PROCEDURES:

The Regional Research Project NE-9 will continue to serve as a conduit for movement of valuable plant genetic resources from worldwide origins to the northeastern states as well as the entire United States (see Tables 6-8). As such, the objectives and the procedures of the project are organized as a continuum. Some objectives (e.g., 1-4) will be performed primarily by personnel of PGRU, whereas latter objectives (4-6) will be responsibilities of the individual cooperators associated with NE-9 activities. Therefore, those responsibilities of, and procedures utilized by, personnel of the PGRU will be presented in greatest detail.

1. To acquire, maintain/regenerate, characterize, document, and distribute plant genetic resources for use in the Northeast and the United States.

Acquisition

Germplasm is acquired in a number of different ways at Geneva. It may be transferred from other domestic repositories, breeder's collections (foreign and domestic), foreign repositories, private individuals, or by means of both foreign and domestic exploration and collection trips. Properly stored and shipped seed or pollen accessions can be accepted at any time, while clonal accessions are preferably obtained as dormant cuttings or rooted plants. Occasionally we receive fruits, from which seeds are extracted and equilibrated before being placed in permanent storage at Geneva. With the recent hiring of a new full-time seed curator, who will begin work in September of 1998, PGRU will certainly increase acquisition of new seed accessions.

Any foreign acquisitions will be brought in via the National Plant Germplasm Quarantine Office in Beltsville or via United States Animal and Plant Health Inspection Service import permits granted to cooperators, e.g., Robert Pool (Cornell University), and Dennis Gonsalves (Cornell University). Depending on the particular crop, seed importation may be subject to quarantine restrictions; clonal accessions always are. Impetus for foreign germplasm explorations comes from the respective Crop Germplasm Committees. The recognition that windows for exploration into certain regions of the world open and close, sometimes unpredictably, catalyzes explorations into specific areas of the world during opportune times. Currently explorations to and exchange with nations in the Caucasus, Central Asia, and China have high priority for PGRU. Collecting trips to one or more of these regions have been conducted annually since 1993 and already are planned for 1998 and 1999. PGRU anticipates that at least one foreign exploration for *Malus* and one domestic collecting trip for *Vitis* or *Malus* will be carried out each year during the period of this project, 1998-2003. Most clonally propagated accessions will be acquired by exploration for and collection of wild material during the next 5 years.

Whenever possible, collection strategies will be based on molecular genetic information. In fact, the exploration to collect *Malus sieversii* in Central Asia in 1996 used a collection strategy that was based in part on results of allozyme research conducted in cooperation with Norman Weeden (Cornell University).

Domestic explorations for germplasm will be conducted by members of PGRU and/or staff of the Plant Exchange Office of the National Germplasm Resources Laboratory. A successful exploration for *Vitis rupestris* was conducted in 1997. An exploration is being conducted in 1998 for *Vitis shuttleworthii* and *Vitis monticola*. Plans for the next five years include hiring a post-doc to continue exploration and collection of native North American grapes to add to the PGRU collection, which was a priority set by the Grape Crop Germplasm Committee.

Maintenance / Regeneration

Regeneration of a seed accession is done when germination falls below 60% or the number of seed available for distribution falls below 5000. Seed regenerations are conducted using the appropriate pollination techniques and pollinators. For example, allogamous (cross-fertilized) species require controlled pollinations to ensure the genetic integrity of the seed produced. Bumble bee, honey bee, solitary bee, fly, or hand pollination is done, as appropriate, in the greenhouse, screenhouse, field, or pollination cages. After extraction of seed from fruits, seed are left at 1 °C and 25% relative humidity until equilibrated. These are then stored under optimal conditions in a freezer room at -18 °C, in heat-sealed, moisture-proof, foil-lined bags. In addition, duplicate seed of many accessions are backed up in cold storage or cryopreservation at the National Seed Storage Lab (NSSL) in Fort Collins, Colorado. Eventually all seed accessions will be backed up in this fashion. During the next five years, PGRU plans to regenerate an average of 300 tomato, 100 *Brassica*, 100 onion, 25 cucurbits, and 30 *Raphanus* (radish) annually. Some of these regenerations will be carried out at the USDA-ARS regeneration site in Parlier, California, which enables PGRU to regenerate a greater number of total accessions.

Clonal crops are maintained, in the case of grapes, as duplicate pairs of plants in a vineyard, grown side by side. All grapes in our collections are own-rooted. In the case of apples, each cultivar is currently maintained as a two plants, one grown on seedling rootstock in one orchard and one on dwarfing rootstocks at another orchard location. During the next five years, two scions of each apple accession will be grafted to EMLA-7 fireblight tolerant rootstock, and the other two orchards (on seedling and dwarfing rootstocks) will be removed. Nearly 70 % of the apple collection is currently cryopreserved at NSSL and/or Geneva in the form of dormant buds, and 98% of the fireblight susceptible apples already are backed up in this way. Non-cold-hardy apples are almost always stored as seed accessions. In the rare case that a non-cold-hardy apple needs to be preserved in clonal form, they are grown in the greenhouse or screenhouse. Secure backup of such material would involve development of a preservation technique such as slow growth in vitro or tissue culture. No method yet exists for secure backup of grape accessions, although work to develop a reliable procedure is in progress. Seed collections of wild germplasm of apple and grape are also maintained at Geneva and backed up at NSSL.

Except under unusual circumstances, we rarely need to repropagate clonal accessions. In the case of grapes, severe winter injury or rarely phylloxera may kill a plant completely. In such cases, layering is used to repropagate a dead plant using the duplicate, if the duplicate is healthy and vigorous. Repropagation from dormant grape cuttings is also commonly done. In the case of

apple, trees are rarely lost, except to fire blight. Repropagation of apple can be achieved via bud grafting of cryopreserved apple buds to the appropriate rootstock. More often, apples are repropagated from dormant cuttings by whip and tongue grafting. Scion material is obtained either from the duplicate in our collection or from external sources. Because of increasing severe outbreaks of fireblight, the entire apple collection is being repropagated on EMLA7 fireblight tolerant rootstock. This will be completed during 1998-2003.

Documentation

All PGRU collections are documented in Paradox databases. For each genus, there are three databases: passport (taxonomy, pedigree, origin, etc.), inventory (number of plants, propagules, or DNA quantities on hand and related information), and characterization (plant characteristics). Most information contained in local databases is also stored in the GRIN (Genetic Resources Information Network) database at the National Germplasm Resources Laboratory at Beltsville, MD. Relevant information about the plant genomes of plants in the Geneva collection are stored in the following plant genome databases: RoseDB (which is designed to hold information about all rosaceous crops including apple and tart cherry), CabbagePatch (*Brassica*), SolGenes (*Lycopersicon, Solanum*), and VitisDB (grape). The GRIN and Plant Genome databases are publicly accessible over the Internet. Plans for the next five years include at least tripling the amount of data contained in these databases, as well as including additional crops, particularly in the RoseDB, which ideally should hold data on *Prunus* (almond, sweet cherry, peach, apricot, plum), *Fragaria* (strawberry), and *Rubus* (raspberry, blackberry, and their relatives). Data for these crops would come from other National Plant Germplasm System repositories.

Characterization

Characterization of PGRU's major crops, apple, grape, vegetable Brassica, tomato, winter squash, and *Allium*, is carried out for characteristics designated by the respective Crop Germplasm Committees. These generally include vegetative characters of leaves, stems, and flowers, and fruit characteristics, such as size, shape, color, flavor, sugar content, etc. Characters are assessed according to international standards, if they exist. For example, grape characterization utilizes the OIV (Office International de la Vigne et du Vin) standard descriptors, rating scales, and values. Ideally, characterization data should be collected from multiple locations to assess genotype by environment interactions. For example, our apple core subset is planted at five locations throughout the U.S. Because, we lack a good deal of characterization data, particularly for our seed crops, we initially emphasize observations made at Geneva, which can serve as a baseline of information for larger scale multi-location studies. Plans are to complete characterization of the apple, tart cherry, and grape collections during the next three years. Characterization of seed crops can only be done during regeneration, i.e., when we have living plants to examine, so that characterization schedule depends upon regeneration schedule for a particular crop. Since the tomato collection is almost completely characterized, emphasis during the next five years will be on the other crops that are planned for regeneration: *Brassica*, onion, cucurbits, and radish.

Distribution

PGRU distributes germplasm in five different forms: seeds, dormant cuttings, pollen, green cuttings, and DNA. Generally, private overnight delivery services (UPS, FedEx, etc.) are used for shipment; no special handling is necessary for seeds (coin envelopes), pollen (microfuge tubes or vials), or DNA (microfuge tubes). Dormant cuttings and green cuttings are shipped in airtight plastic bags, with the green cuttings sprayed lightly with water before packaging. Record keeping for order processing is completely automated. We expect orders for distribution of DNA will increase over the next five years.

2. To ensure the identity of each accession as to species and cultivar

Species identifications are carried out using modern plant taxonomic keys for the appropriate geographic region of the world. Proper identification of hybrids usually depends on obtaining pedigree information about an accession, although some hybrids can be identified as intermediates between parental species. Occasionally, DNA data can shed light on proper species identification. Clonal accessions can be reidentified any year during the growing season, usually when flowers are present. Seed accessions can be reidentified only during regeneration of those accessions, which may occur infrequently.

Cultivars are identified in two ways. First, by considering passport information that accompanied the accessions when they entered the germplasm system. Comparison of plant features to those which are characteristic of an accession assists in determining whether the accession is true-to-type. If an accession is not true to type, it may be difficult to determine exactly which cultivar it is. International and domestic experts are sometimes consulted about the identity of specific accessions.

More and more, we are able to use DNA fingerprints to determine the identity of an accession. Simple sequence repeat DNAs (SSRs) have been particularly useful in this regard, and research in this area will continue at PGRU. So far, using SSRs, we have identified 2 genetic duplicates out of 110 in the grape collection and 7 apparent genetic duplicates out of 66 in the apple collection. The duplicate grape accession was a mixup during propagation, while two of the apple duplicates were incorrectly identified. The other five apple duplicates represented a cultivar and one of its sports. In the time period, 1998-2003, plans are to completely fingerprint the apple, grape, and tart cherry collections using SSRs.

3. To determine the basis for and the extent of genetic variations, the geographic distribution of cultivated species, and their taxonomic relationships with closely related species.

At PGRU we have used and will continue to use whatever methods are appropriate for quantifying genetic variation. Methods have included isozymes, random amplified polymorphic DNAs, simple sequence repeat DNAs (SSRs), and amplified fragment length polymorphisms (AFLPs). Automated fluorescence detection systems have been and will continued to be used in

analyzing for SSRs and AFLPs. This method considerably increases efficiency, reliability, and throughput. These techniques have satisfied a variety of PGRU's needs including: 1) partitioning genetic variability between plants, populations, species, and geographic regions, 2) determining relationships between accessions, and 3) fingerprinting cultivars. We will also utilize the expertise of taxonomic experts in specific crops to assist in the determination of the species identities of some of our accessions. In addition, one of the PGRU staff has extensive taxonomic knowledge which is regularly drawn upon. All plant populations sampled during foreign and domestic germplasm acquisition trips during 1998-2003 will be subjected to analysis of their genetic variation. In addition, all samples will be identified to the proper species, and if appropriate, relationships to other species already in the collections will be determined using cluster analysis, principal component analysis, and other statistical techniques.

4. To characterize and evaluate plant genetic resources for specific desirable traits.

In January of 1998, PGRU hired an apple rootstock breeder to evaluate the apple collection as well as the rootstock collection of Jim Cummins (Cornell University) for desirable traits. The goals of the rootstock breeder are to acquire and evaluate exotic germplasm for potential utilization in breeding, to develop improved breeding techniques and screening methods to accelerate development of germplasm with multiple resistances to biotic and abiotic stresses, and to provide fundamental knowledge on the nature and inheritance of traits necessary to produce horticulturally superior germplasm with commercial potential. Obviously, the activities of this breeder also interface closely with objectives 3, 5, and 6.

Other evaluations are carried out by cooperators and researchers who have the specialized knowledge, equipment, and technology to judge attributes such as disease and insect resistance, phytonutrient concentrations, and tolerance to environmental stresses or extremes. Particular areas of evaluation emphasis in recent years include screening for disease resistances, cold hardiness, leaf hairiness, and anti-oxidant concentrations. The resulting information flows back to PGRU and is entered into the local, GRIN, and, as appropriate, the Plant Genome databases. Evaluation activities depend in large part on the interests of cooperators and their ability to garner funding. The GAO Survey (1997) has recommended that both characterization and evaluation need to be increased in order to facilitate germplasm's use in crop breeding.

5. To determine the genetic mechanisms controlling the inheritance of important traits.

Work in this area is carried out almost exclusively by our cooperators and collaborators and will continue to be done in this way in the future. However, we have been fortunate that some of the same molecular markers we have used for DNA fingerprinting, e.g., simple sequence repeats, have also been able to be mapped in two of our genera, *Vitis* and *Malus*. PGRU's role was to develop marker loci in apple and grape, score the loci in mapping populations, and transmit that data to the owners of the genetic map. PGRU will continue to share such information with the owners of the maps for our crops and ensure that when appropriate it also be included in the Plant Genome databases in Ithaca, New York. Although this activity of PGRU does not directly

address genetic mechanisms, the type of data supplied can assist in mapping projects which do deal with this issue. We are also involved in generating additional molecular markers that will able to be placed on a genetic map of grape, by our participation in the *Vitis* Microsatellite Consortium, whose goal is to generate at least 200 different microsatellite loci in grape. Discovery of desirable traits in the evaluation phase must be followed up by studies that determine heritability and expression of the trait. Creation of populations segregating for disease resistance is one aspect of this objective that PGRU can take part in, and the building of a number of such segregating populations is already planned.

6. To combine genes from diverse sources into germplasm more useful to plant breeders and to breed, release, maintain, and evaluate improved germplasm and cultivars.

Some of the activities of the PGRU apple rootstock breeder naturally work toward meeting this objective, but more often this work is the responsibility of our collaborators and users of our germplasm. PGRU is not directly involved in germplasm improvement, although we are becoming more involved in the creation and maintenance of segregating or mapping populations that are formed by crossing of our germplasm accessions. Greater participation in this arena is being considered because it will contribute to greater utilization of the germplasm. In large part, PGRU's role in achieving this objective is simply to supply germplasm to cooperators in whatever form it is needed, seeds, cuttings, DNA, etc.

EXPECTED OUTCOMES:

Expected outcomes of this work will include:

1. Greater number of accessions, from a wider geographic range, will be incorporated into the germplasm collections at PGRU.

2. Genetically higher quality germplasm collections will be formed containing:
   a. fewer duplicate accessions
   b. fewer accessions that are not true-to-type
   c. seed accessions having greater genetic integrity (via better regeneration procedures)
   d. accessions having higher germination percentages

3. Collections will be made more secure:
   a. a higher percentage of seed accessions will be stored at the National Seed Storage Lab
   b. dormant buds of the *Malus* and *Prunus* collection will be completely backed up in cryopreservation
   c. preservation of *Vitis* collection in an alternative form, such as cryopreservation, tissue culture, slow growth in vitro, etc., will be effected
   d. living apple collection will be less subject to losses from fireblight

4. An increased percentage of accessions with accurate characterization information will be preserved

5. Genetic fingerprints will be available for most clonal accessions (apple and grape cultivars)

6. Core subsets will be established for all major PGRU crops, vegetable Brassicas, tomatoes, alliums, and cucurbits. (Core subsets already exist for apple and grape.) Quality of existing core subsets will be improved.

7. There will be easier access to and more complete information about PGRU's germplasm that will be accessible via GRIN and specific Plant Genome databases.

8. Knowledge of the location of valuable genes will be enhanced, both via characterization activities and by the sharing of marker loci information with the owners of the crop genetic maps.

9. Ordering germplasm will be easier for clients with Internet access, as germplasm catalogs will be on line (apple and grape catalogs already are).

10. More germplasm accessions will be accurately identified taxonomically.

Research results will be communicated via publications in peer-reviewed journals. Accession-specific information will be available in the GRIN database, and, when possible and appropriate, the Plant Genome databases.

ORGANIZATION:

Because of a continual and ever-increasing need for plant genetic resources the NE-9 project lacks a well-defined end point. It also follows that the organization and technical aspects of the project outlines will change only in points of emphasis from year to year.

Regional Research Project NE-9 can be effective only through federal, state, and private cooperation. The federal agency ARS, through acquisition, maintenance, characterization, documentation, and distribution activities, will make plant genetic resources available for evaluation and utilization research. ARS will provide support, staff, facilities, equipment, and specialized technical assistance at both the regional and national levels. The SAES provide facilities, some staff, equipment, utilities, and local assistance.

The NE-9 Regional Technical Advisory Committee will provide technical guidance in this effort. This committee is composed of an Administrative Advisor, Regional Coordinator, plus technical representatives invited to participate from each of the northeastern SAESs plus the District of Columbia. Also included as ex officio members are ARS representatives from the National Program Staff, the National Germplasm Resources Laboratory, the National Seed Storage Lab, and the National Plant Germplasm Quarantine Office. The names, affiliations, and areas of specialization of these individuals are presented subsequently (Attachments 1 and 2).

Other committees contribute to the planning and management and are active participants in the NPGS. These include:

1) The ARS Plant Germplasm Operations Committee evaluates and recommends foreign/domestic exploration proposals, and assists the NPGS, ARS National Program Staff and other officials with plans needed to manage the NPGS.

2) CGCs have been established for about 40 crops (or crop groups) to help advise the NPGS with regard to genetic vulnerability, gaps in current collections, operational procedures, evaluation needs, and current enhancement and utilization research associated with their specific commodity.

ATTACHMENT 1.

A. Project Leaders for the NE-9 Regional Research Project

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Plant Genetic Resources Unit

Northeast Regional Plant Introduction Station and
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ATTACHMENT 2.

RESOURCES ALLOCATION

REGIONAL RESEARCH PROJECT NE-9:
PLANT GENETIC RESOURCE CONSERVATION AND UTILIZATION

Participating State Agricultural Experiment Stations	Objectives						Resources[1]		
	1	2	3	4	5	6	SY	PY	TY
Connecticut									
New Haven				X			0.10	-	-
Storrs	X			X		X	0.05	-	-
Delaware	X		X	X		X	0.10	-	-
Maine	X			X			0.10	-	-
Maryland					X	X	0.25	0.30	-
Massachusetts			X	X	X		0.25	0.50	0.25
New Hampshire	X		X	X	X	X	0.25	0.05	0.50
New Jersey	X			X	X	X	0.30	-	1.50
New York									
Geneva	X	X	X	X	X	X	0.90	1.00	1.00
Ithaca	X		X	X	X	X	0.75	3.00	3.00
Pennsylvania				X	X	X	0.25	0.10	0.15
Rhode Island	X	X	X	X			0.15	-	0.375
Vermont[2]	X		X				0.10	-	-
West Virginia				X	X	X	0.05	-	-
SAES Subtotal							3.60	4.95	6.78

Participating Federal Agencies	Objectives						Resources		
	1	2	3	4	5	6	SY	PY	TY
USDA-Agricultural Res Serv									
Plant Genetic Res Unit	X	X	X	X	X	X	4.00	1.00	5.00
National Program Staff[2]	X						0.10	-	-
National Germplasm Resources Lab	X						0.75	0.50	0.20
National Seed Storage Lab	X						0.03	-	0.59
Federal Subtotal							4.88	1.50	5.79
Total							8.48	6.45	12.57

[1] SY = scientist years, PY = professional years, TY = technician years

[2] Estimate based on previous revision of this project.

SIGNATURES:

CONSERVATION AND UTILIZATION OF PLANT GENETIC RESOURCES

for _____ 7/29/98
Administrative Advisor - J. E. Hunter — Date

for _____ 7/29/98
Chair, Regional Association of Directors — Date

_____ 8/19/98
Administrator, Cooperative State Research, Education, and Extension Service — Date