# 2003 Michigan Report to NRSP-6

**From**

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## INTRODUCTION

At MSU, we conduct a multi-disciplinary program for potato breeding and variety development that integrates traditional and biotechnological approaches. We conduct variety trials of advanced selections and field experiments at MSU research locations (Montcalm Research Farm, Lake City Experiment Station, Muck Soils Research Farm and MSU Soils Farm), we ship seed to other states and Canadian provinces for variety trials, and we cooperate with Chris Long on 17 grower trials throughout Michigan. Through conventional crosses in the greenhouse, we develop new genetic combinations in the breeding program, and also screen and identify exotic germplasm that will enhance the varietal breeding efforts. With each cycle of crossing and selection we are seeing directed improvement towards improved varieties (e.g. combining chip-processing, scab resistance and late blight resistance). In addition, our program has been utilizing genetic engineering as a tool to introduce new genes to improve varieties and advanced germplasm for traits such as solids, insect resistance and disease resistance. We feel that these in-house capacities (both conventional and biotechnological) put us in a unique position to respond to and focus on the most promising directions for variety development and effectively integrate the breeding of improved chip-processing and tablestock potatoes.

The breeding goals at MSU are based upon current and future needs of the Michigan potato industry. Traits of importance include yield potential, disease resistance (scab, late blight and early die), insect (Colorado potato beetle) resistance, chipping (out-of-the-field, storage, and extended cold storage) and cooking quality, bruise resistance, storability, along with shape, internal quality and appearance. We are also developing potato tuber moth resistant lines as a component of our international research project. If these goals can be met, we will be able to reduce the grower's reliance on chemical inputs such as insecticides, fungicides and sprout inhibitors, and improve overall agronomic performance with new potato varieties.

## I. Varietal Development

### Breeding

The MSU potato breeding and genetics program is actively producing new germplasm and advanced seedlings that are improved for cold chipping, and resistance to scab, late blight, and Colorado potato beetle. For the 2003 field season, progeny from over 600 crosses were planted and evaluated. Of those, the majority were crosses to select for round whites (chip-processing and tablestock), with the remainder to select for yellow flesh,

long/russet types, red-skin, and novelty market classes. In addition to crosses from the MSU breeding program, crosses were planted and evaluated from collaborative germplasm exchange from other breeding programs including North Dakota State University, University of Minnesota, and the USDA/ARS program at the University of Wisconsin as part of the Quad state cooperative effort. During the 2003 harvest, about 1800 selections were made from the 45,000 seedlings grown. Following harvest, specific gravity was measured and potential chip-processing selections were chipped out of the field. All potential chip-processing selections will be tested in January or March 2003 directly out of 42°F and 50°F storage. Atlantic (50°F chipper) and Snowden (45°F chipper) are chipped as check cultivars. Selections have been identified at each stage of the selection process that have desirable agronomic characteristics and chip-processing potential. At the 8-hill and 20-hill evaluation state, 400 and 150 selections were made, respectively.

**Chip-Processing**

Excellent chip-processing selections have been identified in the breeding program, despite switching to a more stringent screening temperature (42 vs. 45°F storage) a few years ago. Over 70% of the single hill selections have a chip-processing parent in their pedigree. Of those selections, about 75% have a SFA chip score of 1.5 or less. Based upon the pedigrees of the parents we have identified for breeding cold-chipping potato varieties, we have a diverse genetic base. We believe that we have at least eight cultivated sources of cold-chipping. Examination of pedigrees shows up to three different cold-chipping germplasm sources have been combined in these selections. Our promising chip-processing lines are MSF099-3 (42°F chipper), MSG227-2 (scab resistant 45°F chipper), MSH095-4, MSH094-8, MSH067-3, MSH228-6, MSJ147-1, MSJ126-9Y, MSJ167-1, and late blight resistant chipper MSJ461-1.

Dr. Joe Sowokinos, Univ. of Minnesota, has conducted biochemical analyses of our best chipping lines and has discovered that our lines differ from older varieties in their proteins involved in chipping. His analysis will allow us target specific crosses to find improved chip-processing varieties that will allow processing from colder storage temperatures.

**Tablestock**

Efforts have been made to identify lines with good appearance, low internal defects, good cooking quality, high marketable yield and resistance to scab and late blight. Our current tablestock development goals now are to continue to improve the frequency of scab resistant lines, incorporate resistance to late blight along with marketable maturity and excellent tuber quality, and select more russet lines. From our breeding efforts we have identified mostly round white lines, but we also have a number of yellow-fleshed and red-skinned lines, as well as long, russet type and purple skin selections that carry many of the characteristics mentioned above. We are also selecting for a dual-purpose russet, round white, red-skin, and improved Yukon Gold-type yellow-fleshed potatoes. Some of the tablestock lines were tested in on-farm trials in 2003, while others were tested under replicated conditions at the Montcalm Research Farm. Promising tablestock lines include MSE221-1 as a scab resistant tablestock, while MSE018-1 is a high yielding tablestock with a large oval shape. Boulder (MSF373-8) is a high yielding line with large tubers that also

chip out of the field. Michigan Purple also performs well. In addition, all these clones performed well in the dry land trial at Montcalm Research Farm. MSE192-8RUS and MSE202-3RUS are two russet table selections that have excellent type and scab resistance. MSI005-20Y and MSJ033-10Y are yellow-fleshed lines with smooth round appearance and high yield potential.

**Disease and Insect Resistance Breeding**

Disease screening for scab has been an on-going process since 1988. Results from the 2003 MSU scab nursery indicate that 42 of 190 lines evaluated demonstrated little to no infection to common scab. In addition, 23 other MSU breeding lines showed moderate scab resistance. The limitation of breeding for scab resistance is the reliance on the scab nursery. The environmental conditions can influence the infection each year, thus multiple year data provides more reliable data. A laboratory-based screening process is currently under development that would use thaxtomin in tissue culture to expedite selection of material with potential scab resistance. Secondly, the scab nursery space has been full. In response, we have spent 3 years developing a second scab nursery. In 2004, we will begin early generation evaluation of scab reaction in the breeding program. This additional effort should lead to more clones with scab resistance.

Since the mid-1990's we have directed efforts to identify sources of late blight resistance and use this resistance to breed late blight resistant varieties. At MSU, we have also participated in the national late blight trial and we have conducted our own efforts to use field and greenhouse screening to identify additional sources of resistance that can be used by the breeding community. In the past 7 years the MSU breeding program has intensely evaluated over 1200 crosses between late blight resistant x late blight susceptible parents and have identified parents that transmit strong late blight resistance to the highest percentage of the offspring. This year we added an early generation screen which will improve our ability to select late blight resistant lines.

As of 2003, based upon 7 years of inoculated field experiments, we have at least 8 sources of foliar resistance to the US8 genotype of *Phytophthora infestans* (Mont.) that have different pedigrees from which their resistance is derived. The resistance in Jacqueline Lee has now held resistance for 7 years of testing. MSJ461-1, the chip-processing selection, has the same late blight resistance source Jacqueline Lee and was resistant to a US-17 genotype of *Phytophthora infestans* in New York this year. Our other promising late blight resistant lines that have been tested in replicated agronomic trials are MSJ317-1, MSI152-A, MSJ453-4, MSK136-2, MSL159-AY, MSL179-AY and MSL211-3 (see Potato Variety Evaluation Report for agronomic data). In each of these lines, the resistance is based on a single resistance source. If we rely on a single source of resistance, the varieties developed from this strategy may be overcome by *P. infestans* at some future date that we cannot predict. Therefore, the most effective breeding strategy is to combine resistance from different pedigrees to build a more durable resistance. Our efforts are now focusing on pyramiding the different resistance sources. This year we added a transgenic strategy using the late blight resistant *RB* gene cloned from *S. bulbocastanum*. We should have initial field tests using transplants in 2004.

We also introduced an early generation Colorado potato beetle screen at the Montcalm Research Farm. From this screen we identified 32 individuals with either transgenic or non-transgenic foliar resistance to Colorado potato beetle. Eighteen lines were categorized as moderately resistant and 36 were susceptible.

Single-hill selections in 2003 also had an exciting number of individuals with pedigrees for potential late blight, Colorado potato beetle or scab resistance or some combination of the three. Of the single hill selections, 75% of progeny have at least one late blight parent, 15% have a Colorado potato beetle resistant parent, and 25% have a scab resistant parent in its pedigree.

**II. Evaluation of Advanced Selections for Extended Storage: MSU Potato Breeding Chip-processing Results From the MPIC Demonstration Commercial Storage**

The MSU Potato Breeding Program has been conducting chip-processing evaluations each year on potato lines from the MSU breeding program and from other states. For 4 years we have been conducting a storage study to evaluate advanced breeding lines with chip-processing potential in the Dr. B. F. (Burt) Cargill Potato Demonstration Storage facility directly adjacent to the MSU Montcalm Research Farm. In October 2002, tuber samples from 6 lines in the Montcalm Research Farm trials were placed in the bin to be cooled to 48°F. Tubers from another 9 lines were placed in the bin that was to be cooled then held at 51°F. The first samples were chip-processed at MSU in October and then, each month until June 2003. Samples were evaluated for chip-processing color and quality.

In the 48°F bin, Snowden was the check variety. In April the Snowden and MSH095-4 chips began to go off-color. In contrast only MSG227-2, MSH094-8 and MSF099-3 and W1201 maintained acceptable chip color throughout the storage sampling. Of these lines, MSG227-2, MSF099-3 and MSH094-8 maintained the lightest chip color throughout the storage season. MSG227-2 also has scab resistance.

In the 51°F bin Atlantic and Pike were used as check varieties and both varieties chip-processed acceptably until May. Of the 7 advanced breeding lines evaluated Liberator chip-processed acceptably throughout the storage season until June. Liberator offers chip-processing from storage and scab resistance. MSJ461-1 had the most consistent chip color throughout the storage season until May. MSJ461-1 also offers strong foliar late blight resistance along with the chip-processing quality; however the solids content can be lower than other chip-processing lines. UEC also had good chip color until the May sampling.

In addition, Liberator and MSF099-3 was grown by Sandyland Farms and placed in one of the 500 cwt bins. Despite field frost occurring in MSF099-3's harvested tubers, the potatoes chip-processed successfully out of the bin in April 2003 at Utz. The Liberator bin was sent to Shearers in March and produced a good chip product.

## III. Germplasm Enhancement

In 2003, about 5% of the populations evaluated as single hills were diploid. From this breeding cycle, we plan to screen the selections chip-processing from storage. In addition, selections were made from over 2,000 progeny that was obtained from the USDA/ARS at the University of Wisconsin. These families represent material from South American potato species and other countries around the world that are potential sources of resistance to Colorado potato beetle, late blight, potato early die, and ability to cold-chip process. About 100 selections were made among the diploid material in 2003. Through GREEEN funding, we were able to initiate a breeding effort to introgress leptine-based insect resistance. From previous research we determined that the leptine-based resistance is effective against Colorado potato beetle. We will continue conducting extensive field screening for resistance to Colorado potato beetle at the Montcalm Research Farm and at the Michigan State University Horticulture Farm in 2004.

## Late Blight Breeding and Genetics: Mapping Late Blight Resistance in three Populations

A high priority objective of the breeding program is to identify sources of late blight resistance and use these sources for breeding varieties with late blight resistance. In 1999 we initiated a set of studies to identify the genes in potato associated with late blight resistance. If we can identify the genes that contribute to late blight resistance we feel that we could more effectively breed varieties with durable late blight resistance. A diploid potato population was developed with the objectives to map quantitative trait loci (QTL) conferring resistance to *Phytophthora infestans* (Mont.) de Bary and other agronomic traits using simple sequence repeats (SSR) and isozymes and to examine associations between late blight resistance and other agronomic traits. The mapping population was a cross between a late blight resistant selection of *Solanum microdontum* Bitter and a susceptible diploid advanced breeding clone. A second diploid population derives its late blight resistance from *S. berthaultii*. The third population is tetraploid and the resistance comes from Jacqueline Lee. Based upon field trials at the Muck Soils Research Farm, Bath, MI between 1999 and 2002, we have identified major late blight resistance genes in the three populations. Currently, one chromosome region containing the resistance is linked to a genetic marker has been identified in *S. microdontum*. This past year we identified a major QTL associated with late blight resistance was found in the tetraploid population and multiple QTLs for late blight resistance in the *S. berthaultii* mapping population. These QTLs should be suitable for marker-assisted selection to introgress a new source of resistance to *P. infestans* to the cultivated tetraploid germplasm of potato.

The tetraploid cross for mapping (Jacqueline Lee x MSG227-2) offers more than just mapping late blight resistance genes. This cross has traits such as late blight resistance, scab resistance, chip-processing, specific gravity, maturity all segregating at one time. This summer we screened a sub sample of the population for scab reaction. A number of the progeny showed little scab. In 2004 we hope to screen a greater number of the population.

## IV. Integration of Genetic Engineering with Potato Breeding

### Assessment of Natural (Glandular Trichomes and Glycoalkaloid-Based) and Engineered (*Bt-cry3A*) Potato Host Plant Resistance Mechanisms for Control of Colorado potato beetle: Caged no-choice studies.

The Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), is the leading insect pest of potato (*Solanum tuberosum* L.) in northern latitudes. Host plant resistance is an important tool in an integrated pest management program for controlling insect pests. A field study was conducted in 2003 to compare natural (glandular trichomes (NYL235-4) and glycoalkaloid-based (ND5822C-7)), engineered (*Bt-cry3A*: NO8.8, Atlantic NewLeaf, *Bt-cry1Ia1*: Spunta G2) host plant resistance mechanisms of potato for control of Colorado potato beetle. Six different potato lines representing 5 different host plant resistance lines were evaluated in caged studies (no-choice) at the MSU campus farms. Each cage with 10 plants represented one plot. The cages were arranged in a randomized complete block design consisting of three replications. Twenty egg masses were placed on the plants in each cage. Observations were recorded weekly for a visual estimation of percent defoliation by Colorado potato beetles, and the number of egg masses, larvae, and adults. The *Bt-cry3A* transgenic line and the combined resistance line were effective in controlling feeding by Colorado potato beetle adults and larvae. The high glycoalkaloid line had less feeding, but the beetles clipped the petioles, which led to greater defoliation in the first few weeks. Foliage re-growth occurred by the end of the season. The glandular trichome line suffered less feeding than the susceptible control. Spunta G2 was effective in limiting defoliation, but larval mortality was not as high as in the *Bt-cry3a* lines. Based on these results, the *Bt-cry3A* gene in combination with glandular trichome mechanism is an effective strategy that could be used to develop potato varieties for use in a resistance management program for control of Colorado potato beetle.

### Bt-cry3A-transgenic line Agronomic Trial

In 2001 and 2002, we had extensive field testing for agronomic performance in replicated trials of our most advanced *Bt-cry3A* transgenic lines. Based upon 2001 agronomic performance and 2002 Bt-cry3A protein concentrations in foliage, 12 of 26 transgenic lines were eliminated. In general, the *Bt-cry3A* transgenic lines had similar agronomic and tuber characteristics compared to the non-transgenic parental line. These selections represent a diverse portfolio of *Bt-cry3A* lines that could be commercialized if the intellectual property rights and regulatory requirements could be met. We will maintain these lines in our program. These lines are MSE018-1, NYL235-4, NY123, Jacqueline Lee, Onaway, Norwis and Spunta. If the acceptance of transgenic food crops becomes deregulated, we will consider these lines for commercialization. In 2003 we developed a new Bt-cry3A construct that uses a different gene promoter. We are currently transforming MSJ461-1 and Michigan Purple.

### USAID-funded International project to Develop Potato Tuber Moth Resistant Potatoes

Potato tuber moth, *Phthorimaea operculella* (Zeller), is the most serious insect pest of potatoes worldwide. The introduction of the *Bacillus thuringiensis* (Bt) toxin

gene via genetic engineering offers host plant resistance for the management of potato tuber moth. The primary insect pest in Egyptian potato production, like many other countries in the Middle East, is the potato tuber moth. In the field, the moths lay their eggs on the potato foliage and the hatched larvae mine the foliage and the stems. This feeding damage leads to irregular transparent tunnels in the leaves and weakening of the stem. The larvae attack the tubers through infected stems or directly from eggs, which are oviposited on exposed tubers or where soil cracks allow moths to reach the tubers. Larvae mine the tuber in the field and in storage reducing potato quality and increasing the potential for pathogen infection. Field and storage studies were conducted to evaluate *Bt-cry5* potato lines for resistance to potato tuber moth in Egypt under natural infestations and their agronomic performance in both Egypt and Michigan. From 1997-2001, field experiments were conducted at the International Potato Center (CIP) Research Station, Kafr El-Zyat, Egypt and/or Agricultural Genetic Engineering Institute (AGERI), Giza, Egypt to evaluate resistance to tuber moth.

Two transgenic 'Spunta' clones, G2 and G3, have been identified that produced high control levels of mortality in first instars of potato tuber moth in laboratory tuber tests (100% mortality), and field trials in Egypt (99-100% undamaged tubers). Reduced feeding by Colorado potato beetle first instars was also observed in detached-leaf bioassays (80-90% reduction). Field trials in the U.S. demonstrated that the agronomic performance of the two transgenic lines was comparable to 'Spunta'. We are currently working with USAID, Syngenta and South Africa to commercialize the Spunta-G2 and Spunta-G3 lines. We have also transformed Atlantic, Lady Rosetta and Jacqueline Lee with the *Bt-cry5* gene. We hope to have approval to field test these in Mexico some time in the future.

## V. Variety Release

The MSU breeding program has now named and released its first varieties and is in the process of licensing the new varieties to the Michigan Potato Industry Commission. Three potato varieties were released in 2001: Jacqueline Lee (MSG274-3), Liberator (MSA091-1), and Michigan Purple. MSU is currently licensing the first 3 varieties to MPIC and working out procedures to market these varieties. Boulder (MSF373-8) was released in 2003. Virus-free tissue culture plantlets are maintained at MSU.

## Development of a DNA-based Fingerprint System for Potato Varieties

The ability to quickly and accurately identify potato clones is important to potato breeding programs and to the potato seed industry and commercial growers. Since 1990, the Michigan State University Potato Breeding and Genetics Program has used an isozyme-based fingerprint system to identify potato cultivars. Isozyme analysis has been an economical and effective means of discriminating potato clones; however, they require fresh, healthy tuber or leaf tissue. DNA-based fingerprinting using simple sequence repeats (SSRs or microsatellites) has been shown to discriminate between potato clones.

The objective of this study was to identify those SSR primer combinations that accurately and efficiently distinguish clones on polyacrylamide and agarose gels. SSR primer combinations used were based on polymorphism levels in previous tetraploid studies from PCR amplification products. DNA isolated from 17 potato clones representing chip processing, tablestock, russet and red market classes were visualized on both polyacrylamide and low melting point (Metaphor ®) agarose gel systems. Eighteen SSR primer combinations were screened on both gel systems. Polymorphism was observed in all eighteen of the primer combinations on polyacrylamide (PAGE) and fourteen on agarose gel systems. The PAGE system was determined to be the preferred system for variety identification, but agarose can be used to differentiate lines when specific varietal comparisons need to be made. The primer combination STM0031 with STACCAS3 was able to differentiate all 17 clones on agarose. In addition, five different DNA source tissue types were evaluated (fresh foliar, freeze-dried foliar, fresh tuber skin, freeze-dried tuber skin, and freeze-dried tuber tissue). Amplification products were similar for all five tissue sources used for DNA isolation. This ability to isolate DNA from freeze-dried tissue will allow us to fingerprint varieties when fresh tissue is not available. The SSR fingerprinting system presented here can be used as a practical fingerprint system for cultivated potato. This research will be published in the American Journal of Potato Research in 2004.

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Units: 11 Re-planting of FRN-3 and PL-132.
Units: 75 Early GA treatment experiment:
Units: 10 VERGEO follow-up project:
Units: 2 FRN DNA project with Alfonso.
Units: 32 GA drying vs normal GA treatment for germ comparison:
Units: 328 P-less fen project:
Units: 36 Additions to TUB planting because of low germ.
Units: 44 Good TZ - zero germ material for germ testing.
Units: 13 Ver-hjt Project Planting List.
Units: 107 Additions to the Texas Sugar End Planting List.
Units: 1 Grow to see if it has pear shaped fruit.
Units: 36 Back-Cross planting.
Units: 328 P-less fen project - second attempt:
Units: 77 JAM material for Lisa field for late blight work.
Units: 576 2003 - Original Seedling Selection Project:
Units: 198 2003 - Three-day GA treatment experiment.
Units: 72 2003 - Inbreeding Infertility project.
Units: 118 Benchmark for homogeneity/homozygosity via bandyield.
Units: 2 Diversity check for transplanted material:
Units: 63 Texas Sugar End Planting List.

Boster, Mary HORT
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Units: 13 Plethora of varieties to study tuber flavor and color.

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Units: 2 Planted in screening nurseries for Verticillium wilt and Late blight:
Units: 51 Planted in screening nurseries for Verticillium wilt and Late blight:

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Units: 3 gourlayi accessions for calcium work.

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Units: 5 Accessions to determine if taxonomy is predictive of avr/R-gene interaction
Units: 202 Accessions to determine if taxonomy is predictive of avr/R-gene interaction

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Units: 5 Variety trial.

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Units: 6 Varietal breeding.

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Units: 11 Plethora of varieties to study tuber taste and size:

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Units: 2 S. mat for vergeo/hjertinii project.
Units: 3 Tubers for Isozyme testing.
Units: 2 In bred S. frn for band yield experiment.

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Units: 2 50 seeds of S. pnt for late blight breeding.

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Units: 24 In vitro clones for LB trial.
Units: 9 Foreign varieties for tuber moth work:
Units: 91 Tuber clones for LB trial.
Units: 48 In vitro clones for LB trial.

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Units: 514 Accessions for molecular work.

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Units: 50 Plaisted breeding stocks for breeding work.

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Units: 6 Biochemical genetics.

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Units: 12 Clones to plant in a Florida trial.

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Units: 11 Plethora of varieties to study tuber flavor and color:

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Units: 37 Seeds for tuber trait research stocks:
Units: 8 Tuberization study.
Units: 25 Seeds for tuber trait research stocks:
Units: 4 Seeds for tuber trait research stocks:
Units: 8 Tuberization study.
Units: 24 Predictibility of Sclerotinia resistance based on taxonomic species.
Units: 1416 24 plants of each for tuber trait research:
Units: 178 Predictibility of Sclerotinia resistance based on taxonomic species.
Units: 13 Tuberization study.

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Units: 1 Late Blight breeding.

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Units: 12 Plethora of varieties to study growth rate, flavours and textures:

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Units: 8 Genetics work with Jiming Jiang.

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Units: 12 Seeds for PSTV heat treatment assay.
Units: 156 Seeds for plant vigor evaluation and seed increase:
Units: 10 Seeds for germination:

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Units: 3 Solanum verrucosum seeds.

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Units: 18 Seeds for doing genetic enhancement work:
Units: 64 Seeds for doing genetic enhancement work:
Units: 5 Seeds for doing genetic enhancement work:

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Units: 11 Plethora of varieties to study tuber flavor and color.

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Units: 12 Solanum tubers of chc, phu and tar for nutrition work.
Units: 46 Accessions for tuber family generation for nutrition work.

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Units: 4 Study of signaling pathway of the disease resistance genes.
Units: 5 Study of signaling pathway of the disease resistance genes.

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Units: 10 Tubers to test for tracking A-II isozymes from diploid germasm.

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Units: 2 Accession for TAXON study:
Units: 2 Accession for TAXON study:
Units: 2 Problem accessions for re-identification:
Units: 11 Accession for TAXON study:

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Units: 52 Taxonomic work for David Spooner.
Units: 9 Taxonomic work for David Spooner.

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PHONE: (330) 263-3876 FAX: 330-263-3887

Units: 2 Low Temperature Gene Expression Experiments

Stupar, Robert GENET University of Wisconsin
rmstupar@facstaff.wisc.edu Department of Horticulture
Madison Wisconsin 53706
PHONE: **** FAX: ****

Units: 3 Identification of candidate ploidy-regulated genes.

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Thompson, Dr. Asunta ENTOM North Dakota State University
asunta.thompson@ndsu.nodak.edu Department of Plant Sciences
Fargo North Dakota 58105
PHONE: (701) 231-8892 FAX: ****

Units: 170 Solanum andigena accessions for beetle resistance:

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Tong, Cindy GENET University of Minnesota
c-tong@umn.edu Department of Horticultural Science
St. Paul Minnesota 55108
PHONE: (612) 624-3419 FAX: 612-624-4941

Units: 18 Pink or red skinned varieties:

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Vega, Ms. Sandra GENET University of Wisconsin
svegasem@facstaff.wisc.edu Department of Horticulture
Madison Wisconsin 53706
PHONE: **** FAX: ****

Units: 1 S. gourlayi tubers for cold breeding:
Units: 109 Research stocks of cold breeding material:

**** = INFORMATION NOT PROVIDED BY COOPERATOR