

# OFFICIAL

NORTHEAST REGIONAL RESEARCH PROJECT NE-103 (Revised)

7/98

**PROJECT NUMBER:** NE-103 (Rev.)

**TITLE:** Postharvest Physiology of Fruits

**DURATION:** October 1, 1998 to September 30, 2003

## **JUSTIFICATION**

The North American temperate fruit industry is growing and continually changing to meet new challenges. The total per capita use of commercially produced fruits and vegetables was 686 pounds in 1995 (farm-weight basis), compared with 564 pounds in 1970 representing a 22% increase from 1970-1995. Approximately 80% of this increase occurred since 1982, the year in which an expert scientific panel organized by the National Academy of Sciences published its landmark report on Diet, Nutrition, and Cancer. This report emphasized the importance of fruit and vegetable consumption to reduce the risk of cancer. On a retail weight basis, fresh fruit consumption increased from 96 pounds per capita in 1970 to 120 pounds in 1995. In general, U. S. consumption of fresh and processed apples has trended upward since 1970. Fruit, especially pome fruit (apples, pears) continues to be an important and stable sector of the US agriculture economy. Results reported at the 1997 U. S. Apple Association Apple Crop Outlook and Marketing Conference, held in Chicago, Illinois on August 21-22, 1997, indicate that the 1996 crop, at 246.5 million bushels, was the sixth largest apple crop since records of commercial production have been kept. The record apple crop of 273.8 million bushels was produced in 1994. Total farm-gate value of apples produced in 1996 was \$1.67 billion. Exports of U. S. apples from the 1996 crop increased to approximately 34 million bushels from 29.5 million bushels in 1995. In 1996, production from the West at 164.5 million bushels accounted for 67 % of the national total. The crop in the Eastern and Central U. S. accounted for 33% of the total at 82.1 million bushels. Average production over the five year period of 1992-96, however, indicates that 26% of the crop came from the East, 13% came from the Central States, and 61% was from the West.

A high rate of cultivar turnover has long been accepted by growers of herbaceous perennials (e.g. strawberry). However, other industries that rely on fruit production from woody perennials (e.g. apple), which require a much longer period to realize a return on the investment, recognize the importance of rapid cultivar turnover but have been reluctant to practice it because of the lack of information on new cultivars. This perspective by the apple industry has recently changed due to the success of new cultivars such as Gala, Braeburn and Fuji, which possess eating and storage qualities superior to those of more traditional cultivars such as McIntosh, Jonathan and Cortland. In addition, world over-production of some fruits in some years has meant that fruit producers must consider diversifying in order to remain viable. The fact that some of the successful new cultivars cannot be grown in the northeastern United States reduces grower options and market strength, ultimately reducing the viability of northeast industries. There is a critical need to identify new cultivars of all fruit types which are optimally suited to regional climate and marketing demands.

As production reaches an all-time high and the export market is increasing, the orderly marketing and profitability of fruit-growing enterprises is increasingly reliant on advances in the science and technology of postharvest handling. Managing postharvest losses in perishable commodities such as fruit is the most cost-effective means of increasing profitability, as production inputs have already been realized. Fresh fruits and vegetables, because of the added cost of harvesting and handling, increase several-fold in unit value while moving from the field to the consumer.

One of the primary driving forces behind changes in research needs for the storage of North American fruit has been due to the recent globalization of produce marketing. Management of postharvest diseases and disorders is one of the most complicated aspects of perishable commodity production, especially if producers wish to penetrate and compete successfully on global markets. Some fruit commodities like cherries, raspberries and peaches have a short storage and marketing life with conventional cold storage. Alternately, an increase in the volume of imported fruit has affected the North American market in two important ways: First, importing fruit has exposed the US consumers to a wider variety of high quality produce, and has led to the expectation that a diverse array of unique and desirable products will remain available. For example, the consumer is being presented with new and exciting apple varieties which often provide premium prices for the producers and wholesalers. Second, many of these varieties imported during the off-season have quality attributes that are superior to domestic varieties that have been in storage for six or more months, hence quality standards have increased significantly for North American producers.

Stringent quality requirements compel domestic fruit industries to apply ever more technically advanced and tightly coordinated handling and storage procedures to market fruit that will have a similar table quality to that of imported fruit. Importantly, apples grown in the northeastern US and Canada are at a particular disadvantage in the marketplace given the more traditional mix of fruit being grown in that region. As a result, existing practices are being re-evaluated by all segments of the fruit industry. There is, for instance, intense interest among Northeastern apple producers in developing replacement varieties with superior texture and flavor attributes and improved storability, if only to compete for their own markets with producers from other regions of the US, as well as the rest of the world. Also, the North American fruit industry is threatened with the loss of critically important agricultural chemicals such as DPA, fungicides and methyl bromide, the latter of which is used to disinfest exported fruit crops. Collectively, market, political and safety forces place fruit industries at significant risk as they seek to develop storage technologies that will maintain a high quality, wholesome food product while minimizing its dependence on agri-chemicals.

Another increasingly-important issue is consumer concern about the residues left on produce by postharvest chemical treatments. Fungicides currently registered are quite effective in controlling storage rots of a variety of fruits and vegetables. However, many consumers are becoming concerned about the use of fungicides on fruit and vegetables and are demanding residue-free commodities. Some foreign markets won't accept chemically treated fruits and vegetables. As a result, the use of these treatments is becoming more restricted because of the association, either real or imagined, with human maladies. It is of paramount importance, therefore, to investigate alternate strategies to reduce produce losses in storage.

Of the physiological disorders affecting apple fruit, the one of greatest concern currently is superficial scald, which affects several important varieties of apples and pears during storage. Scald is typified by a brown to black stain-like lesion on the skin accompanied by the collapse of hypodermal cells. In the U.S., scald prevention costs approximately \$.20 per bushel. In addition, the treatment chemical, diphenylamine (DPA), is a drench or dip, which involves drenching load after load of apple fruit with the same batch of DPA. The repeated rinsing of fruits from various orchards leads to a large inoculum build-up in the treatment water, which causes decay problems, making the inclusion of a fungicide such as thiabendazole (TBZ) necessary. According to the 1993 Pesticide Data Program Report of the Ag. Marketing Service of the USDA, the two most frequently found chemical residues on apple fruit are DPA and TBZ, which occur on 51% and 35% of apples, respectively. Perhaps just as important is the disposal problem presented by the relatively large quantities of drench solution needed to treat fruit. At present, the only tenable disposal/treatment alternative is the use of lined lagoons to house the discarded drench material, allowing it to undergo photo-degradation. Efforts in the proposed NE-103 project will be directed toward the control of superficial scald using methods that avoid wetting the fruit and avoid the need for fungicide treatment. If the research proposed here is successful, DPA use could be avoided;

thus the need for TBZ use would be reduced dramatically with a concomitant reduction in the residues frequency of both compounds and the cessation of disposal concerns. Further, considerable reduction in the per bushel storage costs to the grower would result with the potential for savings to be passed on to the consumer.

Previously, justification for NE-103 emphasized apple production in the Northeastern United States, but there are now broader interests in various fruits as well as a greater geographical distribution of State Agricultural Experiment Stations, Federal Laboratories, and Universities contributing to this project. Originally, 6 states participated in the project, but there are currently 12 states and 3 Canadian Provinces. The project continues to concentrate on the problems leading to postharvest losses of fruit grown in the Northeastern United States. However, the other collaborators encounter similar problems, and provide additional climate differences, research facilities, and experience that may facilitate the solution of scientific and technical problems leading to quality maintenance and reduced losses of stored fruit.

The commercial use of CA for fruit storage represents a major success story for NE-103 since the research leading to industry use of this practice was due to the efforts of the members of the NE-103 Technical Committee. This work remains ongoing as new varieties and new physiological and pathological problems dictate the necessity of fine tuning CA regimes to meet these new challenges. More contemporary research by members of the committee involves molecular manipulation of various fruit characteristics to improve storability and quality.

The members of the NE-103 Technical Committee are the leading scientists involved in postharvest research throughout the United States and Canada, and have published over 231 refereed papers, 191 non-refereed papers and 236 abstracts during the 1993-1998 project period. Committee Members were significant contributors to the program at the recent (July, 1997) Seventh International Controlled Atmosphere Research Conference attended by about 300 participants. They were responsible for the research presented in 44% of the posters and papers on temperate fruit storage and chaired two of the three oral sessions. The conference chairman and a member of the organizing committee were members of NE-103. Since the meeting dates of NE-103 are set to coincide with the International Controlled Atmosphere Research Conference or the Gordon Conference on Postharvest Physiology, each of which convenes every four years, many overseas conference attendees have been visitors at the NE-103 meeting.

It is hoped that as a mature research project, NE-103 will be able to sponsor an event such as an international symposium or similar meeting as alternative to the annual project meeting at least once during the next cycle of funding. There are a variety of topics under active investigation that would be suitable choices.

## **RELATED CURRENT WORK AND PREVIOUS WORK**

A search of the Current Research Information System indicated that there were 126 active State Agricultural Experiment Station and Agricultural Research projects related to temperate fruit research. Of these, 45 involved production, fruit processing or plant pathology. Sensory evaluation, mostly from an engineering prospective, accounted for 11 projects. There were 29 projects related to fruit breeding, germplasm or new cultivars, of which 12 were concerned with small fruits. Of the 17 projects related to the pear, peach and apple fruits, only 3 were identified which studied the storage potential and problems of newer apple cultivars, a proposed area of research of the first objective of the new Project. There were 41 projects directly relating to research in the area of postharvest physiology of fruit, and of these, only 9 were being conducted by researchers who were not members of the NE-103 Technical Committee. As of the last Project Revision, there were two Northeast Regional Projects which had some commonalities with NE-103. However, neither NE-87 (Control of Postharvest Decay of Fruits and Vegetables) nor NE-116 (Quality Maintenance and Nutritive Aspects of Vegetables during Processing and Storage) is

currently active. The NE-183 (Multidisciplinary Evaluation of New Apple Cultivars) regional project is almost exclusively focused on the production aspects of new apple cultivars.

A major priority of past NE-103 projects has been to investigate relationships between storage quality and preharvest factors of variety, maturity and growing practices, which are known to markedly affect postharvest performance (Bramlage, 1993). Through studies on the mineral nutrition of apple fruit, we have improved our understanding of the mechanisms of calcium action (Burmeister and Dilley, 1993; Conway and Sams, 1987; Picchioni et al., 1995; Whitaker et al., 1997a) and developed prophylactic measures to treat fruit both pre- and post-harvest (Bramlage, 1993; Conway et al., 1994a,b; Roy et al., 1996). Due in part to the efforts of the NE-103 project, the problem of calcium-related disorders is essentially no longer of commercial significance in North America. In addition, much has been learned about the effect of growing conditions on storage quality, but while we have identified major effects of such conditions on the ability of fruit to withstand postharvest treatments such as low O<sub>2</sub> (Lidster et al., 1985; Park et al. 1993; Lau et al., 1998) and high CO<sub>2</sub> (Bramlage et al., 1977; Burmeister and Dilley, 1995; Lau and Looney, 1978; Watkins et al., 1997), the reasons for this variation are not yet understood. An understanding of this variation is essential, especially for growing regions such as the northeastern US, which experience wide yearly variations in climate. These variations in climate are critical as it can be argued that the limitations to utilization of new technologies and cultural tools lies less with the performance of that technology than with the variable responses of fruit to it. For instance, fruit varieties vary markedly in their response to, and tolerance of, low O<sub>2</sub> (Gran and Beaudry, 1993a,b; Park et al., 1993) and high CO<sub>2</sub> (Burmeister and Dilley, 1995; Lau and Looney, 1978; Watkins et al., 1997). Apparently, responses to both gases are further affected by growing environment (Bramlage et al., 1977; Lau and Looney, 1978; Lau et al., 1998). Variable responses of fruit to storage may be related to effects of growing environment on diffusivity of the skin to CO<sub>2</sub> and O<sub>2</sub> and temperature sensitivity, an area that has received little attention. Varieties also vary widely in susceptibility to storage disorders such as superficial scald, low temperature breakdown and senescent breakdown (Barden and Bramlage, 1994a,b; Barden and Greene, 1997; Wolk et al., 1998) and postharvest factors need to be taken in to account in selection of new varieties for North American apple industries.

A major focus of the proposed studies therefore, will be to evaluate the responses of new fruit varieties to storage in relation to growing region. The geographical composition of the team involved in the NE-103 Project provides a unique situation where responses of any variety to a wide range of growing conditions can be studied efficiently. Such studies provide essential linkages to concurrent studies on productivity and other horticultural factors carried out in the NE-183 Regional Project, whose objectives are to:

1. Evaluate horticultural qualities and pest susceptibilities of new apple cultivars, strains and advanced selections at numerous locations throughout the United States to determine both the limitations and the positive attributes of these cultivars.
2. Develop horticultural and pest-management strategies for new cultivars or cultivar strains that are emerging as commercially-accepted cultivars
3. Compare the costs of production and profitability of new apple cultivars.

The important aspect of postharvest preservation of the quality of new and emerging apple cultivars is lacking in the NE-183 regional project. However, we are presented with a highly promising opportunity for collaboration in that a number of the NE-103 members are presently active in both regional projects. Without question, the varieties tested at the production level in the NE-183 project will vary in their susceptibility to postharvest disorders, decay and pest pressure. It would be highly prudent to examine the responses of selected varieties to low oxygen, high carbon dioxide and temperature as influenced by growing region. Specialist skills within the NE-103 group will allow interdependent activity to occur whereby fruit from different regions can be examined for factor responses in different laboratories

The NE-103 group is currently addressing the problem of superficial scald control through the development of cultural tools to reduce scald, and the improvement of our fundamental understanding of scald development. In terms of the cultural tools being evaluated, the use of low oxygen atmospheres to control the disorder in Delicious apples without DPA application, has been intensively studied as a collaborative project (Lau et al., 1998). The study indicates that this technology cannot be used in all growing regions because of varying effectiveness in controlling scald as well as higher susceptibility to low oxygen injury in some fruit. The protocol, therefore, needs further work and may require refinement on a regional basis. Other approaches include use of ethanol (Wang et al., 1997) and high carbon dioxide shock (Watkins, unpublished) treatments and if successful these will be tested for effectiveness in different growing regions.

Recent studies on the biology and biochemistry of scald development, many of which have been conducted by NE-103 members, have revealed much needed information that has improved our understanding of superficial scald markedly. Our present knowledge of the factors and mechanisms involved in the phenomenon is as follows:

As fruit mature on the tree they become somewhat less susceptible to scald development. Fruit are harvested and placed in cold storage (generally 0 to 2 C) where, after two to three months, symptoms begin to appear (Emongor et al., 1994). The disorder has many of the same elements as other forms of chilling injury (Watkins et al., 1995). Scald is unusual in one aspect, however, the involvement of the isopentenoid pathway, which is activated during ripening (Knee, 1972) and produces farnesyl pyrophosphate (FPP). FPP, synthesized on the endoplasmic reticulum and requiring 5 and 10 carbon precursors produced in the plastids (chloroplasts), is converted to  $\alpha$ -farnesene by farnesene synthase in a single step that does not require farnesol as an intermediate (Rupasinghe et al., 1997). Alpha-farnesene accumulates in the cuticle and epicuticular wax, perhaps by volatilization and diffusion, or alternatively it may be transported in vesicles along with other hydrophobic components of the epicuticular coating. The low temperature of cold storage enhances accumulation of  $\alpha$ -farnesene due to changes in sorption characteristics of the cuticle and epicuticular wax (Wee and Beaudry, unpublished data), and may also stimulate or induce farnesene synthase activity. The  $\alpha$ -farnesene in the cuticle begins to oxidize to conjugated trienols (CTs), which are, for all practical purposes, non-volatile (Rowan et al., 1995, 1996; Whitaker et al., 1997b). Production of CTs from  $\alpha$ -farnesene may occur strictly through autoxidation, as has long been believed, but the recent finding that about 90% of the CT consists of a single isomer of one compound indicates that a lipoxygenase-like enzyme could be involved. The accumulated CTs autoxidize to yield three primary ketone products, 6-methyl-5-hepten-2-one (MHO), acetone and methyl vinyl ketone, which can be isolated from the epicuticular wax of stored apple (Wee and Beaudry, 1996; Whitaker, unpublished data). MH can induce scald-like symptoms (Song et al., 1996a). The oxidation products of  $\alpha$ -farnesene lead to tissue damage. DPA prevents the oxidation of  $\alpha$ -farnesene, and at high concentrations may also inhibit  $\alpha$ -farnesene synthesis. The level of antioxidants and antioxidant enzymes are related to fruit susceptibility to scald (Barden and Bramlage, 1994b; Du and Bramlage, 1995a,b). As fruit ripen, their resistance to oxidative stress declines. Cellular damage takes place. Cellular damage is greatly accelerated by warming fruit to room temperature. This correlates with a burst of MH production as the fruit are warmed (Mir and Beaudry, unpublished). Also, autoxidation of CTs, yielding MHO, is markedly enhanced by increasing temperature (Whitaker, unpublished data). Finally, a genetic basis of scald has recently been demonstrated (Weeden, 1993) and material from crosses between susceptible and resistant material used to show that antioxidant enzyme activities may be associated with its incidence (Rao et al., 1998). Investigations into scald have also been extended to re-examine the ameliorative effect of wounding on scald development (Abdallah et al., 1998).

As a result of the direction and the momentum of research into the nature and control of scald by postharvest physiologists world wide and by members of the NE-103 in particular, there is real promise for rapid and significant progress.

Progress in the more global postharvest areas of decay and pest control in storage environments has been slower, however. Unfortunately, in the past five years, the options for postharvest agricultural chemical use have declined sharply. Agri-chemicals no longer available or under severe restriction for postharvest use include Botran, Benlate, Captan, and Ethoxyquin. For apples in the northeastern US, for instance, there is only one fungicide available for use, thiabendazole. Due to the limited number of registered fungicides, fungicide resistance and the desire to reduce fungicide residues, fruit industries are anxious to obtain effective alternatives for decay control. Fortunately, the sealed environment provided by controlled atmosphere (CA) storages and modified atmosphere packaging (MAP) makes possible the use of biologically active volatiles. Interestingly, the study of the use of biologically active gases has largely remained within the purview of postharvest physiologists, rather than pathologists.

CO<sub>2</sub> is perhaps the best-known volatile to have fungistatic properties. A high CO<sub>2</sub> concentration in the storage atmosphere (e.g., 15 to 20% and above) is able to extend shelf-life by acting as a fungistat. Historically, research into the augmentation of storage atmospheres with CO<sub>2</sub> dates to the 1930's when it was determined that doing so would retard softening and decay under conditions of poor temperature control (a commonly encountered problem at that time) (Brooks et al., 1936; Thornton, 1931). Initially, dry ice was used as a CO<sub>2</sub> source. As the technology for temperature control improved, the necessity of a CO<sub>2</sub> supplement declined. Nevertheless, CO<sub>2</sub> has been shown to inhibit fungal decay of numerous economically important horticultural crops (Li and Kader, 1989; Smith, 1938; Brooks et al., 1936) and is a common practice for some highly perishable, high-value commodities such as strawberries, raspberries and cherries. While not all horticultural commodities can withstand CO<sub>2</sub> levels sufficient to inhibit fungal activity (Kader et al., 1989), a number of highly perishable commodities are not adversely affected. Further research is required to define tolerances as a function of concentration and duration of exposure. Importantly, CO<sub>2</sub> (with and without low O<sub>2</sub>) has also recently been found to have the potential to assist in disinfestation of fruit for export (Mitcham et al., 1997). The loss of methyl bromide in the near future will require the use of alternative quarantine procedures. Research in this area is active and holds significant promise.

In addition to CO<sub>2</sub>, a number of generally recognized as safe (GRAS) natural organic compounds can be used to retard or eliminate decay (Prasad and Stadelbacher, 1973; Vaughn et al., 1993; Wilson et al., 1987). Interestingly, some of these classes of compounds (e.g., aldehydes) are naturally metabolized to aroma compounds (De Pooter et al., 1983; Hamilton-Kemp et al., 1992). The possibility that they can be applied for their antifungal activity, but be removed by the innate ability of the treated fruit to convert them to aroma compounds has been investigated (Song et al., 1996b). No residues of the applied material was detected. Other volatile materials with antifungal potential include H<sub>2</sub>O<sub>2</sub>, ozone and acetic acid. Much needs to be learned, however, before these approaches can be said to have real potential. Subjects for investigation include effects on other pathogenic fungi and bacteria such as *E. coli*, the temperature-sensitivity of this technique, packaging interactions, effectiveness of aroma precursors other than hexanal, and the breadth of fruit species amenable to these treatments.

As indicated above, tolerance of fruit to low O<sub>2</sub> and high CO<sub>2</sub> is also a major focus for researchers in the NE-103. As discussed earlier, there is considerable variation in tolerances of fruit to both gases (Gran and Beaudry, 1993a,b; Lau and Looney, 1978; Lau et al., 1998; Park et al., 1993; Watkins et al., 1997). The importance of CO<sub>2</sub> in apple storage has also been highlighted with both external and internal CO<sub>2</sub> injuries in Braeburn, Empire and Fuji (Burmeister and Dilley, 1995; Elgar et al., 1998; Grant et al., 1996; Watkins et al., 1997). These injuries have affected both local and export marketed fruit. These differential tolerances impact our ability to use these gases to extend storage periods of fruit, to use MAP successfully and/or disinfest fruit. The effects of carbon dioxide on metabolism of fruit has been studied intensively (e.g. Gorny and Kader, 1996a; Ke et al., 1994, 1995; Lange and Kader, 1997). Gene products that are induced or repressed by CO<sub>2</sub> in strawberry have been identified (Watkins and Zhang, 1998).

Ethylene production is critically important to the maturation and ripening processes in climacteric fruit such as the apple. Intensive research has identified key characteristic of ACC oxidase (Dilley et al., 1995; Fan et al., 1996; Gorny and Kader, 1996b; Poneleit and Dilley, 1993; Wilson et al., 1993a,b) and an ELISA system for detection of the enzyme has been developed (Dilley et al., 1996). Rosenfeld et al. (1996) have been studying ACC synthase.

Procedures for the transformation of apples has been developed overseas (Yao et al, 1995) and researchers are producing transgenic apples containing antisense copies of key ripening-related genes. Where appropriate, the apple genes are being tested in transgenic tomatoes (Bolitho et al. 1997). There is also considerable effort in isolating other genes which may have important roles in the storage and/or ripening of fruit, and characterizing the function of the respective proteins (e.g. Reid and Ross 1997). Research following these lines is also being carried out in the US (Hrazdina, unpublished). Fruit will eventually be available for investigation of their horticultural characteristics.

Despite the progress cited here, important postharvest problems such as loss of fruit texture, changes in flavor, ethylene sensitivity, resistance to decay and chilling injury have proven somewhat intractable and research at a fundamental level is required. The use of biochemical and molecular tools also will be essential in the resolution of these problems. Several members of the NE-103 have active programs using these tools. Recent efforts within the NE-103 have touched upon each of these fields and include the discovery of biologically active oligomers of cell wall pectins (Melotto et al., 1994) and genes related to cell wall metabolism (Wu, et al., 1993), the description of CO<sub>2</sub>-responsive genes in strawberry (Watkins, unpublished), the discovery of methylcyclopropene, a specific inhibitor of ethylene binding that has significant commercial potential (Sisler and Blankenship, 1993, 1996), and the discovery that the electron transport capacity of mitochondria may be linked to the sensitivity of fruit to chilling temperatures (Purvis, 1997a,b, 1993)

For fruits, an often overlooked aspect of quality is the volatile compounds associated with aroma and flavor, no doubt due to the demanding techniques necessary for analysis. With the advent of fused-silica capillary gas chromatography, it was possible to build on earlier work and determine not only the nature and amount of volatile aroma compounds within and around apple fruit, but to determine the influence of harvest maturity (Mattheis et al., 1991a) and storage conditions (Mattheis et al., 1991b, 1995) on their occurrence. Further studies identified important aroma compounds in sweet cherries (Mattheis et al., 1992a), as well as when they appear during ripening and what happens to them during storage (Mattheis et al., 1992b; 1997). Recent studies focused on biosynthesis of ester compounds, important fruit "flavor notes". A method was developed to assay for alcohol acetyltransferase (Fellman and Mattheis, 1995) in apples, and used to determine the relationship between enzymatic activity during fruit maturation and storage, and recovery of biosynthetic capacity upon removal from storage (Fellman et al., 1993a,b).

## **OBJECTIVES**

The long term and ultimate objective of this project is to provide wholesome, nutritious, high quality fruit for the consumer at low cost and with minimal postharvest loss.

Specific objectives are:

1. To evaluate postharvest requirements of new and existing temperate fruit varieties.
2. To develop sustainable alternatives to chemical control of physiological disorders, diseases, and pests.
3. To expand fundamental knowledge to improve and create new technologies to assure high quality and wholesomeness of fruit and enhance market opportunities.



## **PROCEDURES**

### **Objective 1. To evaluate postharvest requirements of new and existing temperate fruit varieties.**

As mentioned in the Justification, the critical need to identify new cultivars of all fruit types that are optimally suited to regional climate and marketing demands will be addressed, with a focus on apple fruit. First we will perform postharvest evaluations to complement the existing NE-183 regional project, which conducts production-related evaluations of new apple cultivars, and second, plans are to continue evaluation of existing cultivars in order to establish optimal handling and storage conditions.

Complementing the NE-183 project is our major collaborative and interdependent work plan under this objective. Currently, 26 new cultivars and selections are planted in controlled field trials in over 15 sites across the US. An additional cycle of plantings is planned. In the upcoming season, most of the trees from the first planting will be producing their first full crop. Storage performance is an essential element in evaluating these cultivars/selections. The involvement of the NE-103 Technical Committee members will create a continuum in evaluation of these fruit extending from the field through the storage period and into retail sales, where the consumer is directly affected. Early postharvest evaluation should help avoid the discovery of limiting characteristics after significant investment and planting by the apple industry.

Five stations (MA, MI, NC, NY-I and PA) will evaluate cultivars from various NE-183 sites within their region. MA, MI and NC have single sites available at the respective horticultural experiment stations. Two sites are also available at USDA sites in WV. In NY, three sites are available and evaluations will be made at those other than Ithaca when appropriate. In PA there are two NE-183 sites (Biglerville and State College).

Each station will utilize the same standard protocols for evaluating maturity and storage ability of new cultivars. These protocols will be discussed with members of the NE-183 to ensure that our objectives complement theirs, and where applicable, stations that do not have a current member of the NE-103 group will be invited to participate in our project. At present, basic harvest maturity parameters such as flesh firmness, soluble solids and starch index ratings are made weekly by NE-183 project members. For the purposes of optimizing fruit storage, these data will be complemented with internal ethylene measurements and evaluations of performance under air storage in terms of firmness retention, palatability and disorder incidence. Because of the number of selections, evaluations will be scheduled to ensure that manageable numbers of fruit are assessed each year. At least four selections will be studied in detail each year. As fruit numbers allow, CA storage trials will be initiated. In CA storage trials, quality retention as a function of maturity and condition at harvest and storage atmosphere and duration, will be evaluated. Storage studies that require large numbers of CA storage chambers will be conducted in PA due to the high capacity of their excellent facilities.

Measurements of specific physiological characteristics such as aroma volatile emissions, skin resistance, respiration, and tolerance limits for O<sub>2</sub> and CO<sub>2</sub> will be assigned to specific stations with the appropriate expertise and equipment (MI, NC, NY-I, USDA-MD, WA). NE-103 members have developed and actively shared expertise and protocols for most of the postharvest evaluation techniques needed to address this objective. In situations where regional environmental conditions could influence the outcome of the study but appropriate analytical capability is not available at specific locations, fruit samples will be exchanged among stations. Results obtained from this objective will provide information about the importance of preharvest factors on tolerances of fruit to elevated CO<sub>2</sub> and low O<sub>2</sub>, and is interdependent with Objective 2, in which manipulation of the levels of these two gases will be evaluated for its potential as an alternative to chemical control of diseases, pests and physiological disorders, and Objective 3, in which the



underlying biological principles of fruit responses to these gases are to be investigated. The information derived from this work will prevent large plantings of inappropriate cultivars, and identify best-management practices for worthy new cultivars.

Analyses to determine the composition and amount of volatile compounds will be performed using headspace and purge-and-trap techniques developed by members of the group (MI, WA, USDA-WA). These data will be useful to ascertain the capacity for aroma/flavor regeneration after long-term storage and may assist in the non-destructive detection of physiological stress (related to Objectives 2 and 3).

NY-I and MI will carry out analyses of fruit density and skin resistance on fruit from the NE-103 plantings. These data will be important to quickly identify cultivars that may be more sensitive to low O<sub>2</sub> or elevated CO<sub>2</sub> concentrations. Integrated with this line of research, MI will perform storage tests using a modified atmosphere packaging approach that will determine tolerance limits to O<sub>2</sub> and CO<sub>2</sub> as well as measure rates of gas exchange. Tolerance limits to low O<sub>2</sub> and elevated CO<sub>2</sub> will also be established by non-destructive measurements of chlorophyll fluorescence and ethanol production (NS).

Collectively, this information is needed to identify the suitability of various cultivars for mixing in CA storage rooms. Cultivars that differ markedly in their low O<sub>2</sub> tolerance may require separate storage facilities, or a commitment by commercial producers to create special handling techniques. Data on respiration are needed to estimate cooling demand and relative rates of deterioration. These data may also be useful for modified atmosphere packaging applications in the marketplace.

The second major work plan concerns the postharvest evaluation of existing cultivars. Some overlap of these tasks with those described above exists, as some of the cultivars being evaluated by NE-183 have already been planted extensively throughout North America. Our focus here, however, is to address specific quality issues limiting the storability of several popular cultivars. Again, as the knowledge base expands, information obtained here is expected to integrate with knowledge derived from the fundamentally basic research planned under Objective 3.

Examination of flavor characteristics of Gala apples will be performed as an expansion of previous efforts by NE-103 members. Loss of flavor is a limiting factor for storage of Gala, so a cooperative project between PA and USDA-MD has been established to determine optimum storage conditions to maintain flavor in fruit of this cultivar. Volatile compounds contributing to Gala aroma are being identified in a cooperative project between USDA-W and OSU-Corvallis. USDA-W is also evaluating volatile production and quality retention in Gala fruit following dynamic CA regimes.

Another important project will be reduction of watercore in Fuji apples, as fruit of this cultivar grown in northern North America frequently exhibit this disorder at harvest. Evidence indicates that preharvest temperatures may affect the propensity of fruit to develop watercore. For example, Fuji apples grown in Washington are very susceptible to watercore, whereas those grown in California show only minor watercore, even though they are harvested at a more advanced maturity. Development of protocols to minimize the risk of internal breakdown due to watercore will focus on predictions based on preharvest temperatures (CA, MA, MI, NC, NY-I, PA, WA), and on delay of CA establishment, CA conditions (atmosphere and temperature) and storage duration (WA). Because Fuji is one of the cultivars grown in the NE-183 trial, fruit will be available from a variety of growing locations for assessment of the effects of preharvest temperatures on watercore development of this cultivar. Temperature and watercore development data will be collected from all stations at which Fuji are grown (CA, MA, MI, NC, NY-I, PA, WA) and will subsequently be used to develop a preharvest temperature watercore-prediction model using expertise developed by MA for prediction of superficial scald (see Objective 2).

Collaboration of this type enables us to obtain data on a wide range of temperature conditions quickly for more accurate and rapid model development.

Improving methods to estimate fruit maturity forms the final project under this work plan. The Streif-index [firmness/(starch content x soluble solids)], which has been used successfully in Europe, will be evaluated for its capacity to accurately estimate the optimum harvest window for various cultivars (NS). Two years of commercial evaluation on McIntosh, Cortland and Jonagold in NS has shown it to be a simple, cheap and accurate method to predict final harvest date for each orchard. It is not known if this index will be universally applicable across all climates represented by locations of NE-103 members, but since, under this objective, the requisite data are continuously acquired for many cultivars of apples, its predictive value at different locations should be easy to assess. In addition, MI is developing a promising new method for assessing apple maturity, the 'dip-stick' ELISA for ACC oxidase, which is an immunochromatographic assay similar to the pregnancy test. Prototypes of the ELISA should be available for testing by scientists in other states by the 1998 season. Extensive inter-regional collaboration is expected in evaluating this technology in relation to other maturity indices.

Additional benefits to be realized from progress under this research objective lie in the close coordination with efforts under Objectives 2 and 3. Examination of many different cultivars will facilitate comparative studies of disorder mechanisms, volatiles associated with disorders, composition and regeneration of flavor compounds, molecular biology, and mechanisms of ripening and softening.

**Objective 2. To develop sustainable alternatives to chemical control of physiological disorders, diseases, and pests.**

Within this objective, three avenues of investigation will be undertaken with goals of developing control or management mechanisms for the storage problems of apple and pear scald, fungal decay and insect pests. The focus will be to develop techniques that minimally impact the environment, have the potential to reduce agricultural chemical residues, or provide consumer-acceptable alternatives. Efforts are to be coordinated such that one control strategy does not preclude the use of another. Research for this objective will be coordinated by NE-103 committee members who are recognized leaders in the indicated research areas.

*Physiological disorders:* Control of superficial scald in apple and pear. We have categorized the means for scald control as targeting either scald avoidance or scald reduction. In the proposed project, the priority is to focus on the storage disorder of apples and pears known as superficial scald. Promising scald control mechanisms will be integrated with traditional storage protocols and subjected to a systems evaluation. The criteria for evaluation will be ease of transition, profitability, and sustainability of storage strategies.

Avoidance of superficial scald will be studied primarily through the use of predictive tools. The goal is to minimize postharvest chemical usage by predicting when the use of antioxidants is necessary. Mathematical prediction models developed by the previous project (MA) require further testing and refinement at various locations throughout the U.S. A multi-year cooperative project will be initiated between the eastern and western growing areas (MA, MI, NY-I, ONT-V, PA, WA, NS) to refine these models using the Delicious cultivar grown in varied climates. At MI and ONT-V, the effects of fruit maturity will also be studied. A similar dependency of scald on preharvest temperatures will be investigated for d'Anjou pear fruit (OR). Relationships between preharvest temperature and scald incidence will be used to generate a mathematical model for scald prediction for pear. Variations in the content of scald-related compounds such as antioxidants and/or enzymes may also be used to improve the predictive model developed from the temperature data.

Reduction of scald using non-chemical techniques will be studied using approaches developed by members in the previous NE-103 Project. Continuous low O<sub>2</sub> treatments for scald reduction in commercial conditions requires further examination due to problems with off-flavor development in fruit from some locations, especially the Northeast, and variable efficacy of treatment. In our recently published NE-103 cooperative study (Lau et al., 1998) we used 0.7% O<sub>2</sub> + 1.0% CO<sub>2</sub>. However, Delicious apples in NS were not given the 1% CO<sub>2</sub> treatment and scald was not reduced. Another cooperative scald study (BC, NS, ON-V, CA, OR, MI, WA, NY-I, PA, NC, MA) will be initiated to determine if a minimum level of CO<sub>2</sub> is required for scald control. CO<sub>2</sub> levels to be tested will range from 0 to 2% and the O<sub>2</sub> level will be raised to 1% to avoid the induction of fermentative metabolism. The breadth of cultivars tested also will be expanded to other scald-susceptible cultivars such as Rome Beauty and Granny Smith (MI, CA, NY-I, WA).

A number of independent scald-related projects remain underway with the anticipation that these approaches will be incorporated into larger-scale cooperative projects if promising results are obtained. Important among the more recent findings is that scald control in apple can be achieved by initial low oxygen stress followed by CA storage (MI). In future work, the level and duration of initial low O<sub>2</sub> stress and subsequent CA conditions will be optimized to safely and effectively control scald without compromising fruit quality for apple (MI, NS, NY-I). Conditions and handling prior to and after the low O<sub>2</sub> stress will approximate commercial procedures as closely as is possible.

*Decay control.* We will be continuing our efforts to develop alternatives to persistent agricultural chemicals for postharvest decay control, focusing on the control of *P. expansum* and *B. cinerea*, two of the most destructive postharvest pathogens. Investigation of host/fruit-pathogen interactions will entail a pair of parallel studies using the same molecular biological approach. Work at USDA-MD and CA is examining the interaction of fungal pathogens and fruit with the aim of identifying factors that can limit the development of decay during storage. The focus of this research has been on the polygalacturonase inhibitor protein (PGIP) of apple fruit (USDA-MD) and pear and tomato fruits (CA). The proteins have been purified and the genes for all 3 PGIPs have been cloned. Tests are underway to identify factors that determine which pathogen PG isoforms are selectively inhibited by a specific PGIP. The pear PGIP has been expressed in transgenic tomato plants in order to test whether increased expression of PGIP can influence fruit susceptibility to pathogens. Researchers at USDA-MD and CA will collaborate by exchanging pathogen strains (primarily *Botrytis cinerea*), antibodies and gene probes, as well as information on protocols and results.

Other control measures to be examined will be confined to those with potential to be integrated into modern handling and storage strategies employing sealed storage construction or sealed packages. Investigations will focus on the use mixtures of O<sub>2</sub> and CO<sub>2</sub> (CA, OR, WA), antifungal volatiles (MI, PA), biological control (CA, MI) and heat (OR), separately and in combination (CA). With the exception of CO<sub>2</sub> treatments, these control measures are relatively untested, and are rather exploratory in nature. Therefore, the research does not warrant interaction between numerous participants at this stage. If, however, a technique is considered to have special promise, we anticipate expanding the scale of work and the number of stations involved in efficacy testing.

Optimum levels of O<sub>2</sub> and CO<sub>2</sub> for suppressing fungal growth; fruit toxicity thresholds for the various O<sub>2</sub> and CO<sub>2</sub> atmospheres; and specific atmospheres which will suppress disease development and will maintain fruit quality (CA, MI). Other possible options to the use of fungicides are the use of ozone and/or chlorine dioxide gases in storage atmospheres and during fruit handling operations (WA). Other approaches using reduced or non-chemical control of biotic disorders include use of hexanal and other aldehyde vapor (MI), acetic acid fumigation to control decay of apples (PA), and organic aroma volatiles to control decay of sweet cherries (ONT-V). Decay incidence will be determined as a function of dose. The possibility of applying the gas in

modified atmosphere packages will be explored (MI). Another approach will be the use of extremely high levels of (>20%) CO<sub>2</sub> and heat treatments (CA).

**Objective 3. - To expand fundamental knowledge to improve and create new technologies to assure high quality and wholesomeness of fruit and enhance market opportunities.**

Under this objective, members of NE-103 will conduct various lines of primarily basic research aimed at elucidating the biochemical, physiological and genetic bases of postharvest storage disorders, ripening and softening, and the effects of CAs (both beneficial and detrimental) on quality attributes such as color, aroma, texture and nutritional value. Some of these studies will utilize molecular genetic techniques to address specific problems or questions, and the long-range goal of much of the research will be to introduce into fruits genetic traits which obviate the need to control decay, disorders, and deterioration of quality with chemical treatments that may pose a human health risk and restrict international sales. Research activities under Objective 3 will be distributed among three general areas as follows: 1) Stress-induced disorders and injuries; 2) Biochemical/physiological bases of the effects of O<sub>2</sub>, CO<sub>2</sub> and ethylene on quality of intact and fresh-cut fruits; and 3) Regulation of fruit ripening and softening.

*Stress-induced disorders and injuries.* As indicated in Objective 2, understanding and controlling scald in apple and pear fruits will be a major focus of NE-103 participants. Scald is currently controlled by treatment with DPA and/or by storage in low O<sub>2</sub> atmospheres. DPA treatment is costly, is considered a health risk, is environmentally unsound, and necessitates the use of a fungicide to limit decay in storage. Furthermore, all fruit will not tolerate the low level of oxygen required to prevent scald. Finally, the present control measures are not always effective; sometimes after the fruit are removed from storage scald symptoms arise. Recent research by NE-103 members supports the hypothesis that scald as well as other stress-induced injuries are mediated by active oxygen species (AOS). Also, further evidence was found that development of scald is linked with the synthesis and oxidation of the sesquiterpene -farnesene. Thus, future research efforts by the group will center on: 1) investigating the role of AOS in scald induction and of antioxidative defense mechanisms in scald resistance, and 2) elucidating the pathway of  $\alpha$ -farnesene synthesis and the role of its oxidation products in development of scald symptoms.

In addition to comparison of commercially important apple cultivars that are clearly scald-susceptible (e.g. Granny Smith, Delicious, McIntosh and Cortland) or scald-resistant (e.g. Gala and Empire), this collaborative work will utilize novel susceptible and resistant hybrid lines of Rome Beauty x White Angel available to NY-I. Analyses of the levels of naturally occurring antioxidants (ascorbic acid, glutathione, tocopherols, carotenoids, phenylpropanoids, and simple phenolics) and the activities of AOS scavenging enzymes (superoxide dismutase, peroxidase, catalase, ascorbic acid peroxidase, glutathione peroxidase and glutathione reductase) in resistant and susceptible fruits will be conducted by NY-I, MI, ONT-G, MA, and USDA-MD. Molecular biology protocols will be employed by NY-I and MI to screen for the presence of antioxidant enzyme proteins (by Western blots) and their activities (on non-denaturing PAGE gels), to determine levels of antioxidant enzyme gene expression (by Northern blots using their respective cDNAs as probes) and to determine the number of copies of the various antioxidant enzyme genes (by Southern blot analysis). This approach is expected to identify the biochemical and enzymatic pathways of AOS metabolism that relate to potentiation or prevention of scald development.

The processes by which AOS are generated will be investigated by GA and ONT-G. It is now apparent that the mitochondrial electron transport chain is a major site for production of AOS. Factors that regulate production of AOS in fruit tissue will be examined by isolating mitochondria from scald-resistant and scald-susceptible fruit. These will be used to assess the components which autoxidize and the level of reduction of the component pool which reacts with molecular oxygen to produce AOS. Levels of reduced and total ubiquinones are to be determined by HPLC and production of the superoxide anion will be measured. An exchange of results and protocols

with MD is anticipated, as this station plans to test the ability of new porphyrin-based superoxide radical scavengers to reduce or prevent scald development in highly susceptible Granny Smith fruit. A series of chemically-tailored spin-trapping reagents that can be used to "fix" and then identify the radicals produced in stressed tissue is being developed at WA. Further efforts will be made to develop novel chemical indicators that react with AOS enabling quantification.

The connection between  $\alpha$ -farnesene metabolism and AOS in the promotion of scald has been elusive, but through the efforts of NE-103 participants a breakthrough appears imminent. ONT-G is pursuing the pathway of  $\alpha$ -farnesene synthesis in apple peel tissue, with the ultimate aim of isolating and characterizing farnesene synthase. Using scald-susceptible apple fruit to clone genes that are induced by low temperature but suppressed by low  $O_2$  atmosphere may also provide a cDNA of the farnesene synthase gene (MI). This would enable the eventual testing of antisense transgenics for low  $\alpha$ -farnesene production and resistance to scald. The primary oxidation products of  $\alpha$ -farnesene, conjugated trienes (CTs), are much more closely correlated with scald development than  $\alpha$ -farnesene itself. Therefore, USDA-MD, MD and MI are collaborating to elucidate the mechanism of  $\alpha$ -farnesene oxidation and the subsequent metabolic fate of its CT products. It was recently shown that the CTs which accumulate in apple peel during storage are, surprisingly, comprised mainly of two conjugated trien-6-ol isomers, with one isomer constituting 90% (USDA-MD, MD). This raises the possibility that oxidation of  $\alpha$ -farnesene is enzymatic *in vivo*. USDA-MD and MD plan to devise an assay system using peel tissue from scald-susceptible fruit to test this hypothesis. Should it prove to be the case, the " $\alpha$ -farnesene hydroxylase" would be another target enzyme for genetic regulation to prevent scald. MI has provided evidence for a direct role of a volatile breakdown product of  $\alpha$ -farnesene, 6-methyl-5-hepten-2-one (MHO), in scald development. MHO induced scald-like symptoms in apple peel, tissue sensitivity to MHO increased with time in storage, and *in vivo* MHO production rose sharply after several months at 0 C in air. A joint effort by USDA-MD and MI will ascertain if MHO is a degradation product of CTs. Preliminary results confirm this, and further indicate that autooxidation of CTs is markedly temperature dependent. This could explain why scald symptoms intensify greatly when fruit are rewarmed after long-term storage. Future studies will focus on full elaboration of  $\alpha$ -farnesene metabolism, the precise role of MHO in scald induction, and the effects of low  $O_2$ , ethylene and low temperature on  $\alpha$ -farnesene synthesis and oxidation.

*Biochemical/physiological bases of the effects of  $O_2$ ,  $CO_2$ , and ethylene on quality of intact and fresh-cut fruits.* Comparative studies to identify differences between low- $O_2$ -induced and elevated- $CO_2$ -induced fermentative metabolism, and factors affecting the shift from aerobic to anaerobic respiration in whole and fresh-cut fruits, will be carried out at CA. Also, investigation of the capacity of fruit tissue for repair following stress induced by low  $O_2$  and/or high  $CO_2$  levels will involve collaboration of CA, MD and NY-I. Studies of the roles of pH and copigmentation on stability of internal anthocyanins in strawberries under elevated  $CO_2$ , as well as the effects of  $O_2$ ,  $CO_2$ , and  $C_2H_4$  on phenolic metabolism and browning of fruit tissues, are planned (CA). Other quality-related biochemical studies will include examination of atmospheric composition-time-temperature interactions on nutritional quality, especially on levels of vitamins A and C, and bioactive compounds such as carotenoids and polyphenols (CA).

A major effort will continue in the area of flavor biochemistry. It will center on understanding the metabolic processes involved in the loss of characteristic aroma volatiles in fruit kept in air or controlled atmospheres beyond a certain duration (CA, MI, WA, USDA-WA, PA, USDA-MD).

*Regulation of fruit ripening and softening.* Control of ripening and softening by synthetic and natural bioregulators will be examined (NS, WA, CA, NY-G). The ethylene biosynthesis inhibitor aminoethoxyvinylglycine (trade name ReTain) is now approved in the U.S. to stop fruit drop and retain firmness in apples and pears at harvest. NS and WA will evaluate the possible benefits of ReTain on various ripening-associated changes in fruit quality (especially softening, and retention and regeneration of flavor compounds) after long-term storage and subsequent shelf time.

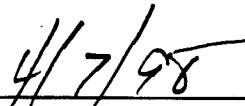
CA will continue to investigate the origin and mode of action of pectin-derived oligosaccharides (PDOs), which promote ripening in tomato. The role of novel pectolytic enzymes in softening-related cell wall metabolism and generation of PDOs will be studied, as well as the possibility that a recently discovered PDO-binding protein (remorin) is part of the signal transduction pathway through which PDOs stimulate ripening. This work will utilize the explanted tomato pericarp system that was devised several years ago. NY-G will take a molecular genetic approach to solving the problem of softening in storage in fruit of important apple varieties such as 'McIntosh.' This softening is the result of cell wall degrading enzymes such as polygalacturonase (PGase), which are controlled by ethylene. Ethylene production depends on the synthesis of its precursor, 1-aminocyclopropane carboxylic acid (ACC), by the enzyme ACC-synthase. Experiments are in progress to transform 'McIntosh' apple with sense or antisense versions of the ACC-synthase gene, with the aim of reducing ACC-synthase activity in the fruit, and thereby reducing ethylene production and consequent softening. A similar strategy will be utilized to achieve a reduction in PGase activity in harvested apple fruit. The overall goal of this work is to attenuate ripening and softening, and to thereby improve storability.

SIGNATURES:

Regional Project Title: Postharvest Physiology of Fruits



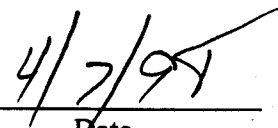
Administrative Advisor - Robert C. Seem



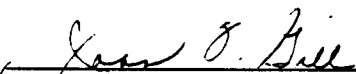
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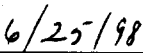
Chair, Regional Association of Directors



Date



Administrator,  
Cooperative State Research, Education, and Extension Service



Date



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# ATTACHMENT A. PROJECT PARTICIPANTS AND PROJECT LEADERS

<u>Location</u>	<u>Department</u>	<u>Participant</u>	<u>Specialization</u>
British Columbia	Pomology and Viticulture	O. L. Lau	Physiology
California	Pomology	C. H. Crisosto A. A. Kader* J. A. Labavitch E. J. Mitcham	Physiology Physiology Physiology Physiology
District of Columbia	Dept. of Biological and Environmental Sci.	Y. Lee*	Physiology
Georgia	Horticulture	A. C. Purvis*	Physiology
Maryland-College Park	Horticulture	T. Solomos*	Physiology
Maryland-Beltsville (USDA, ARS)	Hort Crops Quality Lab	W. S. Conway* B. D. Whitaker	Pathology Physiology
Massachusetts	Plant and Soil Science	W. J. Bramlage*	Physiology
Michigan	Horticulture	R. Beaudry D. R. Dilley*	Physiology Physiology
New York-Geneva	Food Science	G. Hrazdina*	Food Science
New York-Ithaca	Fruit Vegetable Sciences	C. B. Watkins*	Physiology
North Carolina	Horticultural Sciences	S. M. Blankenship*	Physiology
Nova Scotia	Agriculture and Agric-Food Canada Research	R. K. Prange	Physiology
Oklahoma-Lane (USDA, ARS)	Genetics and Production Research	P. M. Perkins-Veazie*	Physiology
Ontario, Canada	Horticultural Sciences Univ., Guelph	D. P. Murr	Physiology
	Horticultural Research Institute of Ontario, Vineland, Station	C. L. Chu	Physiology
Oregon	Mid-Columbia Ag Res/Extension Center	P. M. Chen*	Physiology
Washington-Pullman	Horticulture	J. K. Fellman	Physiology
Washington-Wenatchee	Tree Fruit Res. Center	E. M. Kupferman*	Physiology
Washington-Wenatchee (USDA, ARS)	Tree Fruit Res. Lab	E. A. Curry J. P. Mattheis*	Physiology Physiology

\* Voting Member

# ATTACHMENT B. RESOURCES

## ANNUAL INPUT

<u>STATE OR AGENCY</u>	<u>DEPARTMENT OR DIVISION</u>	<u>SY</u>	<u>PY</u>	<u>TY</u>
California	Pomology	1.00	1.00	1.25
Canada	Agriculture Canada Research Station, Summerland, B.C.	1.00	.00	3.50
	Agriculture Canada Research Station, Kentville, N.S.	.40	1.00	.40
	Horticulture, University of Guelph Ont.	.25	.50	1.00
	Horticultural Research Institute Vineland, Ont.	.80	.00	.80
District of Columbia	Biological and Environmental Sci.			
Georgia	Horticulture	.50	.00	.00
Maryland-College Park	Horticulture	.50	.00	.10
Maryland-Beltsville (USDA, ARS)	Hort Crops Quality Lab	.80	.00	.40
Massachusetts	Plant and Soil Science	.50	.50	1.00
Michigan	Horticulture	1.50	.00	.25
New York-Geneva	Food Science and Technology	.55	.45	.50
New York-Ithaca	Fruit and Vegetable	.50	.20	.50
North Carolina	Horticultural Science	.20	.00	.50
Oklahoma (USDA, ARS)	Genetics and Production Research	.75	.70	.60
Oregon	Mid-Columbia Experiment Station	1.00	.00	.00
Pennsylvania	Horticulture	1.05	1.00	.10
Washington-Pullman	Horticulture			
Washington-Wenatchee	Tree Fruit Research Center	.40	.00	.00
Washington (USDA, ARS)	Tree Fruit Research Lab			
	<b>TOTAL</b>	<b>11.70</b>	<b>5.35</b>	<b>10.90</b>