

NEW YORK FRUIT QUARTERLY

Editorial

The New York Fruit Quarterly Magazine's 10th Anniversary

In the fall of 1992, as the New York State Apple Research and Development Program (ARDP) was getting organized, the New York State Horticultural Society Board led by Rich Erdle, Sheldon Furber, Walter Blackler, Dennis Chaisson, Rod Dressel Jr., Bill Everett, Sayre Fulkerson, Joel Hamlet, George Lamont, Kathy Wafler Madison, Anthony Porphiglia, Peter Russell and Frank Wiles met with the ARDP board and industry leaders at the New York State Agricultural Experiment Station to discuss how to better communicate to growers the results of Cornell research. The research that had been funded by the Apple Research Association (ARA) was at times not communicated well to all growers. With the new research marketing order (ARDP) into its first year of funding, both boards felt that it would be desirable to launch a new publication dedicated to disseminating the results of the grower funded research.

The director of the Experiment Station, Jim Hunter, was enthusiastic and agreed to lend the support of the Station to developing the publication. The NYSHS board agreed to become the publisher of the magazine and the ARDP board agreed to let the new magazine serve as the official publication of ARDP funded projects to fulfill the program's mandate to disseminate the results of funded research to growers. Thus the concept of the *New York Fruit Quarterly* was born.

Jim Hunter asked recently retired vegetable extension leader Bob Becker to become the editor of the publication and to work with the Communications Services group at Geneva to publish the magazine. Under the leadership of Bob Becker, the first issue rolled off the presses in January 1993. The first five articles were authored by Art Agnello, Dave Blanpied, Susan Brown, Dave Rosenberger, and Warren Stiles. Quietly and efficiently, Bob Becker shepherded the magazine from 1993 through 1995, publishing many fine articles detailing the results of research on fruit crops.

Following Bob's untimely death, Dan Donahue of the NYSHS assumed the editorial role for two years. However, in late 1997, the finances of the magazine became unsustainable and a committee of the Horticultural Society led by Frank Wiles and Fran Dellamano developed a plan to reestablish a firm financial footing. It involved selling advertising and finding editors within the Cornell staff. Warren Smith, who had just retired from Extension, volunteered to sell advertising and the current editors volunteered to take over the job of running the magazine.

A new design for the magazine was launched with color photos on the covers and more pictures and advertising. The ARDP board increased their financial contributions and the magazine has again been placed on sound financial footing.

Over the last 10 years, the *New York Fruit Quarterly* has published about 150 articles covering each of the projects funded by ARDP. These have included articles on improving production, harvesting, storage and marketing of New York apples. The magazine has been sent to all apple growers each quarter.

All those involved in making this a useful and quality vehicle to communicate important research results to growers should be proud of the effort they have made to improve the fruit industry of New York state. Growers should also be proud of the high quality research they have funded through the ARDP program and for funding about half the cost of the *New York Fruit Quarterly*.

On this occasion of the 10-year-anniversary of the *New York Fruit Quarterly*, we wish to express thanks to the team that makes this magazine a reality. Thank you authors who have written all of the articles. Thanks Jim Hunter, Bob Becker, the NYSHS board, the ARDP board, Frank Wiles, Fran Dellamano, Dan Donahue and George Lamont. A special thanks to the production team: Linda McCandless, Warren Smith, Elaine Gotham, Gemma Osborne, and Karen Wilson.

Terence Robinson and Steve Hoying
Editors

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FRONT COVER:

These images explore the anatomy of apple russet, from the microscopic to the macroscopic.

Credits: Martin Goffinet

BACK COVER:

Rui Hai Liu, author of two articles in this issue, is a Cornell food scientist in Ithaca whose research program focuses on the beneficial effects of functional foods, and the relationship between diet and cancer. Credit: Cornell University Photography



NEW YORK
FRUIT QUARTERLY
VOLUME 10 • NUMBER 3 • AUTUMN 2002

This publication is a joint effort of the New York State Horticultural Society, Cornell University's New York State Agricultural Experiment Station at Geneva, and the New York State Apple Research and Development Program.



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Anatomy of Apple Russet Caused by the Fungus *Aureobasidium pullulans*

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This research is supported in part by the New York Apple Research and Development Program.

The causes of apple russet are many, but all causes lead to serious concern for growers, storers and sellers of fresh-market varieties. Varietal differences in apple susceptibility are well known. The most published studies of russet inducing factors have been done on the variety 'Golden Delicious', but studies of other varieties are not uncommon. Russet inducing agents are various and include temperature, relative humidity, light quality at the fruit surface, sprays (either active ingredients or adjuvants), and even biological agents that are present in the orchard. Indeed, in the last decade, much evidence has been gathered that implicates several fungi in the production of russet in fruit of a number of varieties in New York

State. Cornell's Department of Plant Pathology has been researching these pathogens along several avenues: surveying New York orchards for presence of these fungi; determining the cycle of inoculum production in season; correlating increase of inoculum and incidence and severity of russet in fruit of many apple varieties; surveying growers and packing houses for information on severity of russet and its economic consequences; planning control strategies that limit or eliminate russet production in the orchard; and determining how the fungus elicits russet formation on the fruit surface at the microscopic level. Some of these findings were reported in a previous issue of this publication (See Heidenreich, et al., *New York Fruit Quarterly*, Vol. 8, No. 2, Summer 2000: pp. 22-24). In this issue, we report on the interaction of the fruit surface with the russet-inducing fungus *Aureobasidium pullulans* and the production of russet as fruits develop.

There are apple varieties that russet naturally and these are even admired by some apple lovers. The skin of such varieties is rough and mottled, but the apple is not deformed. Russetting in other apple varieties is abnormal, so that normally smooth surfaces become crusty, "blistered," or "alligatored," and there may be growth deformities and surface cracks or splits. Figure 1 shows russet in mature fruits of 'McIntosh' (top) and 'Crispin' (bottom) after inoculation with *A. pullulans* spores at an early stage in fruit growth. Less severe russet is often seen (e.g., Figure 2) and may be due to strain of fungus, apple variety, weather conditions, amount of inoculum present in an orchard, and how late in fruit

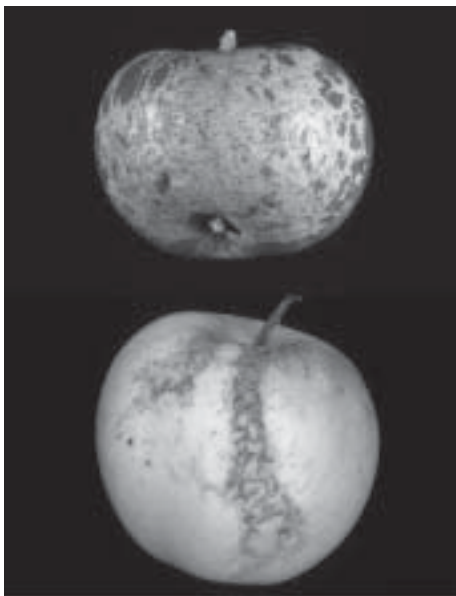


Figure 1. Russet of 'McIntosh' (top) and 'Crispin' (bottom) fruit caused by inoculation with spores of the russet-inducing fungus *Aureobasidium pullulans*.

Apple russet caused by fungal pathogens has been found to be a problem in many New York orchards. The pathogens' life cycles and host-pathogen infection processes are little known. Our research shows that these pathogens feast on the fruit's epidermal cuticle (outer skin), but not on fruit cells themselves. This cuticular "hull breach" initiates the fruit's protective mechanism, namely, russet. Russeted tissue is formed as a response to penetration of the fruit cuticle and exposure of the fruit cells beneath to oxygen. In effect, it represents an attempt to wall-off invading fungi by developing a corky, suberized layer at the site of the cuticular breach.

development inoculation occurs. Older fruits appear less susceptible than those less than a month from bloom. Our inoculation of 'McIntosh' out of winter storage, for example, elicited only a slight reduction in "gloss" of the skin surface.

A. pullulans is a fungus that spreads out after inoculation as a colony of yeast-like cells that produce infective spores (conidia) at the ends of many of the colony's branches (Figure 3, top). The mycelium (the fuzzy, thread-like mat we associate with fungi on a leaf or fruit surface) of this fungus is relatively sparse. The mycelium will eventually produce fungal strands (hyphae) that darken and become compartmentalized as thick-walled spores (arthrospores) (Figure 3,



Figure 2. A less severe form of fungus-induced russet on 'McIntosh' fruit (left), while a nearby fruit is russet free.

bottom). This gives rise to the fungus' common name, "black yeast." Notice the black, crusty spots and cracks in 'Crispin' in Figure 1. This fungus is very common and found on a variety of substrates, including wood, cloth, painted surfaces, many plant species and organs, and even on insects, crustaceans, and man (F.D. Heald, 1933. Manual of Plant Diseases).

We have isolated this fungus and tested various strains of it for its ability to induce russet in apple. We then began a series of anatomical studies to see what happens at the microscopic level when a high concentration of a virulent strain is inoculated onto the developing skin of young fruits. Spore suspensions at the concentration of ten million spores per ml water were inoculated onto 'McIntosh'

fruits as a fine mist (to "run-off") in our early studies. More recently we have been devising small micro-containers that can be applied to young fruits and then filled with the spore suspension using a micropipette. This, we think, will give us a known, small-area sample of fruit skin to examine for russet formation. This is especially important when collecting skin

samples colonized by spores but before russet becomes obvious.

Early trials of spore suspensions misted onto 'McIntosh' fruits at time of fruit set showed us several things. The first is that fruits at this young stage are quite covered with long epidermal hairs (trichomes) (Figure 4, left) and that uninoculated fruit did not russet. Inoculated fruit indeed showed the presence of conidia on the fruit hairs, but also on the developing cuticle of the fruit's epidermal surface (Figure 4, right). It also appeared that these spores and their offspring were capable of digesting the protective cuticle of the fruit, leaving disruptions in that layer (Figure 4, right). Fruits at this stage are developing rapidly, mostly by increase in cell numbers. To

keep pace with the increase in internal volume, the epidermal and hypodermal layers of the fruit (the "skin") must also undergo cell division and cell enlargement, or the skin would be stretched and broken. This is a major reason why fruit inoculated at a young stage often show the most severe russet — the fruit has tried to repeatedly heal and seal a continually fractured epidermal surface as fruit size increases. Normal cell divisions in apple fruit decline to a very low frequency about 4 weeks post bloom, while cells enlarge tremendously after that time.

A cross section of the epidermal cells and overlying cuticle layer in a 'McIntosh' fruit at bloom is seen in Figure 5, top. The fruit was inoculated with *A. pullulans* a few hours before the fruit was collected and processed for this section. Spores are seen on the cuticle layer above an epidermis composed of a single cell layer. Within a week or so of inoculation we find a response of the apple skin to the presence of the fungus. Note in Figure 5, middle, that the spores are then associated with changes in fruit skin organization. The epidermal cell just below the spores has likely been induced to divide to produce an inner and an outer epidermal cell. Also, the hypodermal layers of the fruit have begun to produce a thickened layer of actively dividing files of cells, the periderm tissue. A major function of periderm in plant organs is to

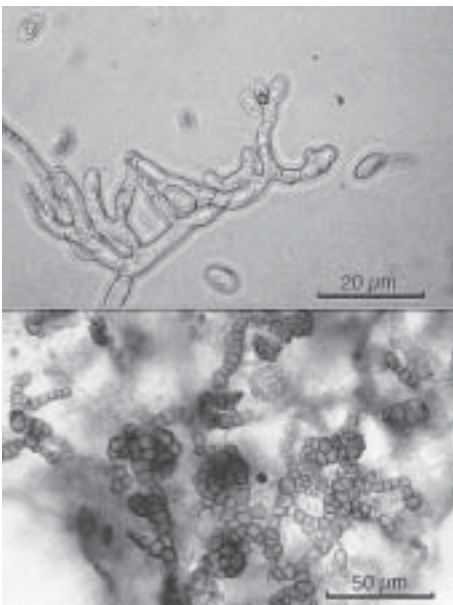


Figure 3. Cultures of the russet-inducing fungus *A. pullulans* showing production of conidiospores from the ends of the branching filaments (top) and chains of dark-staining arthrospores taken from russeted 'Cortland' apple (bottom).

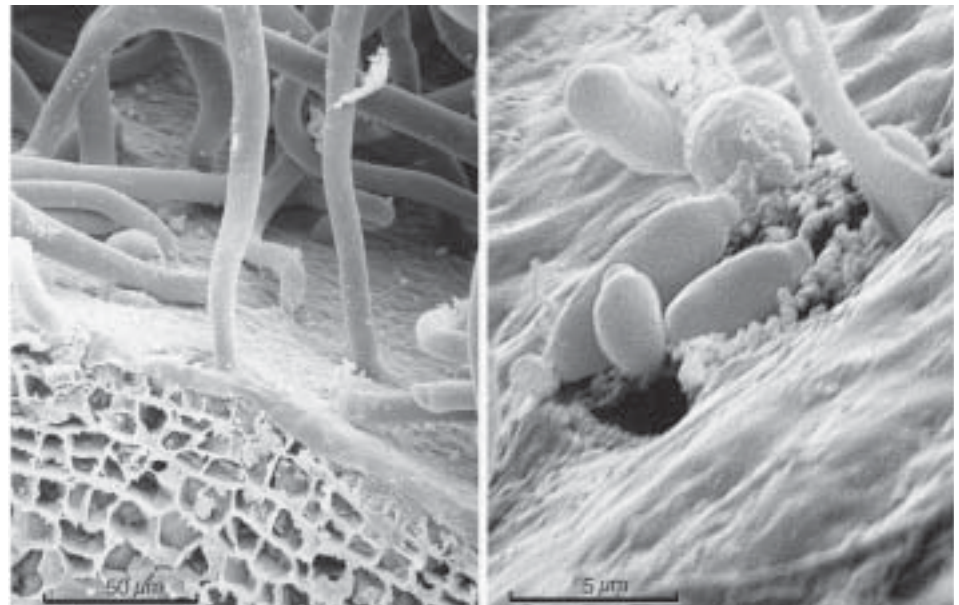


Figure 4. Scanning electron microscope images of 'McIntosh' fruit surfaces at 10 days post bloom, either uninoculated (left) or inoculated (right) with *A. pullulans* spores. Note the dense hairs on the young, intact epidermis (left). The spores are associated with degradation of the protective cuticle in inoculated fruit (right). Spores are about 1/5000 inch (5 µm) long.

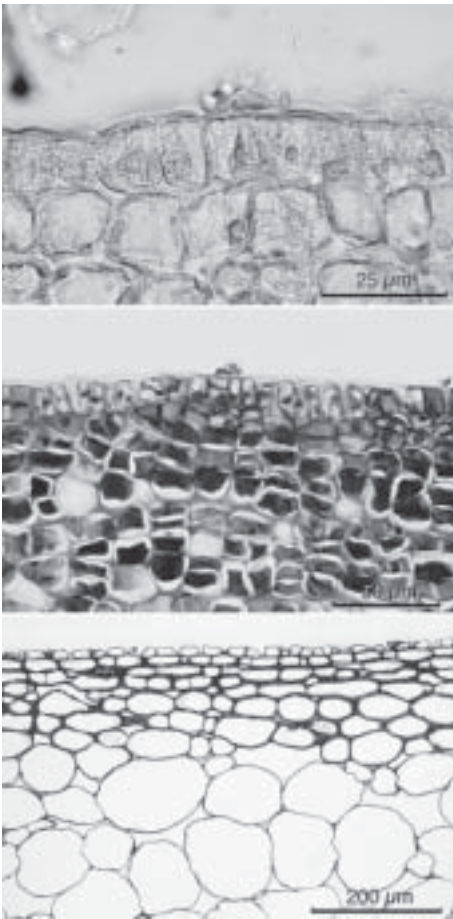


Figure 5. Cross-sections of ‘McIntosh’ apple skin: *A. pullulans* spores attached to the cuticle of the epidermal cell layer (top); spores of *A. pullulans* on the fruit 12 days post bloom and induction of heavy layers of periderm (russet) (middle); and normal apple skin at maturity, without periderm or russet (bottom).

seal off wounds, tears, or cracks in the organ surface. There is evidence that a cell’s exposure to an oxidizing environment (air, in this case) will trigger cell division and, if necessary, periderm formation. The files of periderm cells are usually filled with sealing substances and antimicrobial materials, such as phenolics, that help prevent tissue degradation. A section through a mature fruit’s skin should appear as in Figure 5, bottom. Note that there are no heavy layers of periderm, no files of cells with dark phenolic materials, even though the cells of the flesh have increased tremendously in volume.

As the fruit continues to develop, the small isolated packets of periderm coalesce into small, then increasingly larger, scabby areas (Figure 6). If such activity begins at a stage where much fruit growth has yet to occur, then huge areas of russet result, often with large scabs, cracks, or leathery patches. If only a small

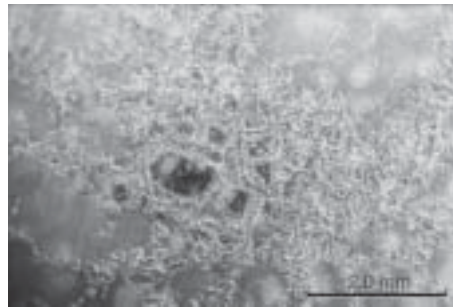


Figure 6. Magnified surface view of ‘McIntosh’ skin inoculated 4 weeks post bloom and examined three weeks later. Note scabby patches of periderm. The later the fruit is inoculated, the less severe the russet, both in surface area russeted and intensity of the russet.

amount of cell division and fruit enlargement remains when fruits are exposed to inoculum, then russeting is more isolated and russeted surface area reduced in proportion to total fruit surface.

In collaborations with Dr. Wolfram Koeller and Diana Parker in the Department of Plant Pathology at the Geneva Experiment Station, the cutin and wax components of the apple fruit cuticle were extracted, isolated, and subjected to inoculation with *A. pullulans*. The fungus was able to digest both crude and purified components of the fruit cuticle and use it as its sole carbon source for fungal growth and differentiation (Figure 7). All *A. pullulans* isolates produce esterases in culture with apple cutin. The esterase produced by the strain used in our studies is a cutinase. This provides supporting evidence for our interpretation that it is the digestion of the waxy protective layer that sets up the russeting process. The cuticle of young fruits is both thinner and simpler than the cuticle of maturing apples. The fungal-induced “hull breach” in the young fruit surface initiates a repair mechanism that is outpaced by growth in fruit volume, while similar breaches in older fruit are repaired at a time when tensile forces in the skin are being reduced. We have not yet determined, however, if production of these cuticle-degrading enzymes by the fungus actually results in russeted fruit in the orchard, but it is highly likely considering the evidence.

In summary, russet formation in apple fruit in the presence of *A. pullulans* appears to be the result of: 1) spore deposition on the fruit cuticle; 2) spore adherence for enough time with the right conditions to begin cuticle degradation; 3) epidermal cell responses, such as phenolic deposition, cell senescence, and

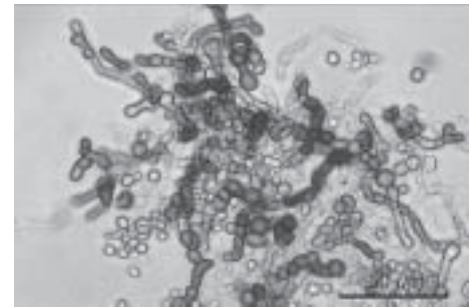


Figure 7. Colony of *A. pullulans* strain YT16 growing and multiplying on isolated cutin taken from the epidermis of a mature apple. The fungus is thus capable of digesting cutin and using it as a food for growth.

death; 4) localized sub-epidermal changes due to loss of epidermal integrity; 5) a tissue recovery period involving cell divisions in the hypodermis (sub-epidermal layer); and 6) production of a corky layer (periderm) that re-isolates the flesh from the desiccating and oxidizing environment surrounding the fruit.

This year we are continuing anatomical studies on ‘McIntosh’ fruit that had inoculum applied to small micro-wells attached temporarily to the fruit surface at about 7–10 days post bloom. This allows a time-course study of a very small area of known exposure to a known concentration of inoculum. And, of course, further field trials are being made of spray materials that may have an ability to retard or prevent russet in a range of apple varieties.

Martin Goffinet is a senior research associate in Cornell’s Department of Horticultural Sciences specializing in the anatomy and morphology of fruit crops. Tom Burr, professor and chairman in Cornell’s Department of Plant Pathology in Geneva, researches bacterial diseases of fruit crops. Catherine Heidenreich served as the research technician and coordinator of the fruit russet study while in Burr’s program. She currently is a research supports specialist in Bill Turechek’s program in fruit diseases in the same department.

We give special thanks to Mary Jean Welser, Research Support Specialist in Goffinet’s anatomy laboratory for excellent histological preparations over several years of this study.



Apple Peels are Rich in Phytochemicals and Have High Antioxidant Activity

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This research is supported by the New York Apple Research and Development Program and the New York Apple Research Association.

The leading causes of death in the United States are cardiovascular disease and cancer. It has been estimated that 32 percent of deaths from cancer could be avoided by dietary modifications. Fruits and vegetables contain many phytochemicals, including phenolics, which may exert chemoprotective effects through a variety of mechanisms. Increased intake of fruits and vegetables has also been associated with reduced risk of coronary heart disease (CHD) and stroke. Flavonoids, a class of phenolic compounds commonly found in fruits and vegetables, have been linked to reduced risk of mortality from CHD. Such findings have led the National Research Council (NRC) to recommend consuming five or more servings of fruits and vegetables a day.

Apples are very significant part of the diet. From a Dutch Food Consumption Survey and previously analyzed flavonoid contents of fruits, vegetables, and beverages, it was determined that apples are the third largest contributors of flavonoids in the Dutch diet behind tea and onions. In Finland, along with onions, they are the top contributors. Twenty-two percent of the fruit phenolics consumed in the United States are from apples, making them the largest source.

Consumption of apples has been linked to the prevention of chronic disease. Apple intake has been negatively associated with lung cancer incidence in two separate studies, and has also been related to reduced cardiovascular disease. Coronary and total mortality, symptoms of chronic obstructive pulmonary disease, and the risk of thrombotic stroke have

all been inversely associated with apple consumption.

Apples are a good source of phenolic compounds. The total extractable phenolic content has been investigated and ranges from 110-357 mg/100 g fresh apple. Previously, our research group found that peeled and unpeeled apples have high antioxidant activity and inhibit the growth of human cancer cells *in vitro*. Vitamin C was responsible for less than 0.4 percent of the antioxidant activity, indicating that other factors, such as phenolics, were the main contributors. The antioxidant and antiproliferative activities of unpeeled apples were greater than that of peeled apples. It is also known that the concentration of total phenolic compounds is much greater in the peel of apples than in the flesh. Both suggest that apple peels may possess more bioactivity than the flesh.

Apple peels are a waste product of applesauce and canned apple manufacturing. The National Agriculture Statistics Service (NASS) reported that 216 million pounds of apples were processed in this manner in New York State in 2000. We estimate that 16 million pounds of peels were generated. If apple peels show potential to improve health when consumed, their utilization should be investigated.

Therefore, the object of this study was to evaluate the nutritional quality of apple peels. Total phenolic content, anthocyanin content, antioxidant activity and antiproliferative effects of apple peels were quantified and the results compared those of the apple flesh and flesh + peel. Four apple varieties (Rome Beauty,

Apples are an important source of phenolic compounds in the European and North American diet, and may exert a chemoprotective effect healthwise.

When studied for nutritional quality, apple peels rate very highly in phytochemical content. In the manufacture of applesauce, peels are frequently considered a waste product.

This study indicates that apple peels show potential as a value-added ingredient because of their high phenolic content, and may possess more bioactivity than apple flesh.

Idared, Cortland, and Golden Delicious) commonly used in applesauce manufacture in New York State were evaluated.

Phytochemical Content

The total phenolic content of the flesh, flesh + peel, and peel of the four apple varieties was determined (Figure 1). Of the peels, the total phenolic content of the Idared peels was highest at 588.9 ± 83.2 mg gallic acid equivalents/100 g peels, followed by Rome Beauty, Cortland, and Golden Delicious apples. The peel phenolic content was not significantly different for Idared and Rome Beauty apples ($p < 0.05$). Rome Beauty flesh + peel phenolics were the highest at 159.0 ± 15.1 mg gallic acid equivalents/100 g flesh + peel. The phenolic content for the flesh + peel of all the varieties was similar. Total phenolic content of the flesh was highest for Cortland (103.2 ± 12.3 mg gallic acid equivalents/100 g flesh), followed by Golden Delicious, Rome Beauty, and

Idared apples. The total phenolic content was highest in the peel, followed by the flesh + peel and the flesh for all four apple varieties. The flesh and flesh + peel values were statistically similar for Idared, Cortland, and Golden Delicious apples ($p > 0.05$).

We have shown that apple peels possessed the highest content of phenolic compounds compared to other edible parts of the apple. The total phenolic contents for the flesh and flesh + peel samples were comparable to those previously reported. Other research groups have also noted that apple peels had higher phenolic contents than the flesh. The nature and distribution of these phytochemicals between the flesh and the peel of the apple is also different. Among others, the flesh contains catechins, procyanidins, phloridzin, phloretin glycosides, caffeic acid and chlorogenic acid. The peel possesses all of these compounds and has additional flavonoids not found in the flesh, such as quercetin glycosides.

The anthocyanins in the apple peels were quantified (Figure 2). Anthocyanins are phenolic compounds with high antioxidant activity that give many fruits and vegetables their red or purple color. The anthocyanin content of the flesh and flesh + peel was not analyzed as apple flesh of these varieties does not contain anthocyanins. The measured anthocyanin contents of the apple peels were related to their appearances. The deep red Idared apple peels contained the most anthocyanins, with 26.8 ± 6.5 mg cyanidin 3-glucoside equivalents/100 g, followed by Cortland and Rome Beauty. The Cortland apples were bright red with green patches; the Rome Beauty apples were pink. The peels from these two varieties had similar values ($p > 0.05$). Golden Delicious apple peels contained only a trace amount of anthocyanins, as expected by their lack of red pigmentation. The red color of apple peels is due to the presence of cyanidin 3-galactoside.

Total Antioxidant Activity

The total antioxidant activity of the apple flesh, flesh + peel, and peel of the four apple varieties was determined using the Total Oxyradical Scavenging Activity (TOSC) assay. The degree of inhibition of an oxidizing reaction by apple extracts was measured in this test. The total antioxidant activity of the peels was greater than that of the flesh or flesh

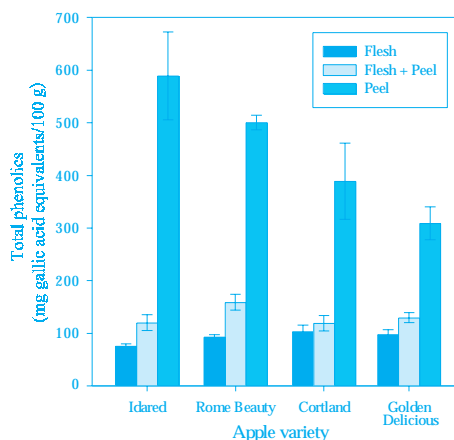


Figure 1. Total phenolic content of apples.

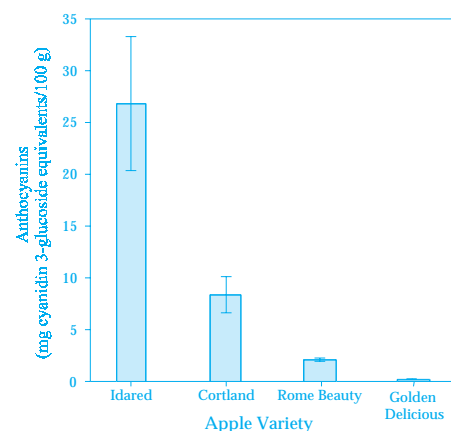


Figure 2. Anthocyanin content of apple peels.

+ peel for all varieties (Figure 3). Idared peels possessed the greatest activity, with 312.2 ± 9.8 μmol vitamin C equivalents/g peel, followed by the peels of Rome Beauty, Cortland, and Golden Delicious apples. The flesh + peel of Rome Beauty apples had the highest antioxidant activity (131.6 ± 0.8 μmol vitamin C equivalents/g flesh + peel) when compared to that component of the other apples. In descending order, the flesh components had total antioxidant activities of 68.0 ± 1.5 (Rome Beauty), 50.4 ± 2.2 (Cortland), 46.9 ± 1.6 (Idared), and 43.5 ± 0.4 (Golden Delicious) μmol vitamin C equivalents/g flesh. The antioxidant activity was highest from the peels in all varieties, followed by the flesh + peel and the flesh.

To our knowledge, this is the first time the total antioxidant activity of apple peels has been measured. The peels of the apple varieties under investigation exhibited high antioxidant activity compared to the flesh and flesh + peel. These antioxidant activities were high in relation to those of other fruits and vegetables tested by our research group. The peels from one average-sized Idared apple have an antioxidant activity equivalent to 820 mg vitamin C. The antioxidant activity of the edible portion of apples has been ascertained in the past using the oxygen radical absorbance capacity assay (ORAC) and compared to other fruits. They have been ranked ninth out of 12 fruits, and eighth out of 20 fruits. Apple antioxidant activity was found to be related to the total phenolic content. Apple extracts have also been shown to have the ability to bind to plasma low density and very low density lipoproteins and inhibit their oxidation. Oxidation of these lipoproteins is thought to be an important step in the progression of atherosclerosis.

Inhibition of Cancer Cell Proliferation

The effect of apple flesh, flesh + peel and peels on the growth of HepG₂ human liver cancer cells *in vitro* was investigated. The cancer cells were exposed to various concentrations of apples in the form of apple extracts and incubated for 96 hours. After this time, their growth was compared to untreated cells. Figure 4 shows the EC₅₀ of the antiproliferative activity of different apple varieties. Lower EC₅₀ values represent higher antiproliferative activity. The peels of each apple variety inhibited the growth of HepG₂ cells more than the flesh or flesh + peel, and the peels had low EC₅₀ values compared to the flesh and flesh + peel components. The flesh + peel and flesh samples showed inhibitory effects in most cases, though they showed much less antiproliferative activity than the peels. Liu et al. likewise reported antiproliferative effects from phytochemical extracts of Fuji, Gala, and Red Delicious apples on human liver cancer cells. Of the four varieties, Rome Beauty apples had the lowest EC₅₀ values of the peel and flesh + peel components at 12.4 ± 0.4 and 26.5 ± 0.3 mg apple/mL, respectively, indicating the most antiproliferative activity of the varieties examined.

Summary

Consumption of fruits and vegetables has been associated with reduced risk of chronic diseases. These benefits are hypothesized to be due to the high content of antioxidants in fruits and vegetables. Apples are commonly eaten and are large contributors of phenolic compounds in European and North American diets. The peels of apples, in

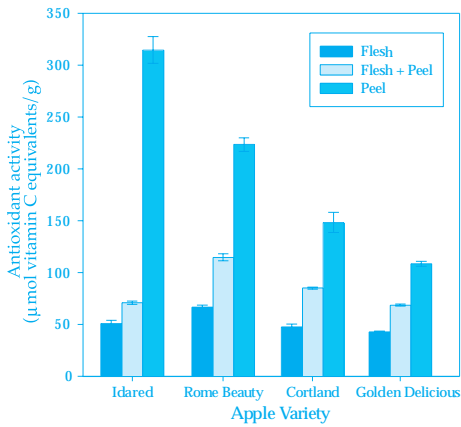


Figure 3. The antioxidant activity of apples.

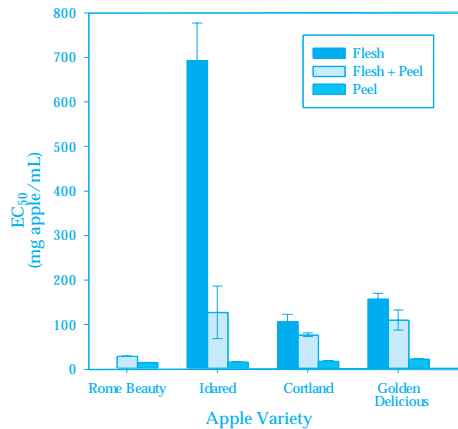


Figure 4. EC₅₀ of inhibition of HepG₂ human liver cancer cell proliferation by phytochemical extracts of apples.

particular, are high in these phytochemicals. During applesauce and canned apple manufacture, the antioxidant-rich peels of apples are discarded. To determine if a useful source of antioxidants is being wasted, the phytochemical content, antioxidant activity, and antiproliferative activity of



Rui Hai Liu directs Kelly Wolfe, a graduate student, on the extraction of apple phytochemicals in Liu's laboratory in Ithaca.

the peels of four varieties of apples (Rome Beauty, Idared, Cortland, and Golden Delicious) commonly used in applesauce production in New York State were investigated. The values of the peels were compared to those of the flesh and flesh + peel components of the apples. Within each variety, the total phenolic content was highest in the peels, followed by the flesh + peel and the flesh. The peels all had significantly higher total antioxidant activities than the flesh + peel and flesh of the apple varieties examined. Apple peels were also shown to more effectively inhibit the growth of HepG₂ human liver cancer cells than the other apple components.

Our results show that eating apple peels may have health benefits for consumers. They are often discarded in the production of other products, but clearly possess high levels of antioxidant and bioactive compounds. Waste apple peels from applesauce and canned apple manufacturing should be regarded as a valuable commodity. We believe they show potential as a functional food or value-added ingredient in the future. As

part of a diet rich in fruits, vegetables and grains, apples and their peels may assist in the prevention of chronic disease.

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Growth Performance of Apple Nursery Trees in Relation to Reserve Nitrogen and Carbohydrates

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This research is supported in part by the New York Apple Research and Development Program.

As apple growers move to high density planting systems, the investment for orchard establishment is considerably increased. The key to achieving early economic return and profitability is to bring the orchard into early production and have sustained high yield of best quality fruit. Tree quality and performance during establishment play a very important role in determining the early productivity of the orchard. If tree establishment is poor, it is very difficult to achieve early productivity.

Currently, nursery stock is graded on the basis of caliper size and the number of feathers. From a physiological standpoint, it is the nutrient reserves that support the initial growth of apple nursery trees during establishment in the orchard. Therefore, a high quality tree needs to reach good size with appropriate root-to-shoot ratio, but, more importantly, to have high levels of nutrient reserves. Among the reserves, carbohydrates and nitrogen are the most important as they provide energy and building blocks for the new growth before any photosynthesis and significant root uptake of nitrogen can take place. Carbohydrates, including both starch and soluble sugars, make up the bulk part of the total reserves in apple trees. In contrast, total nitrogen, including proteins and amino acids, only accounts for less than 2 percent of the dry matter of an apple tree in the winter.

Although both nitrogen and carbohydrate reserves are essential, the

limitations of reserve nitrogen and carbohydrates on the initial growth are not clear. Compared with bearing trees in the orchard, nursery trees only have vegetative growth and the assimilated carbon is only partitioned between growth and storage. Therefore, the requirement for reserve carbohydrates should be readily achievable unless the trees keep growing very late into the season. Nursery trees, on the other hand, may be more dependent on reserve nitrogen for their regrowth during establishment because root uptake of nitrogen is delayed by damaged root system, lack of new roots, or low soil temperatures in the spring.

Understanding the relationship between reserve nitrogen and carbohydrates and the role they play in tree performance has direct practical implications. If the growth potential of apple nursery trees is mainly determined by reserve carbohydrates, cultural practices in the nursery should be optimized to improve reserve carbohydrate status of the tree. In contrast, if the regrowth potential is mainly determined by reserve nitrogen, management strategies directed at optimizing nitrogen storage would improve the regrowth performance of apple nursery stock. In this article, I will describe how tree growth is dependent on reserve nitrogen and carbohydrates accumulated in the tree from the previous year, and how to use foliar urea application in the fall to improve reserve nitrogen status and consequent tree growth the following spring

Nursery tree growth the first few weeks after budburst is almost completely dependent on nitrogen stored in the tree. Combining nitrogen (N) applications in the nursery during the growing season and a foliar urea application after bud set improves reserve N status and 1st leaf tree growth.

Sources for Reserve Nitrogen and Carbohydrates

Mature leaves are the only source for reserve carbohydrates. Growing shoot tips and young leaves are strong sinks for assimilated carbon. When an apple tree is actively growing, most of the assimilated carbon is being used for growth. Only when growth slows down will assimilated carbon accumulate for storage. Therefore, any cultural practices that keep plants growing late into the season, such as heavy N fertilization or over-irrigation, will result in low reserve carbohydrate levels.

For reserve N, there are two sources. One is the root uptake from the soil. The other is mobilization of N from leaves back to the storage tissues during leaf senescence. Mobilization of leaf nitrogen requires a normal senescence process, during which proteins are broken down and the resulting amino acids are translocated back to the stem and root tissues. Approximately 50 percent of the total N in a tree is in the foliage before any significant nitrogen mobilization in the fall. Typically 50 to 60 percent of the leaf N can be mobilized back to the tree during leaf senescence (Oland, 1963; Cheng et al., 2002). However, if too much nitrogen is applied to the soil in the fall, leaf senescence will be delayed and nitrogen mobilization will be reduced. So, in addition to reducing carbon reserve, late N fertilization to the soil may also reduce nitrogen mobilization from the leaves.

In addition to the mobilization of existing nitrogen in leaves, leaves can readily absorb urea in the fall and the nitrogen derived from foliar urea applications can be translocated back to the tree. Our data showed that apple leaves can translocate 80 to 90 percent of the nitrogen absorbed from urea sprays back to the storage tissues (Cheng et al., 2002). After urea is absorbed by leaves, its metabolism involves hydrolysis of urea to ammonium, followed by incorporation of ammonium into amino acids. Some amino acids derived from foliar urea may be directly translocated back to the tree (Shim et al., 1973). In contrast, the existing proteins in the leaves have to break down to amino acids before they are mobilized.

Effects of N Fertilization and Foliar Urea on Reserve N and Carbohydrates

Compared with soil applied nitrogen, foliar urea application after terminal budset has the advantage that it maintains the existing root to shoot ratio and cold tolerance of the trees because it does not stimulate new growth in the fall. As shown in Table 1, apple trees that received foliar urea application have the same level of cold hardness as the control trees.

Both nitrogen fertilization during the growing season and foliar urea application after terminal budset in the fall were used in our study to alter reserve nitrogen and carbohydrate status of apple nursery trees (Cheng and Fuchigami, 2002). Briefly, bench-grafted Fuji/M.26 trees were fertigated with seven different N concentrations ranging from 0 to 280 ppm by using a balanced nutrient solution from June 30 to September 1. There were 30 trees at each N fertigation concentration. Half of the fertigated trees were sprayed with 3 percent urea (25 lbs urea per 100 gallon water) twice at weekly interval in Mid-October, and the remainder were sprayed with water as controls. All the plants were dug in late November after natural leaf fall, and stored at 2°C. One set of trees (five plants) from each treatment was destructively sampled before budbreak for N and carbohydrate content. The remaining plants were used in the regrowth test described below.

N content of the dormant trees increased almost linearly with increasing N fertigation concentration (Figure 1A). Foliar urea application increased reserve

| Treatment | LT50 (December 27) °C | LT50 (February 25) °C |
|-------------------|--------------------------|--------------------------|
| Control | -38.5 | -30 |
| 1.5% Urea 2 times | -39.5 | -28.6 |
| 3% Urea 2 times | -40.8 | -30.8 |
| 4.5% Urea 2 times | -40.4 | -30 |

*Stem tissues were tested. LT50 is the temperature at which 50% of the tissue is killed. Each number is the mean of 6 replications.

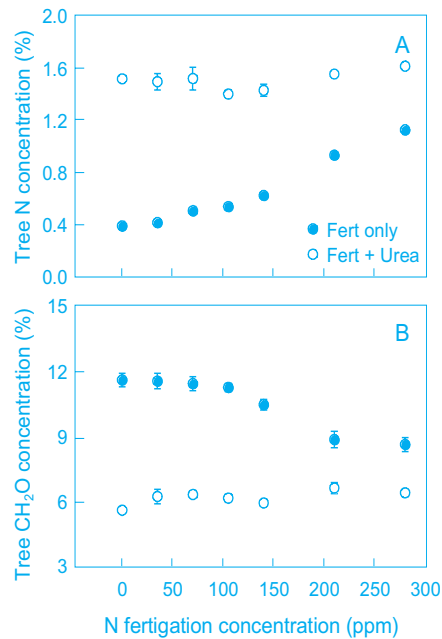


Figure 1. Tree N content (A) and carbohydrate concentration (B) of dormant Fuji/M.26 trees in response to N fertigation during the growing season and to foliar urea applications in the fall. Trees were destructively harvested before budbreak. Each point is mean with SE of 5 replicates. Solid circles represent trees that were only fertigated with seven N concentrations during the growing season, while the open circles are those that also received fall applications of foliar urea.

N content to a similar level across all seven N fertigation concentrations. Carbohydrate concentration generally declined with increasing N fertigation concentration (Figure 1B). Foliar urea application decreased carbohydrate concentration to a similar level across the N fertigation concentrations. On a whole tree basis, total amount of N increased linearly with increasing N fertigation concentration (Figure 2A). Total reserve carbohydrates increased with increasing N fertigation concentration at first, then tended to decrease with further rise in N concentration (Figure 2B). Foliar urea applications increased total N reserve

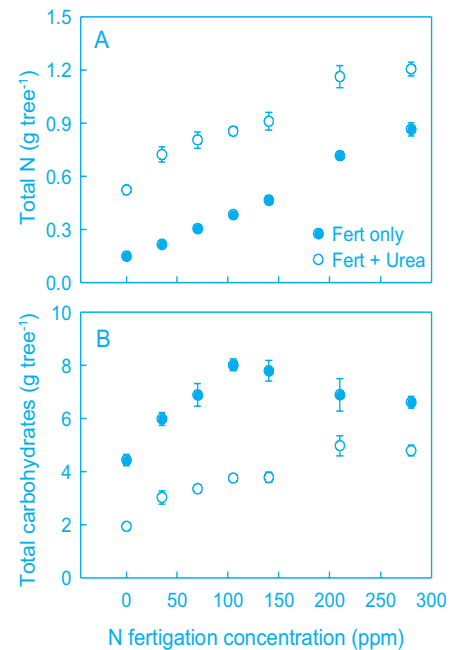


Figure 2. Total nitrogen (A) and total carbohydrates (B) of dormant Fuji/M.26 trees as affected by nitrogen fertigation during the growing season and foliar urea applications in the fall. Trees were destructively harvested before budbreak. Solid circles represent trees that were only fertigated with seven N concentrations during the growing season while the open circles are those that also received fall applications of foliar urea.

and decreased total carbohydrate reserve at each given N fertigation concentration.

Growth in Relation to Reserve Nitrogen and Carbohydrates

For the regrowth test, trees from each treatment were grown under either no N supply or sufficient N supply (140 ppm) in a balanced nutrient solution for two months after budbreak (Cheng and Fuchigami, 2002). ¹⁵N-labeled ammonium nitrate was used as the sole nitrogen source for the regrowth in order to distinguish current uptake of nitrogen

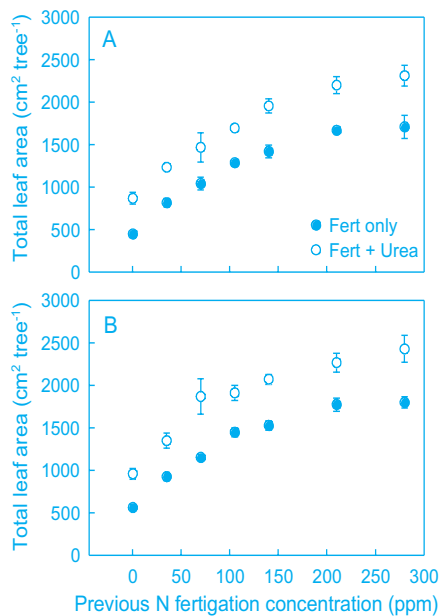


Figure 3. Total leaf area of Fuji/M.26 trees at the end of the regrowth period, in relation to previous N fertigation and foliar urea treatments under no N supply (A) or 140 ppm N supply (B) during the spring regrowth period. Solid circles represent trees that were only fertigated with seven N concentrations during the previous season while the open circles are those that also received foliar urea applications in the previous fall.

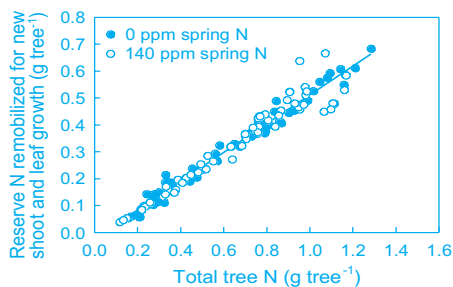


Figure 4. Reserve nitrogen remobilized for new shoot and leaf growth at the end of the regrowth period in relation to total amount of nitrogen accumulated in the tree from the previous growing season. Solid circles are trees that did not receive any nitrogen while open circles are those that received 140 ppm N supply during the spring regrowth period.

from reserve nitrogen. Regardless of N supply in the spring, total leaf area at the end of the regrowth period increased curvilinearly with increasing N concentrations from the fertigation treatment the previous year (Figure 3). Trees sprayed with fall foliar urea produced greater total leaf area at each given N fertigation concentration. Because the fall foliar urea application did not alter the tree size at each given N fertigation concentration, this means that

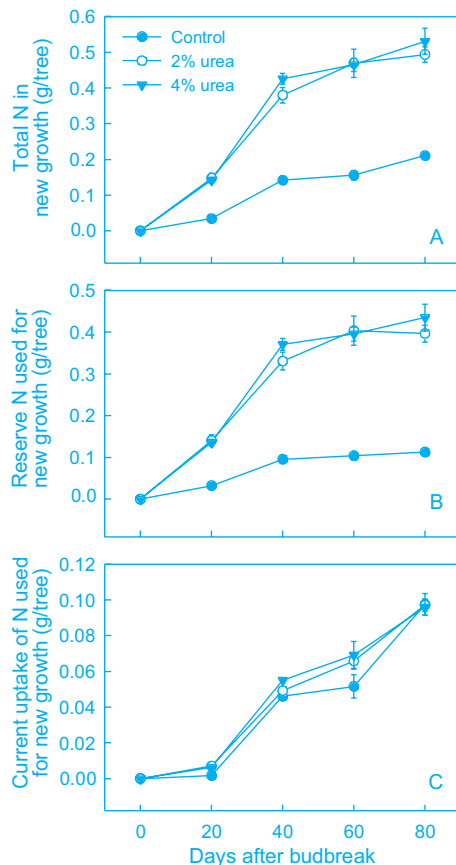


Figure 5. The total amount of nitrogen present in the new shoots and leaves (A), the contribution from nitrogen reserves (B) and that from current uptake of nitrogen in the spring (C) over the course of the spring regrowth as affected by foliar urea sprays in the previous fall.

when tree size was the same, trees with higher reserve N but lower reserve carbohydrates had more new leaf growth than those with lower N reserve but higher carbohydrate reserve.

In addition to the increased quantity of new leaf growth, the quality of the leaf growth was also related to the reserve N status. Regardless of current N supply, trees sprayed with foliar urea maintained a higher level of leaf N content and green color than the controls (data not shown). The effect of N supply in the spring on leaf N content and leaf color was observed mainly on trees fertigated with low N concentrations the previous growing season, and the effect was relatively small.

A single linear relationship was found between the total amount of N accumulated in the tree from the previous growing season and the amount of reserve N used for new shoot and leaf growth in the spring regardless of N supply in the spring (Figure 4). This clearly indicates that reserve N utilization for new shoot and leaf growth was

dependent on the total reserve N available, and was not affected by the current N supply in the spring.

In conclusion, regrowth of apple nursery trees in the spring is mainly determined by nitrogen reserves and is not limited by carbohydrate reserves. Although there are high concentrations of reserve carbohydrates accumulated in the trees with low reserve nitrogen, these trees cannot make the best use of these reserve carbohydrates for new growth unless foliar urea is applied to increase the reserve nitrogen level. So, nitrogen plays a pivotal role in the utilization of carbohydrate resources (Cheng et al., 2001; Cheng and Fuchigami, 2002). Our results also indicate that reserve nitrogen utilization for new shoot and leaf growth is dependent on the total amount of reserve, and is not affected by the current N supply in the spring.

Contribution of Current Uptake of Nitrogen to New Growth

Estimating the contribution of reserve nitrogen and current uptake of nitrogen to the new growth in the spring is important for developing a suitable fertilization program for newly planted trees. We altered the reserve nitrogen levels of Gala/M.26 trees by using foliar urea applications at concentrations of 0, 2 or 4 percent at weekly interval for a total of four times in the fall. These foliar urea sprays increased total tree nitrogen from 0.267g (control) to 0.781 g (2 percent urea treatment) and 0.844g (4 percent urea treatment). The following spring, we supplied the trees with 140 ppm N in a balanced solution for 80 days. During this regrowth period, we destructively harvested trees every 20 days from budbreak to determine the contribution of reserve nitrogen and current uptake of nitrogen to the new growth.

The total amount of nitrogen (including both reserve and current uptake) present in the new shoots and leaves was greater in trees sprayed with 2 or 4 percent urea than the control trees (Figure 5A). The amount of nitrogen from reserves used for new shoot and leaf growth was dependent on the N reserve levels. Trees sprayed with 2 or 4 percent urea remobilized more nitrogen for new shoot and leaf growth than the control trees (Fig. 5B). There was no significant difference in the amount of nitrogen from current uptake used for new shoot and leaf growth between low and high N reserve trees (Fig. 5C). At 20 days after

budbreak, over 95 percent of the nitrogen used for new shoot and leaf growth was from reserves in both low and high N reserve trees whereas current uptake only contributed less than 5 percent of the total amount of N present in new shoots and leaves. Thereafter, the contribution from current uptake increased.

Summary

Although both reserve nitrogen and carbohydrates are essential for the initial growth of apple trees in the spring, the growth potential of apple nursery plants is mainly determined by reserve nitrogen and is not limited by reserve carbohydrates. Therefore, combining optimum nitrogen fertilization during the growing season with foliar urea application after terminal budset in the fall is an effective way to improve reserve nitrogen status yet still maintain enough reserve carbohydrates for the regrowth of nursery trees. Foliar urea application in the fall may also make earlier defoliation of nursery stock possible without reducing the growth potential in

areas where early winter freezing injury is a concern for nursery trees. Because the tree growth during the first couple weeks after budbreak is almost completely dependent on reserve nitrogen, there is no need to apply nitrogen fertilizer until two to three weeks after budbreak for newly planted trees.

Acknowledgements

The research described in this article was supported in part by the Washington Tree Fruit Research Commission, the Northwest Nursery Improvement Institute, and the New York Apple Research and Development Program.

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Health Benefits of Dietary Flavonoids: Flavonols and Flavones

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This work was funded in part by the New York State Apple Research and Development Program.

Flavonoids are a group of phenolic compounds that have been identified in fruits, vegetables and other plant foods, and have been linked to reducing the risk of major chronic diseases. More than 4,000 distinct flavonoids have been identified, but many are still unknown and need to be identified before we can fully understand the health benefits of phytochemicals. More and more convincing evidence suggests that the benefits of phytochemicals in fruits and vegetables may be even greater than currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases.

Flavonoids commonly have a generic structure consisting of two aromatic rings (A and B rings) linked by three carbons that are usually in an oxygenated heterocycle ring, or C ring (Figure 1). Differences in the generic structure of the heterocycle C ring classify them as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, and

isoflavonoids. Flavonols (quercetin, kaempferol, and myricetin) and flavones (luteolin and apigenin) are common flavonoids in the daily diet (Fig. 1, Table 1).

Flavonoids are most frequently found in nature as glycosides, but can occur as aglycones, especially due to the effects of food processing. Many different glycosides can be found in nature as more than 80 different sugars have been discovered bound to flavonoids.

Bioavailability

Human intake of all flavonoids is estimated at a few hundred milligrams (Hollman and Katan, 1999) to 650 mg per day (Kuhnau, 1976). The total average intake of flavonols (quercetin, myricetin, kaempferol) and flavones (luteolin, apigenin) was 23 mg/day, of which quercetin contributed about 70 percent, kaempferol 17 percent, myricetin 6 percent, luteolin 4 percent and apigenin 3 percent (Hertog et al, 1993). In this study, tea was the major source of flavonols and flavones at 48 percent of total intake, followed by onions at 29 percent, and apples at 7 percent.

Flavonoids are usually bound in foods to sugars as beta-glycosides and were considered non-absorbable. Human absorption was greater than was initially thought. Hollman et al. (1995) fed nine ileostomy patients, who lack colons, a single dose of quercetin in onions, which contain mostly quercetin-3-glucoside; pure quercetin-3-rutinoside (rutin), the predominant form of quercetin in tea; and pure quercetin aglycone. The average absorption of quercetin was 52 percent from onions, 17 percent for quercetin-3-rutinoside, and 24 percent for quercetin aglycone. Because the quercetin 3-glucoside present in onions cannot be cleaved by digestive enzymes, it was transported intact into the enterocyte by a sodium-dependent glucose transporter. The same group later fed nine subjects quercetin as a single large dose through onions (quercetin glucoside), apples (both glucose or non-glucose quercetin glycosides), and pure quercetin-3-rutinoside, and monitored plasma quercetin levels over 36 hours (Hollman et al. 1997). Quercetin from onions was absorbed most rapidly, and rutin least rapidly, indicating that quercetin glucoside is likely absorbed from the stomach or small intestine, while quercetin from rutin is probably absorbed from the colon after microbial cleavage of the sugar. Peak time and peak plasma levels were 0.7 h and 224 ng/mL after the onion meal, 2.5 h and 92 ng/mL after the apples, and 9 h and 90 ng/mL after the quercetin-3-rutinoside. Bioavailability of quercetin from apples and pure quercetin-3-rutinoside (rutin) was 30 percent of that from onions. Half-life of quercetin in plasma was about 24 hours, suggesting that accumulation of the compound is possible. In a recent study, six subjects were fed a meal containing fried onions

There is increasingly convincing evidence from in vitro, in vivo and epidemiological studies suggesting the benefits of flavonoids in fruits and vegetables in the prevention of chronic diseases because of their antioxidant, anti-cancer, and cardiovascular-protective effects.

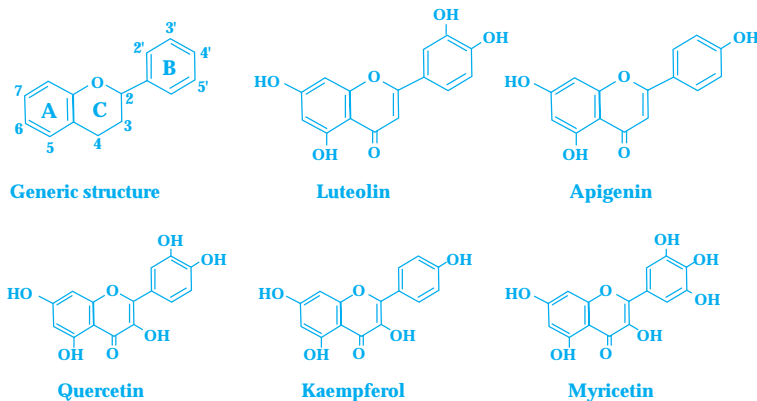


Figure 1. The generic structure of flavonoids, and structures of common flavonols and flavones.

and fresh cherry tomatoes (Boyle et al. 2000). Plasma quercetin levels increased from 16.5 ± 2.7 ng/mL to 104.9 ± 10.42 ng/mL four hours after ingestion and remained elevated at eight hours. After 24 hours, plasma quercetin levels were still higher than the baseline. Therefore, the sugar moiety is important for flavonoid absorption and bioavailability, and conjugation with glucose enhances bioavailability.

Effects of Processing and Storage

The effect of processing on flavonoids depends on the type of food, class of flavonoids being evaluated, and processing and storage conditions. Flavonoids are usually present in foods as conjugates in glycosylated or esterified forms. Different processing techniques may affect flavonoid release from the bound. The effects of heat treatments on quercetin and kaempferol levels in onions, green beans, and peas have been studied (Ewald et al. 1999). Pre-processing of onions (peeling, trimming, and chopping) prior to blanching caused the greatest loss (39 percent) in flavonoids. Subsequent cooking, frying or warm-holding for up to 2 h of the blanched vegetables, resulted in insignificant losses, with kaempferol showing a higher loss compared to quercetin.

In studying the effects of domestic processing and storage on quercetin, myricetin, and kaempferol in five berries, Hakkinen et al. (2000) reported that cooking strawberries with sugar to make jam resulted in minor losses (quercetin 15 percent, kaempferol 18 percent), while a 40 percent quercetin loss occurred during cooking of bilberries with water and sugar to make soup. Cold-pressing was better than steam-extraction in extracting flavonols from black currants. When stored at -20°C for nine months, quercetin content decreased (40 percent) in bilberries and lingonberries, but not in black currants or red raspberries. Under similar conditions, myricetin and kaempferol were more susceptible to losses (Hakkinen et al. 2000). Exposure of lettuce leaf to light after shredding produced significant losses of flavonoid moieties in green oak leaf (94 percent), green batavia (25 percent), iceberg (36 percent), lollo biondo (24 percent), red oak leaf (43 percent) and lollo rosso (6 percent) samples (DuPout et al, 2000). Storage of lettuce and endive heads in the dark at 1°C and 98 percent humidity for seven days also caused losses of total flavonol

| Major food sources of flavonols and flavones | | |
|--|--------------------------------------|--|
| | Flavonoids | Major Food Sources |
| Flavonols | Quercetin Kaempferol Myricetin | Onion, apple, broccoli, cranberry, grape, red wine* Kale, broccoli, parsley, leek, apple, berries, tea Parsley, cranberry, grape, red wine, berries, spinach |
| Flavones | Luteolin Apigenin | Celery, green pepper, spinach, green bean Celery, parsley, apple, grape, leek, onion |

* Listed in order of content in foods (Arai et al, 2000; Hertog et al, 1993; Hollman and Arts, 2000).

glycosides in the range of 7-46 percent (Dupont et al, 2000). Kaempferol conjugates, including kaempferol 3-O-glucoside, kaempferol 3-O-glucuronide, and kaempferol 3-O-(6-O-malonyl) glucoside have been found in endive varieties. Analysis of kaempferol levels in nine berries before and after they were used for jam preparation indicated that fresh and jam samples did not change much, indicating the effect of jam processing was little (Amakura et al, 2000). However, the effect of processing on the bioavailability of flavonoids has not been investigated.

Health Benefits

More and more convincing evidence from in vitro, in vivo and epidemiological studies suggests the benefits of flavonoids in fruits and vegetables in the prevention of chronic diseases is because of their antioxidant, anti-cancer, and cardiovascular-protective effects.

Cancer: Evidence suggests that dietary antioxidants can reduce cancer risk. Block et al. (1992) established this in an epidemiological review of approximately 200 studies that examined the relationship between fruit and vegetable intake and cancers of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas and ovary. In 128 of 156 dietary studies, the consumption of fruits and vegetables was found to have a significant protective effect. In persons whose intake of fruits and vegetables was low compared to those with high intake, the risk of cancer was twice as much. Significant protection was found in 24 of 25 studies for lung cancer. Fruits were significantly protective in cancers of the esophagus, oral cavity, and larynx. In 26 of 30 studies, there was a protective effect of fruit and vegetable intake in respect to cancers of the pancreas and stomach and in 23 of 38 studies for colorectal and bladder cancers. A prospective study involving 9,959 men and women at ages 15-99 in Finland

showed an inverse association between the intake of flavonoids and incidence of all sites of cancer combined (Knekt et al, 1997). After a 24-year follow-up, the risk of lung cancer was reduced to 50 percent in the highest quartile of flavonol intake. Consumption of quercetin in onions and apples was found to be inversely associated with lung cancer risk in Hawaii (Le Marchand et al. 2000). The effect of onions was particularly strong against squamous cell carcinoma. Boyle et al. (2000) showed that increased plasma levels of quercetin following a meal of onions was accompanied by increased resistance to strand breakage by lymphocyte DNA and decreased levels of some oxidative metabolites in the urine.

Carcinogenesis is a multi-step process and oxidative damage is linked to formation of tumors through several mechanisms. Oxidative stresses induced by free radicals cause DNA damage, and when left unrepaired can lead to base mutation, single and double strand breaks, DNA cross-linking and chromosomal breakage and rearrangement. This potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables. Studies to date have demonstrated that flavonoids in common fruits and vegetables can have complementary and overlapping mechanisms of actions, including modulation of detoxification enzymes, scavenging oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects.

Cardiovascular disease: Dietary flavonoid intake was significantly inversely associated with mortality from coronary heart disease, and an inverse relation (weaker but significant) with incidence of myocardial infarction (Hertog et al, 1993b). In a study in Finland, the intake of apples and onions, both high in quercetin, was inversely

correlated with total and coronary mortality (Knekt et al. 1996). In a recent Japanese study, the total intake of flavonoids (quercetin, myricetin, kaempferol, luteolin and ficetin) was inversely correlated with the plasma total cholesterol and low density lipoprotein (LDL) cholesterol concentrations (Arai et al. 2000). As a single phytochemical, quercetin intake was inversely related to total cholesterol and LDL plasma levels.

Mechanisms for the prevention of atherosclerosis by antioxidants have been proposed. For example, oxidized low-density lipoprotein (LDL) cholesterol is known as the atherogenic factor that contributes to heart disease. Oxidized LDL is typically taken up by macrophage scavenger receptors, thus promoting cholesterol ester accumulation and foam cell formation, which promotes atherosclerotic disease. Dietary antioxidants that are incorporated in LDL are themselves oxidized when these LDL are exposed to prooxidative conditions before any extensive oxidation can occur in the sterol or polyunsaturated fatty acids (Sanchez-Moreno et al., 2000). In addition, phytochemicals have been shown to have roles in reduction of platelet aggregation, modulation of cholesterol synthesis and absorption, and reduction of blood pressure.

Summary

In summary, dietary modifications by increasing the consumption of fruits, vegetables, and whole grains is a practical strategy for consumers to optimize their health and reduce the risk of chronic diseases. It is recommended that consumers follow the USDA/USDHHS Dietary Guidelines to meet their nutrient requirements for health improvement and disease prevention. Antioxidants are best acquired through whole food consumption.

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