small cone planter for single row seeding for Clare Coyne in the Cold Season Legume Program.

Start use of the “Potting Shed” for the Greenhouse program.

Continue monitoring of the particulate matter in the cleaning room with Brad Baugh, and hopefully compare previous numbers to those after a new air filtration system is in place.
Continue monitoring the fall beet plantings with Alan Hodgdon to see if the stretch wrap configuration on the cold frames makes a difference in winter hardiness.

Start design and possible construction of a mechanical field harvester for small lines of grasses with Vicki Bradley and R.C. Johnson to replace “Hand Harvesting” with student help.

Start installation and operation of drip irrigation on some accessions on the Observatory Hill Farm here at Pullman, WA.

GREENHOUSE PROGRAM, PULLMAN
William L. Luna

This year has passed getting familiar with the responsibilities and duties that are associated with the greenhouse position. Time was spent learning about the different crops that are propagated at W6. This included varieties of *Allium*, *Beta*, *Lactuca*, *Phaseolus*, *Pisum*, and *Vicia*. Close association was spent with the farm manager, as well as the curators, and each of their technicians. This established a familiarity with each of the stations’ five greenhouses and respective operations. Also, this association allowed a combined effort to effectively control three of this stations' common greenhouse pests; western flower thrips, two spotted spider mites, and the greenhouse whitefly.

Activities

After falling in step with the rhythm set by the rest of the staff, the first thing accomplished was to condition enough recycled soil for spring planting in the greenhouses and screen houses. The conditioning process was expedited due to farm manager, Wayne Olson, refurbishing the soil trailer so that it would function correctly. Optimal conditioning temperature is now at 220° F. The soil trailer is attached to a blower system, and steam is forced through the trailer for approximately four to six hours. Our research plant pathologist, Frank Dugan and technician, Shari Lupien, determined that our reconditioned soil is pathogen free when it leaves the soil trailer.

A greenhouse pest monitoring program established and is actively being refined. Part of that refinement now includes advice from research entomologist, Steve Clement. Steve's participation will aid in establishing an effective, standardized, monitoring program. This program will allow us to control greenhouse pests, reduce the spread of insect pests, and reduce our overall pesticide applications. Currently our attempt in controlling insect pests includes monitoring one greenhouse per day and limiting our travels to only that greenhouse on that particular day. Daily pest monitoring will aid in defining a pest population threshold. Once this threshold is defined, it will then be decided if and when it is critical to apply pesticides.
A pesticide applicator license was obtained after successful testing. This allows for the legal application of pesticides in licensed categories of Washington State Laws and Regulations, Weeds, and Insects.

Some pesticides that have been used in the past years are currently being phased out of our inventory. This includes any pesticides with the signal word "danger" on the label. The goal is to reduce the number of pesticides used in the past and the number of pesticide applications. We also want to keep the chemicals updated, and not on the shelf for longer than two or more years. Currently, "softer" products have been added to our inventory. These are pesticides that are compatible with an IPM program that is on-going in two of the stations' greenhouses. "Softer" products include pesticides with the signal word "caution" and "warning". We are also paralleling our pesticide program with the plant growth facilities at Washington State University.

We have added digital hygothermographs to each greenhouse. These devices record temperature and relative humidity data that is downloaded and printed in graph form. By comparing the graphs to the greenhouse thermostat settings, it helps determine if the greenhouse temperature control system is working correctly. The recorder has been helpful in trouble shooting mechanical failures.

This December Angie Kuno, our weekend waterer, graduated and moved out of the area. Angie worked for the station since April of 99. Jeff Bonnett was hired in her place. Jeff has been employed by the station since January 1999 and has added the weekend and holiday watering to his responsibilities.

Committees, Training, Other Assignments, Activities and News

<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 18</td>
<td>Attended respirator training and fit test required by USDA.</td>
<td></td>
</tr>
<tr>
<td>Jul. 18</td>
<td>Lift Operator Certification</td>
<td></td>
</tr>
<tr>
<td>Oct. 3</td>
<td>First Aid; Basic</td>
<td></td>
</tr>
<tr>
<td>Nov. 2</td>
<td>Supervision of Temporary Employees (WSU Leadership Dev. Module)</td>
<td></td>
</tr>
<tr>
<td>Nov. 21</td>
<td>Delegation (WSU Leadership Dev. Module)</td>
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<tr>
<td>Dec. 5</td>
<td>Coaching (WSU Leadership Dev. Module)</td>
<td></td>
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<tr>
<td>Dec. 12</td>
<td>Corrective action and discipline (WSU Leadership Dev. Module)</td>
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<tr>
<td>Dec. 14</td>
<td>Conflict Management (WSU Leadership Dev. Module)</td>
<td></td>
</tr>
<tr>
<td>Dec. 19</td>
<td>Influence and Power (WSU Leadership Dev. Module)</td>
<td></td>
</tr>
</tbody>
</table>

Plans for 2001

Continue with soil reconditioning to meet the needs and requests of the curators and technicians at the station.
Utilize the new potting shed as housing facilities for pot storage and pot cleaning. Maintain pot inventory count and maintain the various sizes of pots. Supervise and coordinate the pot washing with the technicians.

Continue to develop the pest monitoring program. This will included further interaction with the curators, technicians, and entomologist as well as the other staff members. Ideally this will lead to a reduction of the number of pesticide spray applications.

Older pesticides in the chemical storage inventory will continue to be removed.

The relationships between common occurring greenhouse pests and their natural predators will continue to be learned. Accomplishing this will be way of reading of trade magazines, journals, publications, and web sights found on the internet. Relevant information found on entomology, IPM, and greenhouse management will be used to enhance the greenhouse biological program at the station.

Find alternate distributors of natural predators so that the station can expand the selection of choices in order to maintain development of a biocontrol program.

Find the current output of greenhouse lights by means of a light meter. The results will be used to determine if maximum output is being achieved. If results show that bulb output is not optimal then light bulbs will be changed as needed. This will increase the efficacy of plan propagation.

**CENTRAL FERRY RESEARCH FARM MANAGER**

Kurt Tetrick

**Activities**

In late February we began construction on the drip irrigation system that enabled the farm to have drip and sprinkler irrigation at the same time. We poured a concrete pad and put in place the two-ten thousand gallon tanks. We then plumbed and wired the low volume pump into the existing system. Wired the pump station to accept a variable frequency drive and automatic tank fill. After that we made manifolds for each nursery and plumbed in the adapters on the appropriate risers. The first stage (operational) was completed by the end of May, which ran the farm into irrigation season.

We had an open house for many of the research projects being done here. The weather was cloudy, rainy and cold. So we had approximately 50 people, 25 were research staff. Wayne Olsen and myself represented WRPIS.

Germplasm increases at the Central Ferry Research Farm included, but were not limited to: .25 acre of peas and .5 acre of chick peas for Clare Coyne’s program research; a total of .5 acre in
several locations for isolation of aliums, leeks and lettuce for Barbara Hellier’s research; one acre of safflower, 19 acres for the 1999-2000 grass nursery, and 22 acres for the 2000-2001 grass nursery for Vicki Bradley’s program (The amount of land used for grass nurseries is to accommodate isolation. The actual nurseries themselves were 3 acres each); and, there were 64 lines each of beans, greenhouse and three crops for Molly Welsh’s *Phaseolus* program.

**Research Activities**

Research activities included, but were not limited to: a three acre chickpea core collection evaluation by Clare Coyne; perennial wheat insect resistance research by Stephen Clement; and, research on on the ornamental aspects of 80 lines of safflower by Vicki Bradley.

Kim Campbell, USDA Wheat Genetics, used two acres for club wheat breeding; Steven Jones used one acre for his winter wheat breeding project; David Bragg, WSU extension (Garfield County), work on pesticide research with winter, wheat, spring wheat and barley; Mark Nelson, WSU Prosser, investigated hops pathology, fungicide work for powdery mildew in hops on two and one-half acres; and, Robert Thornton, WSU Horticulture, used four acres for his early generation potato selection research. Many of these research projects are at the Central Ferry farm because of its isolation from similar crops grown on the farm.

**Talks and Presentations**

On June 12, 2000, I had an open house and gave a presentation of the importance of germplasm, a brief look at the whole system, and Central Ferry farms history and plans for the future.

**Travel, Invitations, Special Awards and/or Assignments**

- **Jan. 5** Attended the Central Basin Ag Equipment Show at “The Track” in Pasco, WA.
- **Jan. 25-26** Attended Eastern Washington Ag Recertification Courses at the University Inn/Best Western in Moscow, Idaho
- **Nov. 13-14** Traveled to Pasco, Washington, to attend the Pacific Northwest Vegetable Association Annual Conference and Trade Show.

**Plans for 2001**

Irrigation: To have the irrigation system working automatically turning the individual manifolds on and off.

Constructing fertility injection carts to be towed by four wheeler to manifolds.

Expand the uses of the weather station and irrometers to help timing of irrigation.
Activities

The NTFLGR is involved in the maintenance, accession acquisition, evaluation and distribution of the NPGS temperate forage legume germplasm collections which contain over 11,000 accessions representing current and obsolete cultivars, landraces, wild species and genetic stocks. Additionally, we carry out research in support of our germplasm conservation objectives.

Collection Maintenance

The table below summarizes the number of accessions increased at Prosser, WA, in 2000.

<table>
<thead>
<tr>
<th>Genera</th>
<th>Over wintered ‘99</th>
<th>Started Spring ‘00</th>
<th>Started Fall ‘00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lotus</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medicago</td>
<td>39</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Trifolium</td>
<td>29</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Misc. Legumes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Astragalus, Vicia,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lathyrus, Onybychis)</td>
<td>36</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Total:</td>
<td>104</td>
<td>298</td>
<td>50</td>
</tr>
</tbody>
</table>

In addition, we carried out germination testing on recent increases, and obtained seed counts on original seed of Medicago for approximately 250 accessions. The information supporting the germplasm collection is as essential as the seed itself. Reflecting this, we also spent time on documentation activities. A brochure entitled "Germplasm Collection User Guide- Alfalfa, Clover, Medic" was developed and made available. It provides instructions for accessing germplasm information in GRIN. An information poster that describes the objectives and activities of the National Temperate Forage Legume Germplasm Resource Unit was also developed. The poster is displayed in the lobby of Hamilton Hall, Prosser and in a corridor of Johnson Hall, Pullman.

Accession Acquisition

In collaboration with scientists from Kazakhstan and Russia, Dr. Stephanie Greene and Dr. Rich Hannan, collected a large number of alfalfa relatives from northwestern Kazakhstan in August and September. Overall, we traveled 1180 km and made collections at 89 sites. A total of 432 accessions were collected, of which approximately 135 represented species closely related to cultivated alfalfa. This area had not been previously collected from and our collection has a
significant gap in alfalfa relatives. Local site description included photos, monthly rainfall and temperature, and soil and vegetation classification. Additionally, a GIS database was developed to support the mission and allowed us to compare past observations with present observations, and understand the occurrence of species in relationship to temperature, rainfall and soil factors.

**Germplasm Evaluation**

We revised the entire set of GRIN Trifolium descriptors to ensure the format supports effective accession queries. The proposal was presented to the Clover and Special Purpose Legume CGC subcommittee. All CGC-funded evaluation data sets received during 2000 were uploaded into GRIN.

**Germplasm Distribution**

In 2000 we distributed the following number of seed packets: Lotus, 598; Medicago, 4420; Trifolium, 615. We also answered numerous requests for information.

**Research Activities**

In collaboration with Dr. George Vandermark, USDA, ARS, Prosser, we planned and supervised the FAO-sponsored training of two Indian scientists in germplasm evaluation and patenting. The scientists were in Prosser from September 18 to December 18, 2000. They learned how to isolate DNA, carry out PCR reactions, produce and analyze RAPD marker data. We also spent time teaching them how to use computer resources to carry out research via the internet, use on-line library resources, Microsoft Power Point and NTSYS. Additionally, we organized a trip where they received training in NPGS activities through site visits to Georgia, Beltsville and Pullman, and visited forage research programs in Beltsville and Ohio.

Another area where considerable attention was focused was on examining the correlation of genetic (RAPD markers) and morphological variation in red clover collected in the Caucasus Mountains, Russia in 1995, with ecogeographic variation as described by GIS-derived attributes. We found a very strong correlation between GIS-derived ecological description and morphological variation, and a much weaker association between ecogeographic data and RAPD marker variation. We are preparing to submit a manuscript to a peer-reviewed journal to report on this research.

Another area focused on was a review of the parentage of 500 cultivars of alfalfa developed in the United States over the last 100 years. We were interested in identifying the contribution PI material has made in cultivar development in the United States. We found that approximately 76% of the 500 cultivars reviewed, had significant ancestry that could be traced to a PI accession.

**Talks and Presentations**

Jun. 16 Greene, S.L., M. Gritsenko, R.C. Johnson, G. Vandemerk. ‘Predicting genetic
differentiation using GIS-derived data: case study in the Caucasus Mountains, Russia’ Invited talk at International Conference on Science and Technology for Managing Plant Genetic Diversity in the 21st Century, Kuala Lumpur, Malaysia.

Jul. 15 Greene, S.L. ‘Status and management constraints in the Medicago collection’. Talk at Alfalfa Crop Germplasm Committee Meeting, Madison, WI.


Nov. 5 Greene, S.L. ‘Annual report to the Clover and Special Purpose Legume Germplasm Committee’, Minneapolis, MI


Dec. 19 Greene, S.L. ‘Collecting wild relatives of alfalfa in Kazakhstan’. WSU-IAREC Department seminar, Prosser, WA.

Travel, Invitations, Special Awards and/or Assignments

Jan. 2-7 Traveled to Moscow, Idaho, to attend the workshop on the Metapopulation of Animals and Plants at the University of Idaho.


Jan. 25-26 Traveled to Pasco, Washington, to attend the Washington State University Grower’s Association Annual Hay Conference.

Feb. 14-17 Traveled to Albany, California, to attend the Pacific West Area new scientist orientation.

Mar. 3-6 Andrew Bell traveled to Eugene, Oregon, to attend the Farmer’s Genome Cooperative-NPGS meetings.

May 9-10 Traveled to Pullman, Washington, for a site visitation.

Jun. 9-17 Traveled to Kuala Lumpur, Malaysia, to present a paper at the IPGRI conference.


Jul. 15-18 Traveled to Madison, Wisconsin, to present a paper at the 37th National Annual Alfalfa Improvement Conference and attend the Alfalfa CGC meeting.

Aug. 23-Sep. 13 Traveled with Richard Hannan to Kazakhstan on a plant exploration/collection trip.

Oct. 14 Traveled to Pullman for WRPIS site visit.

Nov. 4-9 Traveled to Minneapolis, Minnesota, to present a paper and attend the ASA-CSSA-SSSA meetings, and CSPL CGC meeting.

Nov. 15-16 Traveled to Pullman for WRPIS site visit.

Committees, Training, Other Assignments, Activities and News

Coauthored with Dr. Gary Bauchan the CGC report, “Status of Alfalfa germplasm in the United
States’.
Chair, CSPL CGC subcommittee on descriptor revision.
Member, Crop Science Crop Registration Subcommittee - misc. legumes.
On graduate committee of Mercy Neumann, who graduated in May 2000 with M.S. degree.

Mar. 8       Program visited by Dan Skinner, R.C. Johnson, who collected leaf tissue of various legume taxa.
Apr. 6-7     Program visited by Hailu Aynalem, from the Volcani Institute, Israel.
Jul. 5       Program visited by James de Barro, De Barro Agricultural Consulting (alfalfa seed production), Keith, South Australia.

We now have high quality lab space through sharing with WSU Hops Genetic program.

Alfalfa standard check cultivars were transferred to Pullman. We have inventoried the seed lots and continue to distribute seed to support variety development in the US.

Plans for 2001

The unit is planning to increase between 300-400 accessions, focusing predominately on Medicago and Trifolium. We will be experimenting with new regeneration protocols that follow the optimal standards published in Sackville-Hamilton and Chorlton (1997), 'IPGRI Handbook for Genebanks No.5 Regeneration of accession in seed collections: a decision guide'. We will also be developing a better way to gather and input regeneration data with the use of bar codes and a handheld pocket pc with built in scanner.

In addition to increasing seed, we will positively identify the Medicago Kazakhstan germplasm by obtaining chromosome counts and keying out using morphology. We will also be getting passport data including site images into GRIN. I am trying to have Dr. Alexandr Afonin and Nicolai Dzubenko come to Prosser to help identify Kazakhstan material and discuss further collaboration for carrying out research on the Kazakhstan germplasm. I also plan to develop and submit an exploration proposal to collect forage legumes in Turkmenistan in 2002. I will also assist the CSPL CGC with revising their Germplasm Vulnerability Report.

The documentation projects we plan to work on in 2001 include developing a Unit web site, and completing a documentation upgrade of Lotus collection and G number, which will subsequently be uploaded into GRIN. In terms of research papers, I plan to write a summary paper of the 2000 Kazakhstan collecting trip, submit a manuscript on the Caucasus red clover project and complete and submit a manuscript on the orchard grass work carried out with Marina Gritsenko in 1998.

NATIONAL ARID LAND PLANT GENETIC RESOURCES UNIT, PARLIER, CA
M. M. Jenderek, Horticulturist/Curator

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Activities

The Parlier site processed a total of 1,148 different accessions of 36 diverse genera and 98 different species. All accessions were propagated for other NPGS stations and harvested seeds were sent to respective curators. A back up collection for *Corylus avellana* (116 accessions) was established. A *Bassia prostrata* germplasm nursery of over 80 accessions, (collected in Kazakhstan) has been initiated. Cuttings of 46 different *Simmondsia chinensis* accessions, collected in Maricopa Agricultural Center in Arizona, were successfully rooted and are ready to be transplanted to field. Evaluation for 21 different descriptors for 50 *Lupinus albus* accessions was submitted to the respective curator. A new perennial plant collection was established. Our site environment and cultural practices have proven to be appropriate for *Hordeum* and *Triticum* sp. germplasm seed production. This was a successful year for germplasm propagation at our station.

Two new Biological Science Technicians were recruited and two Bio. Sci. Aids for a 1 year term appointment were employed.

The following structures and infrastructure improvement projects were completed - head house to both screen houses, north pole barn extension, air temperature regulation in screen house #1, pavements of field roads adjacent to field #33 and #43, irrigation systems on field #23 and #33 were completed, all asbestos containing floor tiles in former Yarazoni house were replaced and two rooms were renovated and converted to a specimen preparation lab and technician’ operating room.

Research Activities

Seed producing ability for over 30 garlic germplasm accessions was evaluated for the second time (manuscript in preparation). Fertile plants are in great demand by domestic and international industry, growers and researchers working on garlic variety improvement and genetics; hence the data present a valuable source of information o publicly available fertile germplasm. A part of the results were presented at the 3rd International Alliums Symposium in Athens, GA, Garlic Symposium in Tulsa, OK and on a Garlic Day, organized by UC Davis extension in Santa Clara, CA.

F$_1$ seed families were developed for selected *Allium sativum* germplasm accessions. The material and the F$_2$ progenies (in progress) have been prepared for a genetic analysis of inheritance patterns of selected economic traits, as e.g. male sterility, outer skin color, and morphological characteristics. Few of the developed families will be used for studies on synteny with other monocotyledonous species, for establishing molecular markers and gene mapping.

Some *Parthenium argentaum* do not produce seeds. Since the species has been assigned to Parlier as its primary conservation site, studies on promoting rooting on cuttings of three different accessions were conducted. Out of three different chemical agents applied (Rootone, IBA and Hormex), the treatment with concentrated Hormex solution produced the highest
number of cuttings with sufficient root mass able to support plant growth and development. The study will be repeated in CY 01 with a wider group of chemical agents.

Station Visitors

Sep. 14 Dr. Minnie Wang, DeFrancesco and Sons, Inc., Firebaugh, California.
Sep. 29 Dr. Larry Robertson, PGRU, Geneva, NY.
Dec. 11 Dr. Minnie Wang, DeFrancesco and Sons, Inc., Firebaugh, California.

Talks and Presentations

Jul. 11 “Garlic fertility in the USDA Germplasm Collection”, presentation (invited), The 3rd Annual Garlic Field Day, UC Davis-BAREC Santa Clara, CA.
Oct. 12-13 “Characterization of garlic (Allium sativum L.) clones producing true seeds”, presentation (invited), Garlic is Life Symposium & Garlic Growers Conference, Oklahoma State University Tulsa, OK.

Travel, Invitations, Special Awards and/or Assignments

Feb. 14-17 New SY Orientation, Albany, CA.
Jul. 11 The 3rd Annual Garlic Field Day, UC Davis-BAREC Santa Clara, CA.
Jun. 25-27 W6 TAC, Wasilla, AK.
Jul. 18-21 PGOC, Beltsville, MD.
Oct. 12 Western Region International Plant Propagators’ Society, Modesto, CA.
Oct. 13-14 Garlic is Life Symposium & Garlic Growers Conference, Oklahoma State University Tulsa, OK.
Oct. 14-16 Crop Germplasm Committee for New Crops, St. Louis, MO.
Oct. 29- Alliums 2000, The 3rd International Symposium on Edible Alliaceae, University of Georgia, Athens, GA.

Special Assignments: Collection of jojoba germplasm in Maricopa, AZ, April 24-26, 2000.

Committees, Other Assignments, Activities and News

American Society for Horticultural Science, member
Society for In Vitro Biology, member
California Native Plant Society, member
Crop Germplasm Committee for New Crops, member
Leafy Vegetable Crop Germplasm Committee, member
Root and Bulb Vegetable Crop Germplasm Committee, member; worked on selection of 10 top garlic descriptors
Plant Germplasm Operations Committee
W6 Technical Advisory Committee
Mentored a Reedley high school student in a Research Apprenticeship Program, eight weeks
Advised/consulted callers, via e-mail and visitors on in vitro virus free plant production, field cultivation and production of true seeds of *Allium sativum*.

**Plans for 2001**

**Structures:**

The most important and at the same time dreaded plan is to obtain laboratory and office space in the newly constructed USDA, ARS, San Joaquin Valley Agricultural Science Center or to obtain funds for laboratory and office construction for the NPGS Parlier program. Until now, all efforts initiated were unsuccessful.

In screen house No. 2, the damaged by time, external protective plastic covers should be replaced and the hand operated system should be changed to a motorized one.

**Personnel:**

GRIN training for one technician will be arranged at Beltsville and at the Parlier site. One of the sites biological science aids will be trained to carry out germplasm evaluation according to established descriptors.

**Germplasm:**

Since Parlier has received an increased number of cereal accessions from regeneration, an experimental planter, small grain harvester/binder and a thresher will be purchased. For germplasm assigned to Parlier as its primary preservation site,

a) a status report of seeds available, seed germination rate and immediate needs for regeneration in CY 01/02 will be written,

b) source for species with very low or no representation in the NPGS should be identified and selected perennial species will be planted at the Parlier screen house or nursery,

c) we will work with Dr. Waldron on identifying *Bassia* accessions with a uniform ploidy level, representing unique populations.

The Parlier NALPGRU group will continue efforts to attract other NPGS sites to use our location for regeneration of long day, extended season plant species.
Research:

Since many genera assigned to Parlier are wind pollinated, evaluation on seeds productions capability in prototype isolation structures with positive air pressure, in comparison to canvas cages, using Spinacia oleracea as the experimental material, will be evaluated. Also, a pollen (grain size ca. 20 microns) permeability test for a new kind of canvas will be carried out.

Development of F2 families for selected garlic accessions will be continued.

NATIONAL ARCTIC PLANT GENETIC RESOURCES UNIT, PALMER, ALASKA
Nancy L. Robertson, Research Plant Pathologist

The National Arctic Plant Germplasm Resources Unit (NAPGRU) was designated as a new unit within the USDA National Plant Germplasm System in 1998, and is part of the Western Regional Plant Introduction Station, Pullman, WA. A plant pathology program was initiated Sept. 1998 and a laboratory established at the Alaska Plant Materials Center (AKPMC) in Palmer, AK. The laboratory was moved to new facilities in Palmer on Sept. 2000 at the University of Alaska-Fairbanks Experiment Station. The NAPGRU curator’s facilities and germplasm collection remain at the AKPMC. This report will outline the research activities and accomplishments of the plant pathology program for 2000.

Activities

Surveyed plants in the greenhouse and field for plant pathogens that were part of the NAPGRU and AKPMC; selected plants were assayed for virus infections in the laboratory. No plant pathogens were isolated and unhealthy appearing plants were concluded to be affected by herbicides and/or physiologically induced.

Assayed diseased tomato plants from private greenhouse grower for tomato spotted wilt virus and other viruses; identified tobacco mosaic virus to be the causal agent.

Harvested seed from greenhouse grown virus indicator host plants that included 12 species to be used in future research projects.

Research Activities

In 1999, I found diseased Pyrola asarifolia and Lupine nootkatensis plants growing in the Hatcher Pass region. These plant species are included in the NAPGRU. Both diseases reappeared in 2000 in the same locations and in contiguous areas that had not been previously surveyed. The following describes what I have learned on-site and in the laboratory and greenhouse about each of these new diseases: (1) Pyrola asarifolia. Symptomology: Leaves may have vein-clearing giving a mosaic appearance, or chlorotic blotches. Virus Purification and protein/nucleic acid analysis: Two unique protein species appear either together or alone, and are
~36-37kDa and ~18-20kDa. The low molecular wt. is most dominant in the early spring and late fall, which implies a photoperiod and/or temperature effect. No distinct nucleic acid detected by electrophoresis or particles seen by EM. Transmission: No movement of the causal agent by mechanical inoculation of diseased sap or by grafts. Geographical Distribution: Diseased plants detected throughout Hatcher Pass, appearing in patches. Plants collected near Delta Junction and along the Dalton Highway did not have symptoms or the unique proteins. (2) *Lupinus nootkatensis*. Symptomology: Leaves may have prominent vein-clearing or chlorotic mottling, and plants may be stunted. Virus Purification and Protein/Nucleic Acid Analysis: Distinct spherical particles (~26-30nm) seen in partially purified samples and from leaf sap. A distinct protein species (~39-40 kDa), presumed to be the virion protein coat, and a ssRNA genome (~4.3 Kb) have been identified. Random segments of the genome have been cloned and sequenced. Based upon these parameters, the virus is a new member of the Tombusviridae family. Serology: Polyclonal antiserum was made against partially purified virions and used to confirm plant infections. Transmission. No mechanical transmission occurred in commonly used viral indicator plant hosts. Several *Lupinus nootkatensis* seedlings inoculated with diseased leaf sap have consequently tested positive for the virus by western analysis. Seed transmission tests are now being conducted from both seeds that were derived from diseased and healthy plants. Soil transmission of the virus is being examined by growing seedlings in soil/roots collected from roots of diseased plants. Nematodes have been detected from the root/soil region, and are being studied for viral transmission. Geographical Distribution: Other lupine plants collected from the Dalton and Richardson Highways, the AKPMC, and Palmer Botanical Garden did not have the virus; virus disease appears to be confined along stream banks in Hatcher Pass.

Barley yellow streak mosaic virus (BaYSMV) studies were initiated into my research program in 1999 after I found the virus in barley growing on the UAF Experiment Station. The virus is now maintained in *Nicotiana benthamiana* plants and partially purified virions are stored at –80 C. In 2000, barley plants in Delta Junction were infested with the brown wheat mite, *Petrobia latens* - the only vector and natural mode of transmission in nature for the virus. Diapausal eggs of the mite were found on rocks and collected for BaYSMV transmission studies; this is the first report of the mite in Alaska.

Disease surveys in barley were conducted at the University of Alaska experimental plots in Palmer, Delta Junction, and Fairbanks. Additional observations were made in Delta Junction’s growers field of barley. Visual detection of scald, net-blotch, and smut was not unusual with some frost damage in Delta Junction.

A survey for plant pathogens in native plants along the Steese and Dalton Highways to Prudhue Bay took place between July 25-August 2. Twenty-one plant genera were collected and analyzed in the laboratory for viruses. No viruses were detected from the minipurification procedure used in the laboratory.

Surveys in the Hatcher Pass region took place between May and October for diseases in native plants with emphasis on *Pyrola asarifolia* and *Lupinus nootkatensis*. Twelve additional plant
genera since the 1999 survey were analyzed for viruses, and also tested for the previously identified lupine virus; none were found.

The Georgeson Botanical Garden at the UAF Experiment Station was surveyed for virus diseases. A native Alaskan plant, *Delphinium glaucum*, had chlorotic mosaic pattern, and definite virus particles extracted from leaf tissue. Tentative viral transmission to *Nicotiana benthamiana* test plants was confirmed by the development of mosaic symptoms and matching size of the viral protein; viral characterization is under investigation.

Studies on the development of an effective monocot gene expression vector were conducted with modified foxtail mosaic virus constructs. A foreign gene was successfully inserted, transcripts synthesized, and systemic viral movement obtained in barley seedlings. Assays are now under investigation for gene expression in barley plants.

**Talks and Presentation**


Apr. 6 Presented “Do Viruses Exist in Native Plants” at the Alaska Rare Plant Forum, Fairbanks, Alaska.

Jun. 15 Presented “Virus-Infected *Lupinus nootkatensis* found in Hatcher Pass, Alaska” at the Pacific Division APS meeting in Victoria, British Columbia.

Aug. 13 Presented “Plant virus Survey of Native Plants and Barley in Alaska” at the annual APS meeting in New Orleans, Louisiana.

**Travel, Invitations, Special Awards and/or Assignments**

Feb. 14-17 Traveled to Albany, California, to attend the Pacific West Area new scientist orientation.


Apr. 5-9 Traveled to Fairbanks, Alaska, to participate in the Alaskan Rare Plant Forum, present a paper, meet with University Faculty and do library research.

May 7-11 Traveled to Des Moines, Iowa and Lincoln, Nebraska to discuss a CRADA with Pioneer Hi-Bred Company.

Jun. 3-4 Traveled to Fairbanks, Alaska, for plant exploration purposes.

Jun. 16-22 Traveled to Victoria, British Columbia, to attend the joint meeting of the Pacific Division of the American Phytopathological Society and the Canadian Phytopathological Society and present paper.


Jun. 28- Traveled to Fairbanks, Alaska, to survey experimental plots for disease.
Jul. 6-13 Traveled to Fort Collins, Colorado, to visit NSSL and attend the American Society for Virology Annual meeting.

Jul. 25- Traveled to various locations in Alaska for plant exploration and collection purposes.

Aug. 3 Traveled to New Orleans, Louisiana, to attend the American Phytopathological Society meeting, and presented paper.

Aug. 11-18 Attended the 23rd Annual Alaska Agriculture Symposium in Anchorage, Alaska.

Nov. 10-11 Attended the 23rd Annual Alaska Agriculture Symposium in Anchorage, Alaska.

Committees, Other Assignments, Activities and News

Affiliate Associate Professor of Plant Pathology, University of Alaska-Fairbanks.
Member, Search Committee for selection of a tenure track Assistant professor of Science, Matanuska-Susitna College, 2000-01.
Tour/hands-on demonstration of my research laboratory for students enrolled in Natural Resources-Fbks 313, Introduction to Plant Pathology, April 19, 2000.

Plans for 2001

Research

Selected crops and native plants in Alaska will be surveyed for plant pathogens. This is an ongoing annual project that is especially important for documenting plant viruses. Surveys that were conducted in 1999 and 2000 found three new diseases in Alaskan native plants and an Alaskan isolate of barley yellow streak mosaic virus (BaYSMV). The following studies of these diseases will continue in 2001, and specifically will target:

1) the epidemiology and biology of virus-infected Lupinus nootkatensis plants in Hatcher Pass, Alaska, and genomic characterization of the virus isolates, 2) the biology of diseased Pyrola asarifolia in Hatcher Pass, Alaska, 3) the biology and identity of virus from infected Delphinium glaucum, and 4) the biology and genomic characterization of the Alaskan isolate of BaYSMV, and transmission studies of the mite vector, Petrobia latens.

A new project will involve surveying streams and rivers in Alaska for water-borne plant viruses and phages. This will overlap with the virus-lupine epidemiology study, and some aspects will be in collaboration with Cynthia Eayre’s water surveys in California (USDA, ARS, Fresno, CA).

The development of a monocot gene expression vector using foxtail mosaic virus will be ongoing during the fall/winter season.

Plant Germplasm activities
Survey plants at the AKPMC site that are part of the NAPGRU for plant pathogens

ARCTIC PLANT GENETIC CURATOR, PALMER, ALASKA
David C. Ianson, Horticulturist/Curator

Activities

Germplasm Activities

The 2000 season marked the real beginning of field planting for the National Arctic Plant Germplasm Genetic Resources Unit. 272 accessions were planted comprising 75 genera. The initial focus has been on planting and establishment of genera transferred from the WRPIS in Pullman Washington. The bulk of the accessions planted came from the following genera: *Poa* (38 accessions), *Juncus* (27 accessions), *Carex* (73 accessions), *Calamagrostis* (16 accessions), *Artemesia* (12 accessions). The rest of the collection that was planted for increase ranged from cuttings of native medicinals like *Oplopanax horridus* (Devils Club, Alaska Ginseng) to seeding six accessions of *Crepis* (Hawk’s-beard). The emergence and/or survival of all accessions taken together was 25%. At first observation this doesn’t seem to be a good number but when looked at in the light of the viability and/or ecophysiology of these native plants 25% survival is not unexpected. Many of the accessions sent to us were old and non-viable. In addition, it is not uncommon for native plants to have physical and chemical inhibitors that prevent germination under unfavorable conditions. This is borne out by the fact that some of the accessions that were given up for dead, but not thrown out are beginning to germinate after one year. We have finished conducting viability tests based on Tetrazolium staining for metabolism and have found that many of the accessions had extremely low viability.

Growout Activities

We received seed of *Brassica oleraceae var. botrytis* (Cauliflower) from NE-9, Geneva, New York for grow-out under Alaskan conditions. The cool season vegetable curator in Geneva felt that these particular ten accessions may be better suited to grow under Alaska’s cooler summer weather and 20 h daylength. Two of the shorter season accessions did produce seed under our conditions when planted out in mid June of 2000. We feel that putting them out sooner (mid to late May) will result in excellent seed production under our conditions as the vegetative portion of the plants did well under the cooler weather and longer days. We also received seed of *Schizandra chinensis* (Strawberry Vine) from the National Clonal Repository in Corvallis, Oregon during the second quarter of 2000. These five accessions are currently growing in our greenhouse following a five month cold stratification.

Research Activities
An experiment involving the effects of acid scarification, cold stratification temperature and time on *Oplopanax horridum* (Devils Club) was started in August. It was a 3 way, multifactorial with the independent factors being: 1. Duration of acid (HCl) scarification; Duration of cold stratification at 4 C; and actual germination temperature. The combination of factors resulted in a 24 way factorial with 3 repetitions for a total of 72 experimental units.

Results: The results of this experiment were that none of the *Oplopanax horridus* germinated under these experimental conditions.

We also set-up an experiment in which we transplanted taxa of the following genera: *Carex, Festuca, Poa, Polemonium, Scirpus, Spartina, and Calamagrostis* either into specially designed and portable miniature wetland beds (Fig. 1) or into 6" round pots. The above taxa were split up; and 75% of each accession placed in the portable bed. The remaining 25% of each accession were left to overwinter in the greenhouse (4 - 10 C)

Survival and vigor data from this experiment will be gathered this spring.

Talks and Presentations
Jul. 19-20 Presented results of the first years efforts of the Germplasm Transfer Subcommittee to the PGOC in Beltsville.

Travel, Invitations, Special Awards and/or Assignments

Jan. 14-17 Traveled to Albany, California, to attend the New Scientist Orientation.
Jun. 23-25 Traveled to Palmer, Alaska, to attend the Willow Workshop Seminars.
Jul. 18-22 Traveled to Beltsville, Maryland, to attend the PGOC Meeting.
Oct. 22-27 Traveled to Baltimore, Maryland, to attend the USDA-GRIN meetings.

Committees, Other Assignments, Activities and News

Chairman of the Plant Germplasm Operations Committee subcommittee on transfer of germplasm between stations.

Given numerous tours at the National Arctic Plant Germplasm Resources Unit including a contingent of farmers from Austria and Plant Science and Horticultural Sciences classes at the University of Alaska, Anchorage.

Spoke to the Palmer branch of the Kiwanis (11/14/2000) on the mission and activities of the NPGS and on the National Arctic Plant Germplasm Resources Unit in particular.

Assembled and hosted the 2000 W6 meetings in Wasilla, Alaska (June 26-28).

Plans for 2001

Establish Palmer as a grow out site for short season cauliflower (*Brassica oleraceae*) in order to help the NE9 priority site

Seed production on a number of taxa collected from Attu in Aleutians

Establishment of Palmer as the priority site for rhubarb (*Rheum*) accessions

GRIN Updating - Getting the Palmer site firmly established as the priority site for those species in GRIN that are economically important or are genetically and/or chemically related to economically important crops, are circumpolar in nature, and are not currently being maintained elsewhere within the NPGS system.

Establish Palmer as a viable grow out site for those crops, which by their nature cannot produce viable seed elsewhere within the NPGS system.

Continue to update the infrastructure of the site in order to produce seed and maintain the germplasm assigned to us as best we can.

Establishment of a Circumpolar Crop Advisory Committee made up of scientists and growers from academia and the private and governmental sector.