

Fertilizing Crops for Functional Foods

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Foreword

The topics described in the following papers were originally presented at the “Symposium on Fertilizing Crops for Functional Food,” November 11, 2002. The Symposium took place during the 2002 annual meetings of the American Society of Agronomy (ASA), the Crop Science Society of America (CSSA), and the Soil Science Society of America (SSSA), in Indianapolis, Indiana.

Consumer and producer interest in healthy food is growing. The concept of “functional food”, or food with enhanced levels of health-promoting phytochemicals, gives fresh perspective to the mineral nutrition of plants. The symposium was planned to review the impacts of plant mineral nutrition on functional food components—nutraceuticals—in crop products. While past soil fertility research has considered impacts on traditional quality parameters such as protein, oil, and vitamins, the possibilities for functional foods and nutraceuticals represent a new area of discovery.

Research is continuing on many fronts and there is still much to learn about this fascinating topic. However, the papers in this proceedings provide insight into the wide range of possibilities related to fertilizing crops for functional foods.

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Introductory Remarks

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Welcome to the “Symposium on Fertilizing Crops for Functional Food.” It is a joy to have the privilege of moderating this symposium. Fertilizing crops for the functionality of food—for its nutraceutical properties—can be one part of the full set of production practices needed to deliver increased value to producer and consumer alike. This is the first time for such an event at the annual meetings of the tri-societies, ASA/CSSA/SSSA.

The increasing media attention to organic food is received differently in the science community than in agri-business. Scientists look at the principles and definitions of organic farming, note correctly that its goals cannot be met with its methods, and tend to dismiss it. Agri-business, however, is more familiar with another principle – utterly non-scientific yet highly functional – and that principle is that “the customer is always right.” The customer in this case isn’t right, but is expressing an unmet need. Consumer demand for organic food points to a perception that agronomy has failed to meet a need.

People choose organic foods for good reasons. One of them is a desire for foods that contribute more to

their health and longevity. There’s no scientific basis for their expectation that organically produced food will meet that desire. But science exploring functional foods and nutraceuticals is identifying not only new compounds of general benefit to health, but also those that are specific to the needs of specific people. The need expressed for food that is organic, can be met by food that is functional!

The literature contains plenty of evidence of an impact of mineral nutrients on the classical nutritional categories of vitamins, minerals, proteins, and oils. Decades if not centuries of research have documented such impacts. But little has so far been published on how mineral nutrients impact these newly discovered nutraceutical compounds. That is the topic of today’s symposium. It is my hope that this symposium will be one small step advancing such science. The small step, I hope, will contribute to a giant leap forward in improving the perception of agriculture. Both producers and consumers should see that agriculture rightly claims to nourish the soil to nourish the world.

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Can Potassium Application Affect the Mineral and Antioxidant Content of Horticultural Crops?

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Abstract

As consumers become increasingly aware of the importance of diet in avoidance of chronic diseases, the content of vitamins, minerals, and antioxidants in fruits and vegetables can greatly affect consumer demand. In humans, potassium (K) is needed to regulate enzyme function, enable muscle contraction, and transmit nerve impulses. Increased K intake may lower hypertension. Good sources of K from fruits and vegetables are bananas, melons, tomatoes, potatoes, squash, citrus, and spinach. Potassium has multiple enzymatic and catalytic functions in plants. Increased K fertility has been reported to decrease beta-carotene and increase lycopene content in tomato, and enhance the levels of vitamin C and carotenoids in carrots, tomatoes, and citrus. Additionally, the U.S. Food and Drug Administration (FDA) has approved the use of K as a health claim if the food contains at least 350 mg K, less than 140 mg sodium (Na), no more than 20 mg cholesterol, no more than 3 g saturated fat, and no more than 15% of calories from saturated fat per serving. Several fruits and vegetables qualify for this health claim. The effect of K fertility on watermelon was studied to determine if modification of commonly accepted farm practices could enhance K content and lycopene of fruit without negative effects on quality. Application of K at high rates (>280 kg K₂O/ha) decreased yield and fruit tissue pH. The K content of leaf petiole sap, length to diameter ratio of fruit, and fruit rind thickness increased with high application of K.

Introduction

Nutritional imbalances can cause or contribute to many human diseases. These include chronic conditions such as osteoporosis, obesity, heart disease, and mineral or vitamin deficiency such as anemia (iron deficiency) or vitamin A-deficient blindness. Potassium is used to maintain electrolyte balance in the body and may help prevent bone demineralization by preventing calcium loss from urine (Tucker

et al., 1999; He and MacGregor, 2001). Potassium can lower blood pressure, especially when used as a substitute for Na in Na-sensitive patients (Appel, 1999). These benefits resulted in approval by the FDA for a K health claim. This claim can be used on approved foods if the following criteria are met: 1) a serving contains at least 10% of the recommended daily intake (RDI) of 3,500 mg K; 2) a serving contains no more than 140 mg sodium; 3) the food contains no more than 3 g saturated fat; 4) the food contains no more than 20 mg cholesterol; and 5) total saturated fatty acids contribute no more than 15% of the total caloric intake per serving (FDA, 2000).

Many fruits and vegetables meet the FDA criteria for low fat and for high K (**Table 1**). Additionally, fruits and vegetables contain other vitamins and minerals, and non-nutritive compounds (phytochemicals) (Hasler, 1998; Craig and Beck, 1999). These phytochemicals appear to have health-beneficial properties, such as glucosinolates found in broccoli, lycopene found in tomatoes and watermelon, and quercetin in onions (Gerster, 1997).

In the past, identification of foods high in specific nutrients has meant little to most except those individuals careful to tailor their diet for existing medical conditions. Fortunately, the swell of consumers interested and willing to pay for a diet designed to prevent chronic disease has created a new market niche for health-functional fruits and vegetables (Shaffer, 2001a,b).

The benefit of K as a soil amendment (fertilizer) to increase yields of crops has been known for several hundred years, as reviewed by Mengel and Kirkby, 1980. Studies from the 1930s through the 1960s were done to establish the amounts of K needed for best crop response on K deficient soils. Requirements for K depend largely on the soil type, as crops grown on soils with high K fixation show less response to applied K. The recommended rate for many fruits and vegetables grown on sandy or sandy loam soils is usually 100 to 300 kg K₂O/ha.

Potassium is considered a macronutrient in plants. It is used in many photosynthetic and metabolic processes, and has a more general use rather than the specific cofactor or enzymatic uses of other minerals such as iron or magnesium. Potassium acts as an

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Table 1. Potassium content of fruits and vegetables, and contribution to % RDI of 3,500 mg.

Crop	Serving size	Weight of serving size (g)	Potassium per serving (mg)	% RDI per serving
Squash	1 cup	205	896	26
Potato	1 potato	202	844	24
Spinach	1 cup	180	840	24
Beets	1 cup	170	509	14
Sweet potato	1 potato	146	508	14
Banana	1 banana	118	461	13
Muskmelon	1 cup	160	494	14
Papaya	½ fruit	152	390	11
Watermelon	2 cups	304	352	10
Tomato	1 medium	123	273	8

Source: USDA Nutrient Database for Standard Reference, Release 14, Potassium Content of Selected Foods per Common Measure. 2002.

Table 2. Reported effects of potassium on horticultural crops.

Crop	Type of K application	Response	Reference
Grape (<i>V. labruscana</i> , var. Concord L.)	Field, 0 or 450 kg K ₂ O/ha	increased juice pH, K increased 15 to 50%, reduced uneven ripening	Morris and Cawthon, 1982
Grape (<i>V. Vinifera</i> , var. Shiraz, Sultana L.)	Greenhouse, perlite, with 0.25 or normal K application as Hoagland's solution.	Increased juice pH and titratable acidity increased K content of grapes	Hale, 1977
Grapefruit (<i>Citrus paradisi</i>)	Field, fertigation, foliar sprays	increased lycopene, b-carotene, vitamin C	Patil, 2002
Pistachio (<i>Pistacia vera</i> L.)	Field application, 0 to 330 kg/ha	decreased nut stain	Zeng et al., 2001
Strawberry (<i>Fragaria ananassa</i> Duch.)	Field application, matted row system	increased titratable acidity relative to no K	Haut et al., 1935;
	Greenhouse sand culture	increased soluble solids, titratable acidity, earliness, relative to no K	Ricketson, 1966;
Strawberry (<i>Fragaria Vesca</i> var. Semperflorens Duch.)	Field application, annual hill system (KCL, K ₂ SO ₄ , KNO ₃ , 60 to 180 lb/A)	increased titratable acidity with increased K	Saxena and Locasio, 1968
	Greenhouse sand culture (0 to 15 meq/L K)	K increased, titratable acidity increased, nonvolatile acids increased with increased K	Choureitah and Bünemann, 1972.
Tomato (<i>Lycopersicon esculentum</i> L.)	Hydroponic (0 to 10 meq/L K)	Lycopene, total carotenoids increase up to 8 meq/L	Trudel and Ozbun, 1971
Tomato	Field, fertigation (0 to 400 kg/ha)	decreased pH, increased titratable acidity with increased K	Fontes et al., 2000;
	Field, KCl or K ₂ SO ₄	less white and yellow color disorder	Francis, 2002
Watermelon (<i>Citrullus lanatus</i>)	Field, KCl (0 to 209 kg/ha)	increased resistance to rind rupture, increased rind thickness with K	Sundstrom and Carter, 1983

osmoticum in plant cells to regulate water and solute uptake, and is used for ATP formation, respiration, and enzyme activation. Most reports on K amendment for horticultural crops have centered on the yield response rather than quality. Potassium effects on composition and quality have been documented in strawberries, grapes, grapefruit, pistachio, watermelon, and tomatoes (**Table 2**). Potassium appears to most affect acidity, pH, and carotenoid content, and may promote disease resistance in the plant.

In most instances, additional K will decrease the fruit pH and increase acidity. This may be due to a general increased synthesis of organic acids from

up-regulation of the tricarboxylic acid pathway (TCA), as has been suggested for tomatoes (Hobson and Davies, 1971). In grapes, about 70% of the total mineral content is K, which increases as grapes ripen (Hale, 1977), and can combine with tartaric acid to form K tartarate. Tartaric and malic acid are the primary organic acids in wine grapes, and these and their salts determine the titratable acidity and pH of grapes. Hale (1977) concluded that K increased malic acid concentration through increased membrane permeability and subsequent release of malic acid from the vacuole, rather than from increased acid synthesis.

In watermelons, K content just meets the 10% RDI in a 2 cup serving size (**Table 1**). Preliminary results indicate that K can differ substantially among watermelon varieties (900 to 2000 mg/kg edible flesh), but does not appear to change with ripeness. Lycopene is the carotenoid imparting red color to watermelons and tomato (Perkins-Veazie et al., 2001). In tomatoes, increasing K hydroponically increases K content, flesh color, and lycopene content (Trudel, 1972). While Sundstrom and Chisholm (1982) found that increased K could increase rind thickness in a small sample of watermelons, they found no change in subjectively rated flesh color. The following study was done to determine if K content of watermelon could be increased by increasing soil K content, without detrimental effects on melon quality.

Materials and Methods

Plant material

Seeded watermelon ‘Sangria’ transplants were planted in a Bernow silty loam soil (fine-loamy, siliceous, thermic Glossic Paleudalf, CEC of 4 meq/100 g) with in-row spacing of 91 cm, 274 cm between rows, and 610 cm between plots. The field, previously in bermudagrass, and unamended with fertilizer, had a base level of 34 kg/ha available K. Treatments were arranged in a randomized complete block design, with four blocks per treatment, and consisted of 34, 140, 280, 560, and 840 kg K₂O /ha using KCl as the source. Potassium was applied preplant by spreading fertilizer over the plot area then tilling in. Nitrogen (a total of 120 kg/ha) was applied as split applications, with a broadcast application of 60 kg/ha preplant, then sidedressed with 30 kg/ha at vine running and 30 kg/ha at fruit set. Recommended fertility rates for this soil type and crop was 280 kg/ha K₂O. Phosphorus was applied preplant by broadcast at a rate of 160 kg P₂O₅/ha.

Leaf petiole sap K

Eight to ten leaves (most recently expanded leaf per vine) from each plot were harvested weekly from watermelon plants starting 30 days after planting, placed in plastic bags, held on ice until returning to the lab, and processed within an hour following the method of Hochmuth (1994). Stages of growth over the sampling period represented vines 20 cm long to fruit at first harvest. Leaves were gently rinsed in deionized water to remove excess soil and the petioles were cut from the leaves. Petioles were crushed in a garlic press and expressed sap placed on a portable K meter (Cardy Ion Meter, Spectrum Technologies, Plainfield, IL). Values for petiole sap K were in the 3,000 to 5,000 mg/kg range reported for watermelon (Hochmuth, 1994).

Melon measurements

Total melons, including those sunburned, poorly pollinated, or otherwise unmarketable due to injury or blossom end rot were counted in each block. Melons free of injury were harvested from plots, weighed, and length (blossom end to stem end) and

circumference measured around the center of the melon. Melons were cut in half transversely and thickness of the rind (between outer epidermis and pink colored flesh) measured with calipers at the ground spot and directly opposite the ground spot. Diameter of the cut surface (including epidermis) was measured with a tape measure marked in cm. The ratio of length to diameter, a gauge of melon shape, was determined from above measurements. Flesh samples (about 300 g) were taken from the center of each melon (locule and heart) and held at -80°C until analyzed. Frozen samples were thawed, homogenized, pH of puree measured by electrode, and total soluble solids content measured by digital refractometer (Atago, Plainfield, NY).

Mineral Analysis of Leaf and Fruit Tissue

Leaves used for petiole sap extraction were dried at 60°C. A 1 to 2 g sample was then ashed at 500°C in a muffle furnace for 12 hours and nutrients were extracted using a double acid Mehlich III extractant. Tissues from both leaves and fruit were analyzed for K using an atomic absorption spectrophotometer at 769.9 nm wavelength.

Results and Discussion

The K content of leaf petiole sap ranged from 2,700 to 5,000 mg/kg, depending on stage of development (**Figure 1**). These levels were similar to those reported by Hochmuth et al. (1994) for watermelon. Leaf petiole sap K content was highest in leaves from high K application, and lowest in leaves from plots with no K applied. The K content of leaf tissue was similar to that of petiole sap (data not shown).

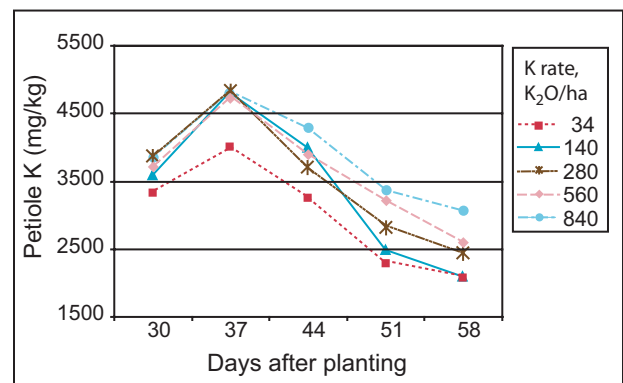


Figure 1. Petiole sap potassium content (mg/kg) of watermelon leaves harvested at intervals of growth.

The number of watermelons per plant decreased as K application increased over 280 kg K₂O/ha (**Figure 2A**). The length/diameter of watermelons increased in plots with more than 280 kg K₂O/ha (**Figure 2B**). This difference was due largely to the smaller diameter of the melons with increasing K rate (data not shown). At least 140 kg K₂O/ha was needed for full fruit set and growth of watermelons. Sundstrom and Carter (1982?) found reduced watermelon yield at K application above 139 kg K₂O/ha,

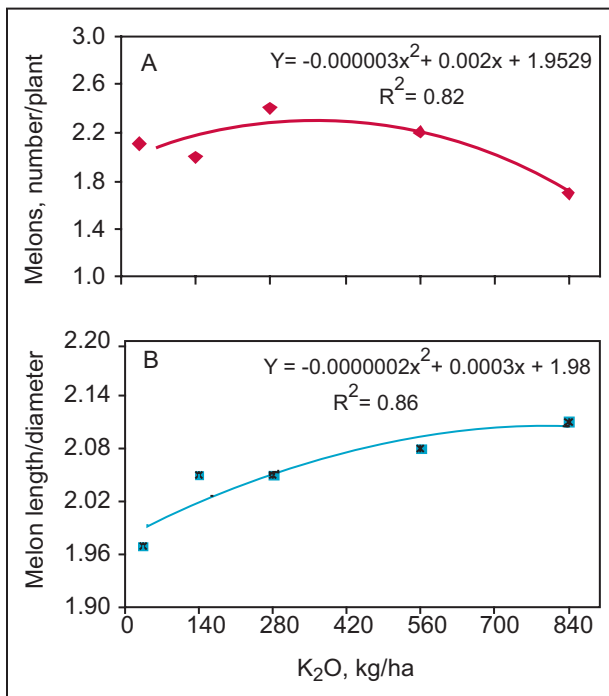


Figure 2. The relation of potassium application (kg/ha) to (A) yield (watermelons per plant), and (B) melon size, measured as the ratio of cm length to cm diameter.

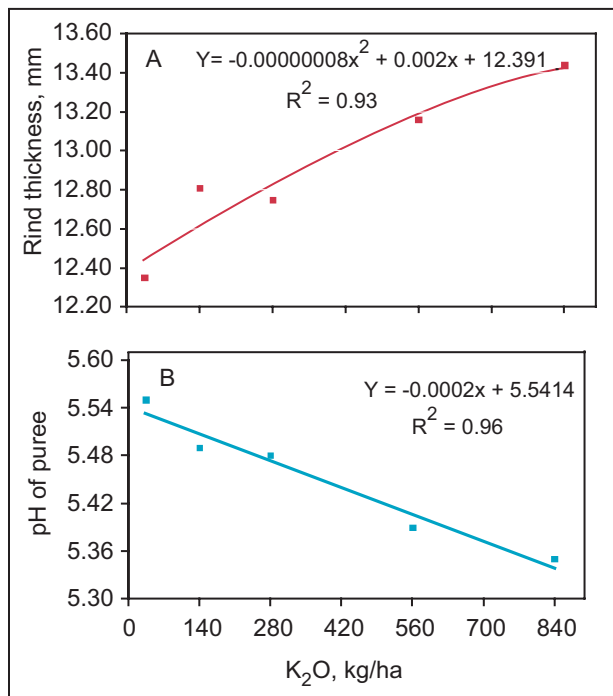


Figure 3. The relation of potassium application (kg/ha) to (A) transverse rind thickness (mm) of watermelons and (B) puree pH of watermelon placental tissue (heart and locular tissue).

and Zeng et al. (2001) found decreased pistachio nut yields when K application exceeded 220 kg K₂O/ha. Our results show that K rates above 280 kg K₂O/ha are detrimental to watermelon yield and size.

Rind thickness increased with K treatment (Figure 3A). The puree pH decreased as K treatment increased (Figure 3B). Normally, rind thickness and pH decrease with ripeness in watermelon. These results indicate that the watermelons from plots with 560 or 840 kg K₂O/ha K may have been less ripe than those from other plots. However, total soluble solids content, also a measure of ripeness, was similar among all treatments (about 11%). The K content of fruit flesh increased slightly (68 to 82 mg/100g) as K fertility increased from 31 to 280 kg K₂O/ha, but did not increase as fertility increased from 280 to 840 kg K₂O/ha (data not shown). It is possible that the chloride form of K used may have caused some toxicity to the watermelon plants.

Conclusions

Potassium contributes to the health of plants and humans. Use of K in hydroponic or soil applications increased the carotenoid and vitamin C content of tomatoes and grapefruit, and increased the K content of grapes and strawberries. The ability to use K as a health claim on fruits and vegetables offers new opportunities to explore environmental, genetic, and

production manipulation for increased K content. Results from our study with watermelons indicate that application of K above recommended rates may effectively alter fruit characteristics and composition, but yields may be reduced.

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Note: Missing in reference list:

Gerster. 1997. (pg. 2-1).

Sundstrom and Chisholm. 1982. (pg. 2-3).

USDA Nutrient Data Base. (pg. 2-2, Table 1).

From Soil to Snake Oil: Possibilities and Limitations in Food Functionality*

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Abstract

Plants are the foundation for a significant part of human medicine. Monomolecular drugs have been widely adopted, obscuring the collective wisdom of many traditional remedies. Plants contain secondary compounds that have the potential to influence human health. Functional foods deliver physiological benefits beyond nutrition. However, the functional constituents of many foods may be unknown. Uptake and assimilation of nutrients by plants may dictate the kinds and amounts of functional secondary compounds in crops. For example, sulfur (S) uptake may dictate the health functionality of *Brassicaceae* and *Alliaceae* crops. Competitive S and selenium (Se) uptake may further enhance or diminish the health functionality of these crops. Fertilization of *Brassica* crops with sodium selenate (Na_2SeO_4) can increase Se accumulation, but reduce glucosinolate concentration, thereby diminishing the benefits of these S-based compounds. Luxuriant fertility levels generally do not result in vitamin or secondary compound increases in crop plants, although mineral increases have been noted. The potential for improving and/or limiting food functionality through fertility practices will be considered from the perspective of nutrient uptake and assimilation.

Introduction

Food and health are inextricably connected, but their relationship is difficult to understand. If one judges a scientific field on the level of interest among the general public, nutrition and health come close to the top of the list. And in fact, many consumers are highly interested in matters of food and health. Some of this may stem from their desire to improve their health through dietary modification, and others may be interested in the potential healing benefits of foods. Unfortunately, there is so much conflicting information about diets and health that many readers find themselves overwhelmed by contradictory suggestions, unproven claims, and marketing efforts that seem to lack scientific foundations. It is not surprising that today's consumer faces a myriad

of confusing recommendations and suggestions that do little to sort out fact from fiction. In fact, the modern field of food functionality has at times seemed to resemble the snake oil claims sales that characterized a number of natural product–health marketing efforts over the years. How much of this has a scientific foundation and the potential to positively impact human health?

Fortunately, the origin of many of our edible crop plants (and particularly horticultural crops such as vegetables) is connected to their use as both food and medicine. The medicinal or therapeutic value of a number of vegetable plants may have been an important factor in their domestication. For example, rice has been used by cultures throughout the world as a symbol of fertility. Throwing rice at weddings may have originated with the belief that rice protected the fertility of the newly married couple. Tomatoes were originally used to treat eye diseases and other ailments in addition to being a food crop. The vegetable alliums, including onion, garlic, and their relatives, have been used by cultures throughout the world to improve blood circulation, as well as additions to flavor food. This is also true of carrot and related members of the vegetable *Apiaceae*. Thus, plants and their use as both food and medicine are deeply woven into the fabric of many human cultures. Although we do not have information on the details of domestication history for these crops, it is possible that their potential value as foods enabled humans to make dual use of them, thereby improving their chances of becoming adopted by cultures.

Medicine, horticulture, and botany intersected in the recognition that plants can prevent, treat, and cure disease (Janick, 2002). The origins of all the healing arts begins with plant-based products, and thus horticulture and medicine have been linked since the beginning of these practices. The priest, physician, healer, shaman, botanist, and horticulturist were connected by their natural interest in plant materials. They also incorporated a magical or dimension of superstition to their healing practices. Janick (2002) has pointed out that the Egyptian word *pharagia*, which means making magic, is the origin of the Greek *pharmakon* and Egyptian *pharmaki*, serving as the origin of the word pharmacy.

However, the development of the scientific method transformed medicine into a scientific discipline by the 17th century. It was at this time that medicine and horticultural botany began to separate. Prior to

*Portions of this paper can be found in Goldman, 2003.

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their separation, documents known as herbals described the healing properties of many plants. Today, the herbal is a reminder of the primary linkage between horticulture, medicine, and human health, as it combines information about medical remedies, botanical features, and horticulture. As chemical methods of synthesis improved, highly purified monomolecular drugs became the norm (Lawson, 1998). Within the last 50 years, these pharmaceuticals have become standard in preventing, treating, and curing disease in the U.S. In my opinion, this has been a tremendous boon to public health and a fantastic success of modern medicine. However, it has also displaced some elements of our collective wisdom with regard to matters of food and health.

Many of the compounds that have been identified as health-functional in vegetable crops have also been associated with defense against pathogens. Compounds such as flavonoids, carotenoids, terpenes, glucosinolates, isoflavones, and thiosulfinates may, through their inherent toxicity, confer significant opportunities for pest control. In addition, they may also deliver some of the bitterness and pungency to vegetables that we have come to associate with some crops, such as certain salad greens, peppers, vegetable brassicas, and vegetable alliums. It is possible that the astringency of these compounds, which protect the plants in their growing environment, may also protect humans from pathogens.

Onions and Cardiovascular Health

Onion was domesticated in the mountains of Asia from a wild progenitor that was likely perennial and formed many small bulbs. Selection pressure for a single, large, apically dominant bulb with a biennial life cycle enabled humans to develop a propagule that could serve as a storage vegetable (Goldman, 2001). The biennial life cycle requires storage of certain compounds during the over-wintering period, and the formation of the alk(en)yl-L-cysteine sulfoxides (ACSOs), also known as flavor precursors, may have been one of the ways in which onion bulbs stored S.

Tissue disruption initiates lysis of the ACSOs and causes formation of a variety of organosulfur compounds including the lachrymatory factor, which causes the familiar tearing in those chopping fresh onion, and thiosulfinates. Thiosulfinates are very unique molecules because they are responsible for a variety of activities, including both the flavor and medicinal properties that derive from alliaceous vegetables (Block, 1992). For example, the thiosulfinates have been shown to be important in onion-induced antiplatelet activity (Briggs et al., 2000). In addition, organosulfur compounds produced by alliums, particularly those that develop from disruption of the ACSOs, likely play a unique role in pest control and inhibit predation by would-be predators (Fenwick and Hanley, 1986).

Thus, the formation of thiosulfinates and other organosulfur compounds has a multiplicity of roles for onion: (i) they serve to deter pests, (ii) they

confer desirable flavors to food and mask undesirable flavors; and (iii) they confer unique medicinal properties to onion consumers, many of which have yet to be fully understood.

At least 23% of the current U.S. population has some form of cardiovascular disease (AHA, 2000), and most of the U.S. public suffers from some narrowing of coronary arteries due to the presence of arterial plaque (discussed in Goldman, 2001). Cardiovascular disease, including heart attack and stroke, is the leading cause of death in the U.S. for men and women from all ethnic and racial groups studied. The primary forms of the disease include high blood pressure, coronary heart disease, and stroke. Persons with risk of heart disease are often prescribed antiplatelet agents such as aspirin to maintain blood flow and prevent thromboembolic events caused by aggregating platelets in the coronary arteries. Platelets play a key role in thrombosis and acute coronary syndromes because they facilitate blood coagulation. Platelets also play a major role in the development of atherosclerotic narrowing of coronary and cerebral arteries. The inhibition of platelet aggregation is directly associated with the maintenance of coronary arterial blood flow.

In 1998, we formed an interdisciplinary team to investigate cardiovascular health claims for onion. We have (i) determined allium-derived thiosulfinates differentially inhibit human platelet aggregation *in vitro* and are more potent than aspirin at similar doses (Briggs et al., 2001), (ii) developed a model reaction mixture for large-scale preparation of allium-derived organosulfur compounds such as thiosulfinates (Shen et al., 2000), which should facilitate clinical trials; (iii) determined that a chromosome region on linkage group E in onion accounted for a significant amount of the phenotypic variation for pungency, soluble solids, and *in vitro* antiplatelet activity (Galmarini et al., 2001), (iv) demonstrated that even minimal cooking times eliminate the *in vitro* antiplatelet potential of onion extracts, suggesting that fresh onion may be the only efficacious preparation for inhibition of platelet activity, (v) determined that S fertility at high levels (beyond 2 mM sulfate) in solution culture was not able to influence onion-induced antiplatelet activity (Orvis et al., 2001).

John Folts, one of our team members, has developed a well-accepted and widely used canine model for investigation of *in vivo* platelet activity and platelet-vessel wall interactions. Through this model, our team has been able to begin realistically testing whole foods for their suspected health functional characteristics. Our work shows that surprisingly low doses of fresh onion administered both intravenously and intragastrically abolish platelet-mediated thrombosis in stenosed canine coronary arteries (Briggs et al., 2001). This finding suggests the potential for significant cardiovascular health benefit. However the magnitude, duration, and bioactive principles remain unclear. Furthermore, preliminary tests with cooked

onion revealed no abolishment of platelet-mediated thrombosis, suggesting fresh onion may be the only dietary strategy for platelet inhibition from onion.

Cultivar differences for antiplatelet activity have been well documented (Goldman et al., 1995, 1996). One possible explanation for this finding is variation among candidate genes in the S biosynthetic pathway. Orvis (2000) used more than 25 cloned genes from this pathway from a variety of organisms including *Arabidopsis*, barley, maize, and onion as restriction fragment length polymorphism (RFLP) probes in an onion population segregating for antiplatelet activity, pungency, and soluble solids. Very few polymorphisms were detected among segregating progeny, indicating widespread conservation of the S biosynthetic pathway genes across disparate species. This finding suggests differences among cultivars may be due to factors other than differences in S biosynthetic genes.

Galmarini et al. (2001) identified a quantitative trait locus (QTL) that explained phenotypic variation for antiplatelet activity in a segregating onion population. The probe that revealed this QTL showed high homology to an invertase. Invertases are responsible for hydrolyzing fructans, which are primary storage carbohydrates in onion. Fructan polymerization and hydrolysis affect the osmotic potential of onion bulb cells and, as such, are associated with the bulbing process. Thus, one possible explanation for genetic variation in onion for antiplatelet activity may be variation in carbohydrate genes, which can influence water uptake, bulb size, and the concentration of organosulfur compounds in the bulb. More research must be conducted to determine if carbohydrate genes are indeed candidate genes for medicinal traits such as antiplatelet activity.

Selenium and Chemoprevention

Carcinogenesis involves prolonged and cumulative cell injury resulting in the development of a malignant cell or cells. Chemoprevention is the attempt to intervene in the precancerous stages of carcinogenesis by introducing natural or synthetic compounds (Greenwald, 1996). In chemoprevention, emphasis is placed on the beginnings rather than the endings of human disease. Many standard chemotherapeutic agents are toxic to both cancerous and healthy cells, but chemopreventative compounds must be nontoxic and free of side effects since they are intended to be administered to healthy individuals over long periods of time (Greenwald, 1996). Vegetables and fruits, already an integral part of the human diet, have offered a wealth of phytochemicals with potential in chemoprevention. The vegetable *Brassicas*, for example, contain dithiolthiones which in synthetic form inhibit lung, colon, mammary gland, and bladder tumors in laboratory animals. Like other natural chemopreventatives, this synthetic compound activates enzymes found in the liver, which detoxify carcinogens. The isothiocyanate sulforophane, recently isolated from broccoli, is

thought to function in a similar manner.

Organosulfur compounds from allium species have been shown to inhibit carcinogenesis by acting as blocking agents and effectively preventing carcinogen activation (Wattenberg, 1992; Fenwick and Hanley, 1985a,b,c). The similarity in biochemical activity between S and Se led to manipulation of nutrient uptake to produce seleniferous Alliaceous plants, which in turn may be useful in supplementing Se in humans and animal diets. Selenium may be more active than S analogues in chemoprevention in studies using Se-enriched garlic. Further investigation revealed Se-enriched garlic was superior to unenriched garlic in suppression of mammary tumors in cancer-treated mice (Ip et al., 1992). Selenoamino acids have been identified in Se-enriched and unenriched garlic, and in Se-enriched onion (Cai et al. 1995a,b). Cai et al. (1995b) proposed enhanced levels of selenocysteine were responsible for a reduction in mammary tumor growth in carcinogen-treated mice fed a Se-enriched garlic diet, although clinical evidence has yet to be produced.

Sulfur and Selenium Relationships

Sulfur, an important constituent of the glucosinolates that form in *Brassica* species, may have an inverse relationship with Se uptake. For example, Charron et al. (2001) found that Na_2SeO_4 fertilization increased Se accumulation and decreased glucosinolate concentration in rapid-cycling *Brassica oleracea*. For hydroponically cultivated onion, Barak and Goldman (1997) found that increasing levels of sulfate (SO_4)-S decreased selenite (SeO_4)-Se. When the antagonistic relationship between these two elements was expressed as a molar ratio, S/Se in plant dry matter was nearly identical to S/Se in solution culture with a slight preference for S when S/Se in solution culture is low. Thus, SO_4 and SeO_4 uptake must be considered jointly when attempting to produce a reliable source of organoselenium compounds in plants. Such SO_4/SeO_4 antagonistic uptake interactions have been noted in single cells (Smith, 1976), excised roots (Legett and Epstein, 1956), and whole plants (Bell et al., 1992), even at the field level (Severson and Gough, 1992).

Kopsell and Randle (2001) selected for high and low Se accumulation in a rapid-cycling *Brassica oleracea* population. They conducted two cycles of selection and found gains of 4.8% and 4% per cycle for high and low Se accumulation, respectively. These findings demonstrate the potential for manipulating Se uptake in rapid-cycling plants through breeding. Kopsell et al. (2000) found that increasing selenium in solution culture was associated with increases in leaf Se, potassium, and S, while leaf boron, iron, and phosphorus decreased. This indicates that plant mineral composition may be affected by Se in solution culture.

It is interesting to speculate how Se might influence onion flavor, since it will substitute for S in the ACSO's or flavor precursors. Kopsell and Randle

(1999a,b) found that Se did not affect the content of total ACSO in several onion cultivars grown in nutrient solutions. However, it did affect the levels of several individual ACSOs. They found that Se decreased gamma-L-glutamyl-S-(1-propenyl)-L-cysteine sulfoxide and trans(+)-S-(1-propenyl)-L-cysteine sulfoxide content in all four cultivars tested. They also reported that (+)-S-Methyl-L-cysteine sulfoxide content was higher, while (+)-S-propyl-L-cysteine sulfoxide content was lower with the added Se for two cultivars. Barak and Goldman (1997) found that Se lowered total bulb S content in all onion cultivars, and increased the percentage of total S accumulated as SO_4^{2-} in three cultivars. The effect of Se on the flavor pathway was similar to that found when onions were grown under low S-concentrations. Kopsell and Randle (1999a,b) showed that root, stem, and leaf tissues of rapid-cycling *Brassica oleracea* responded in positive, linear fashion to increasing Se.

Conclusions

Fertilization of crops for improved health functionality may be possible. However, certain challenges have already been identified. For example, competitive S and Se uptake has the potential to either enhance or diminish the health functionality of vegetable alliums and brassicas. Fertilization of *Brassica* crops with Na_2SeO_4 can increase Se accumulation, but reduce glucosinolate concentration, thereby potentially diminishing the benefits of these S-based compounds. Luxuriant fertility levels generally do not result in vitamin or secondary compound increases in crop plants, although mineral increases have been measured and may be worth pursuing. In general, it would appear that strategies designed to improve the health functionality of crops must take into account the entire balance of nutrient uptake in the plant. Focusing on a single nutrient or a single pathway in order to enhance secondary compounds may have significant tradeoffs for other biosynthetic pathways. Functionality must be examined in an interdisciplinary context in order for significant progress to be made in improving human health.

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Factors Affecting the Concentration of a Nutraceutical Lignan in Flaxseed

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Abstract

There is considerable interest in the use of naturally occurring plant components to prevent or treat a variety of human diseases. One such phytochemical, secoisolariciresinol diglucoside (SDG), can be isolated and purified from flax (*Linum usitatissimum*). SDG has been shown to have potential therapeutic benefits in some hormone dependent cancers, heart disease, diabetes, and even in some auto-immune diseases such as lupus nephritis. SDG is bio-synthesized from the amino acid phenylalanine and accumulates in the mature flaxseed as part of a larger chemical complex. As many as 12 varieties of flaxseed grown at locations in both Manitoba and Saskatchewan for up to 11 years have been analyzed for SDG content. Both variety and year had significant effect of SDG concentration in defatted flaxseed meal. Studies of shorter duration and with fewer varieties where soil nitrogen (N), phosphorus (P), sulfur (S) and/or potassium (K) levels were supplemented have also been conducted. In general, nutrient supplementation had little effect on SDG concentration. Where N boosted flax yield, reductions in SDG were observed, but much smaller proportionally than the increase in yield.

Introduction

Flax has long been grown as a source of linen fiber and linseed oil. In the last decade there has been a great deal of research on the seed component called SDG. This phytochemical is but one example of a larger group of compounds referred to as lignans. SDG has been shown to have potential benefits in some forms of cancer, heart disease, lupus nephritis,

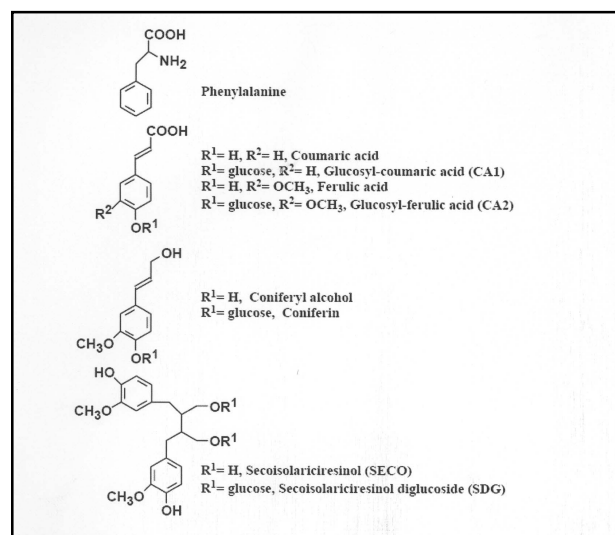


Figure 1. Chemical structure of flax lignans.

and diabetes (Clark and Ogborn, 2003; Prasad, 2000, 2001, 2002; 2003, et al., 2000; Rickard-Bon and Thompson, 2003).

The biosynthesis of some lignans, including secoisolariciresinol, the aglycone of SDG, have been extensively studied (Ford et al., 2001; Xia et al., 2000). The pathway is summarized in **Figure 1**. As illustrated, the amino acid phenylalanine is converted into cinnamic acid that undergoes ring oxidation to coumaric acid and then both oxidation and methylation to ferulic acid. Ferulic acid is reduced into the corresponding alcohol, coniferyl alcohol. Two coniferyl alcohols are enzymatically oxidized and dimerized followed by some additional enzymatically controlled rearrangements and glucosylation which forms SDG. In flax seed, SDG is not found free but is incorporated into a larger complex (Ford et al., 2001; Kamal-Eldin et al., 2001). Careful chemical

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degradation of this complex also yields the cinnamic acid derivatives, glucosyl-coumaric acid (CA1) and glucosyl-ferulic acid (CA2).

It was demonstrated by Muir et al. (2000) that coniferin, the storage form of coniferyl alcohol, was at maximum concentration at about 5 days after start of flowering. Coniferyl alcohol reached maximum concentration a few days later while SDG reached maximum concentration in the seed by about 20 days after start of flowering.

Based on chemical analysis of eight varieties of flax seed that were collected from seed grown at four locations in 4 consecutive years, it was reported previously that flax seed meal contains between 9 and 30 mg of SDG per gram of defatted meal. The main source of variation noted from this sample set was year with secondary effect due to variety. The influence of location was of lesser significance (Westcott and Muir, 1996). It is known that CA1 and CA2 concentrations also vary with variety. Vimy has a very low combined concentration of the cinnamic acids, 1.3 mg/g meal, while other varieties contain between 8 to 16 mg/g defatted meal (Unpublished, Westcott and Muir).

In the above sample set, soil nutrients were not specifically measured or adjusted. It was of interest therefore to examine the effects of added soil nutrients on the concentration of SDG, CA1, and CA2. Seed was obtained from previously conducted fertility research studies that had been designed to measure other agronomic indicators.

1. Phosphorus study. The study was conducted in 1995 and 1997 using the variety AC McDuff. The 10 and 30 kg P₂O₅/ha rates were applied only in 1997. The least square means (presented) adjust for the effect of year on those two treatments, since the 1997 crop had lower SDG and yield levels than the 1995 crop. The yield was weakly correlated to CA1 and CA2 (r=0.27; p<0.10). Intermediate rates of P resulted in lower SDG levels than either zero or higher rates, while yield did not respond to P in these soils (Table 1).

Table 1. SDG, CA1, or CA2 concentrations and yield in response to applied P.

P ₂ O ₅ applied, kg/ha	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
0	11.8	0.94	1.41	2330
10	11.1	0.90	1.37	2634
20	11.4	0.89	1.32	2396
30	11.3	0.91	1.32	2368
40	12.0	0.95	1.42	2398
Contrasts:				
linear p	NS	NS	NS	NS
quadratic p	0.04	0.06	0.05	NS

2. Nitrogen by P study. Conducted 1996 and 1998, this was a full factorial design with four rates of each of N and P. Interactions between N and P were not significant (NS; p>0.05) for all four attributes (Table 2). The yield increased with N rate, but at a diminishing rate, as indicated by significant

linear and quadratic components. The concentrations of SDG and the cinnamic acids decreased linearly with increasing N rate. The level of P had no effect, except that intermediate rates of P produced the highest levels of CA2 and the lowest yields.

Table 2. SDG, CA1, or CA2 concentrations and yield in response to applied P and N.

N rate, kg/ha	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
0	17.6	1.74	1.17	1579
40	17.5	1.73	1.13	1874
80	17.4	1.63	1.01	2027
120	17.0	1.57	1.03	2097
Contrasts:				
linear p	0.024	<0.0001	<0.0001	<0.0001
quadratic p	NS	NS	NS	0.0007
P ₂ O ₅ rate, kg/ha				
0	17.3	1.64	1.09	1945
15	17.5	1.71	1.10	1905
30	17.4	1.67	1.12	1819
45	17.2	1.67	1.03	1909
Contrasts:				
linear p	NS	NS	NS	NS
quadratic p	NS	NS	0.029	0.044

3. Cultivar by N study. The study was conducted in 1997 to compare three cultivars at four N rates. The yield increased with increasing N but with a diminishing response at higher rates (Table 3a). The glucosyl-ferulic acid (CA2) increased with increasing N up to 60 kg/ha. However, SDG and glucosyl-coumaric acid (CA1) were not affected by N rate.

The Flanders cultivar had a modest increase in SDG as N increased, while the other two decreased (Table 3b). This was the only cultivar-N interaction with statistical significance at p<0.01. The difference in response between the low-SDG cultivar and the two that were generally higher in SDG is interesting.

Table 3a. SDG, CA1, or CA2 concentrations and yield in response to applied N.

N rate, kg/ha	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
0	17.6	1.74	1.17	1579
0	13.5	1.8	1.5	1675
30	13.2	1.8	1.6	1991
60	13.6	1.8	1.7	2039
90	12.9	1.8	1.6	2014
Contrasts:				
linear p	NS	NS	0.033	0.0019
quadratic p	NS	NS	NS	0.021

Table 3b. SDG in mg/g as affected by cultivar and N rate.

N rate, kg/ha	AC Emerson	AC McDuff	Flanders
0	16.4	13.4	10.6
30	16.2	13.4	10.1
60	16.1	12.3	12.4
90	14.7	12.4	11.6

4. NPKS study. This study was conducted 1996-1998 in Indian Head and 1996-1997 in Lemberg for a total of 5 site-years. Seed yields were significantly increased only by N (**Table 4**). Nitrogen significantly decreased the concentration of CA1 and CA2, but the decrease in SDG was only marginally significant. The yields were increased by a greater proportion (36%) than SDG, CA1, or CA2 were reduced (10%-14%). It should be noted that in 1998 at Indian Head, yields were highest of all 5 site-years but there was no effect of fertility treatment on any of the phytochemical attributes.

Table 4. SDG, CA1, and CA2 concentration and yield in response to applied NPKS fertilizers.

Treatment	SDG, mg/g	CA1, mg/g	CA2, mg/g	Yield, kg/ha
Check	15.8	1.48	0.99	985
N	15.2	1.28	0.88	1340
NP	14.8	1.27	0.85	1440
NPK	15.1	1.31	0.86	1391
NPS	15.1	1.39	0.88	1422
NPKS	14.9	1.28	0.83	1476
Contrasts:				
N (N-check)	0.11	<0.0001	0.0001	<0.0001
P (NP-N)	NS	NS	NS	NS
K (NPK+NPKS-NP-NPS)	NS	NS	NS	NS
S (NPS+NPKS-NP-NPK)	NS	NS	NS	NS

Conclusions

Added nutrients applied at time of seeding were not effective in increasing the concentration of SDG in flax seed. It is possible that application of added nutrients nearer flowering time may cause an increase in SDG concentration. These studies confirm earlier observations that there is significant variability in the SDG concentration in registered cultivars of flax seed. Thus, it might be possible to increase SDG concentrations using conventional breeding strategies.

Acknowledgments

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Phosphorus Fertilization and Biosynthesis of Functional Food Ingredients

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Abstract

The disease-preventive and health-restorative effects of fruit and vegetable consumption have been related to the action of several nutraceutical components, positively affecting the physiological functions in humans. Two classes of the major nutraceutical components studied in fruits include flavonoids and isoprenoids. Flavonoids such as quercetin and catechin are strong antioxidants and powerful inhibitors of calcium (Ca) second messenger function. Isoprenoids such as lycopene and carotene are also strong antioxidants. In general, these components are believed to be the principal agents in fruits, vegetables, and their processed products that impart anticancer properties and cardiovascular protection in humans. Phenolic components from fruit wines acted as strong superoxide and hydroxyl radical scavengers. As well, phenolic components from red grape wine inhibited the proliferation of breast cancer cell lines. Therefore, enriching the fruits and vegetables with nutraceutical components could have beneficial effects to the consumers. Both the flavonoid biosynthetic pathway and the isoprenoid pathway are heavily dependent on phosphorus (P)-containing metabolites such as ATP, NADPH, and sugar phosphates derived through the pentose P pathway. Thus, P fertilization may have a direct effect on the levels of these metabolites, and potentially in the levels of the end products such as flavonoids and lycopene. To examine this possibility, we have subjected tomato plants and apple trees to supplemented soil and foliar P (superphosphate, Hydrophos, Seniphos) fertilization. Evidence gathered so far suggests that increased P fertilization can enhance the anthocyanin levels in apples and lycopene levels in tomatoes. Further studies are in progress.

Introduction

Functional food ingredients (nutraceuticals) in fruits and vegetables such as flavonoids, terpenes, lycopene, etc. are biosynthesized through pathways that use reducing power in the form of NADPH, and ATP. As well, the biosynthetic origin of many of the

functional food ingredients occurs from the pentose phosphate pathway, which is a key metabolic pathway also involved in the antioxidant defense system. Higher levels of nutraceuticals will increase the nutritional quality of fruits and vegetables since the nutraceutical components have been found to exert beneficial effects on health of the consumers. The effect of P fertilization on the levels of functional food ingredients has not been investigated. To our knowledge, this is the first systematic study into the physiology and biochemistry of nutraceutical biosynthesis. In this study, we have used two varieties of apples (Red Delicious and McIntosh), and several varieties of tomatoes to evaluate the effects of P fertilization. Phosphorus fertilization was provided either as superphosphate or as foliar spray (Hydrophos and Seniphos; Phosyn plc, UK).

Apples

Objective

To evaluate the effect of soil and foliar P supplementation on the post harvest quality of apples (*Malus domestica* Borkh. cv. 'McIntosh' and cv. 'Red Delicious').

Methods

Five P treatments were applied to 20 trees (4 reps): control (no P), superphosphate (soil applied, 0-20-0) at a low dose (420g/tree) and a high dose (420g x 3/tree, in 20 dry intervals), and foliar applications of Hydrophos (8g P, 1.2g magnesium (Mg) in 4L) and Seniphos (11.6g P, 1.5g Ca in 4L).

For foliar treatments, one side of the tree was sprayed throughout the season at four 20-day intervals. Superphosphate treatments were applied to the entire tree, but apples were still separated into 'sprayed' and 'non-sprayed' sides to reduce other field effects. Apples were harvested at optimal maturity and stored in air at 0°C for 4 months.

Results

Phosphorus fertilization did not affect the fruit weight, firmness, starch, and internal ethylene of either 'McIntosh' or 'Red Delicious' fruit at harvest (data not shown). The soluble solids content of 'McIntosh' fruit was increased slightly by superphosphate and Hydrophos (**Figure 1**).

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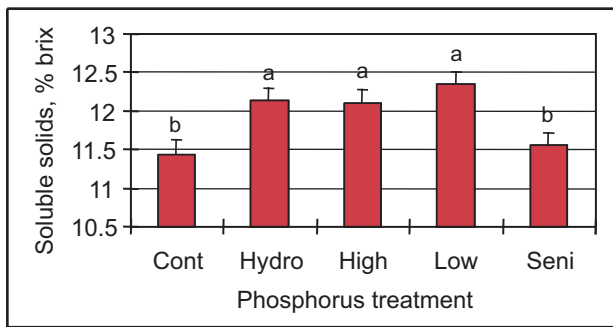


Figure 1. Mean (n=20) and standard error of soluble solids content of 'McIntosh' at harvest. Values with the same letter are not different ($p < 0.05$);

Phosphorus fertilization increased the percentage of red skin on both varieties at harvest (Figures 2 and 3) as well as the intensity of the red color in Red Delicious fruit at harvest (Figure 4). Fruit from sprayed sides of the trees with foliar treatments had more red color than those from the non-sprayed side (data not shown).

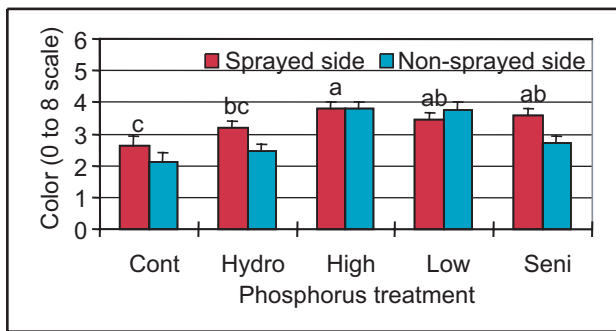


Figure 2. Color of 'McIntosh' at harvest (higher values indicate more red color). Letters compare 'sprayed' sides of treated trees with 'sprayed' and 'non-sprayed' sides of controls. Values with the same letter are not different ($p < 0.05$); mean (n=15) + S.E.

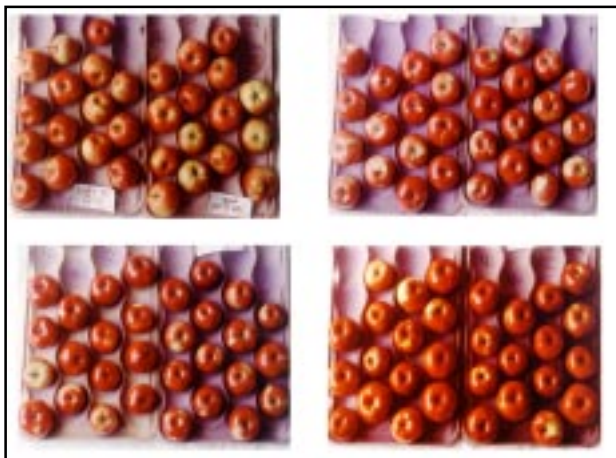


Figure 3. 'Red Delicious' apples at harvest.

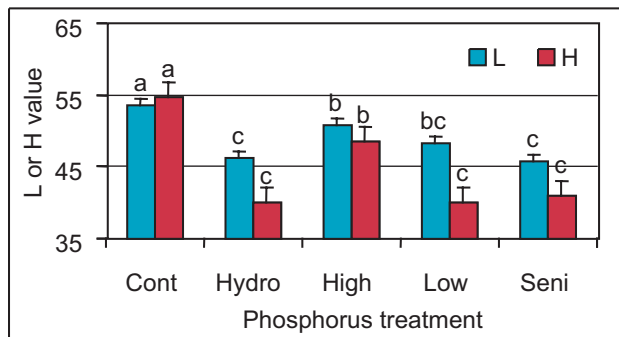


Figure 4. Evaluation of red color of 'Red Delicious' at harvest by colorimeter. Lower L-values indicate a darker (deeper red) color and H-values differentiate between red and green, with lower values indicating red. Values with the same letter are not different ($p < 0.05$); mean (n=15) + S.E.

Phosphorus fertilization decreased the incidence of superficial scald in 'McIntosh' (Figure 5), but not 'Delicious' (data not shown) after 4 months of air storage.

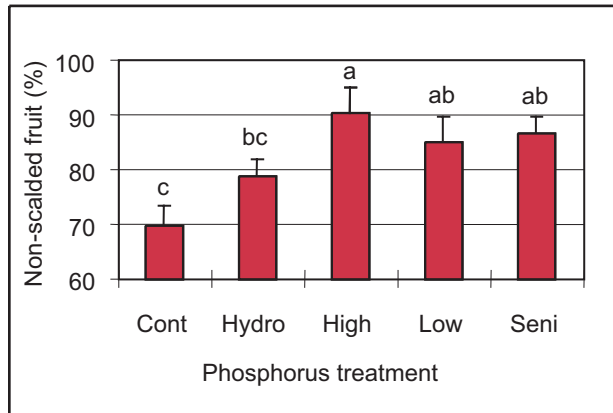


Figure 5. Evaluation of superficial scald on 'McIntosh' after 4 months of air storage plus 7 days at room temperature. Values with the same letter are not different ($p < 0.05$); mean (n=15) + S.E.

Discussion

This experiment was repeated in 2000 with 'McIntosh', but no differences were observed in color or soluble solids at harvest (data not shown). The 1999 season ended with warm sunny days and cool nights—conditions which stimulate anthocyanin production. The 2000 season was more cloudy and not as cool in the nights. After 4 months of air storage some P treatments developed red color (data not shown) to a greater extent, perhaps because of exposure to the cold storage conditions.

Under certain conditions, P fertilization demonstrates some improvements in initial quality and storability; however, a number of environmental factors strongly influence P effects. In particular, day/night temperatures and light exposure influence scald susceptibility and color development.

Tomatoes

The consumption of tomato products is increasing in light of its potential in preventing several forms of cancer, and the antioxidant properties of lycopene, xanthophylls, and lutein.

The objectives of the project were:

- 1) To evaluate the effect of soil and foliar P supplementation on lycopene biosynthesis, its compartmentation, and levels in tomatoes.
- 2) To establish the relation between P supplementation and antioxidants, as well as the activities of antioxidant enzymes in tomato.
- 3) To evaluate the relation between the antioxidant status and the stability of lycopene in processed tomato products.

Materials and Methods

Tomato seeds (*Lycopersicon esculentum* Mill. H9478, H9997, Heinz processing varieties) were germinated in potting soil in the greenhouse. Four-week old plants were transplanted in early June at the Cambridge Research Station of the University of Guelph. Tomato seedling were planted in 1.8 m x 3 m plots, each plot containing 24 plants. Each plot was separated from the others by a minimum distance of 5 ft. Fertilizer applications were based on Ontario Ministry of Agriculture and Food (OMAF) recommendations. The regular P (RP) plots received 50 kg P₂O₅/ha supplied as 5-20-20 and N as 80 g of ammonium nitrate at the time of planting. The low (LP) and high (HP) P-supplemented plots received an additional 120 and 240 kg P₂O₅/ha, respectively, as superphosphate (0-20-0). Two foliar sprays manufactured by Phosyn were applied in separate treatments to plots fertilized at the RP rate: Hydrophos (440 g P₂O₅/L, 74 g K₂O/L, and 60 g/L Mg) at 15 kg P₂O₅/ha, and Seniphos (310 g P₂O₅/L and 40 g/L Ca) at 21 kg P₂O₅/ha. The sprays were applied two times at 15-day intervals during the growing period, diluted in water at 7,400 L/ha. A no P (NP) control was included, which received no P but normal rates of other nutrients. The Olsen soil test P level was 50 mg/L, which is considered high. There were four randomly selected replicates for each treatment.

Hydrophos (Hydro) and Seniphos (Seni) were also applied at a lower dilution (high concentration), which is originally recommended by Phosyn. For the high-concentration treatments, the rate of water application was 200 L/ha. The solutions were sprayed using a hand-held bottle sprayer.

Analysis of Quality Parameters

Physicochemical analysis: The following parameters were analyzed: soluble solids or °Brix, non-soluble solids (NSS - %), ash (%), acidity (%), color Hunter LAB system (L [brightness], a+ [redness], b+ [yellowness], and a/b [color stability] values), lycopene content (mg/100 g of juice), sedimentation stability (precipitate weight ratio [PWR -%], gross viscosity GV [Brookfield viscosity -mPa.s], and serum density [SD -mPa.s]).

Soluble solids (°Brix): The soluble solids content of all samples was measured at room temperature with a hand held refractometer (Fisherbrand, 0 to 25% Fisher Scientific Co.). Readings were expressed as °Brix (AOAC methods 9.32.14 1995). This parameter was measured during processing for all the samples before and after boiling and sterilization.

Total acidity: 10 g of tomato juice was accurately weighed into 250 mL beakers in duplicate. To each sample 200 mL of distilled water was added. The resulting mixture was titrated with 0.1 N NaOH to a pH value of 8.0 in an Accumet Basic AB15 - pH meter (Fisher Scientific Co.). Total acidity was calculated as percentage of citric acid on a fresh weight basis (AOAC Methods 1995; Postlmayr et al., 1956; Nielsen, 1998).

Ash content: 10 g of tomato juice was accurately weighed in pre-dried crucibles. The samples were ashed for 16 hrs. in an Isotemp muffle furnace at 550°C. The ash content was calculated using the formula in AOAC International, 900.02A, 1995.

Phosphorus analysis: A known weight of the fruits was transferred to a crucible, and heated in an oven at 500°C to obtain the ash. The ash was dissolved in 6M sulfuric acid and the phosphate content determined by reacting with molybdate reagent and spectrophotometric estimation.

Non soluble solids (NSS): 10 g of juice and sauce was weighed in pre-dried crucibles. The samples were dry at 105°C for 12 hrs. NSS were measured and calculated according to AOAC Methods 926.08, 1995.

Color: Tomato juice and sauce processed were analyzed using a Minolta CR-300 Chroma meter (Minolta, Ramsey, N.J.) calibrated with a white standard tile. Twenty-five mL of each replicate was transferred to petri plates and the color measurements were performed on the surface of the liquid five times at different places. The chromaticity parameters L, a+, b+, and a/b recorded were the average of five measurements for each replicate (Cheng & Shewfelt, 1988; Baldwin et al., 1991).

Consistency (Brookfield viscosity): Jars containing 250 mL of juice and sauce samples were analyzed using a Brookfield Synchro-lectric viscometer (Brookfield Engineering Laboratories, Stoughton, Massachusetts) with a No. 4 or a No 5 spindle. Positions as well as settings of the viscometer were prepared to obtain precise measurements. Speed of 10-rpm was used. Readings were taken at the 10th cycle (Takada & Nelson, 1983).

Serum viscosity (SV): Fifteen samples of serum were used. The serum was obtained by centrifugation of the juice at 12,800 rpm for 30 min at 4°C followed by filtration of the supernatant through Whatman # 1 filter paper. A water bath (VWR Scientific) was set at 24°C (room temperature). An Ostwald-Cannon-Fenske capillary viscometer (size #50) for transparent liquids was used for this purpose; 10 mL of serum were weighed to determine the density at 24°C; 7 mL were deposited into the

viscometer previously submerged partially into the water bath and left for 2 min to equilibrate temperature. Serum was sucked up until the line of measure with a manual vacuum valve and two readings were made for each sample. The flow time was recorded. The kinematic viscosity (ν) is expressed in centistokes (cSt or mm²/s) and calculated as:

$\nu = Ct$, where:

C = calibration constant of the viscometer, cSt/s and

t = flow time, s,

The dynamic viscosity is calculated as follow:

$\eta = \rho\nu$, where:

η = dynamic viscosity, centipoises (cP) or millipascal-second (mPa.s).

ρ = density, g/mL, at the same temperature used for measuring the flow time t, and

ν = kinematic viscosity, cSt (mm²/s), (Caradec and Nelson, 1985).

Sedimentation analysis: For determining precipitate weight ratio, approximately 40 g of tomato juice prepared through hot break process, as accurately weighed into a 50 mL pre-weighed glass centrifuge tube. The sample was centrifuged at 12,800rpm x g, for 30 min at 4°C. After centrifugation, the supernatant was removed from the precipitate. Samples were replicated two times. The precipitate with the tube was then reweighed accurately and the precipitate weight ratio was calculated by using the equation of Takada & Nelson (1983).

$$\text{PWR}\% = \frac{(\text{Precipitate} + \text{tube weight}) - (\text{tube weight})}{(\text{Initial sample} + \text{tube weight}) - (\text{tube weight})} \times 100$$

where : PWR = Precipitate Weight Ratio

pH: The pH of all samples was measured at room temperature with a pH meter (Fisher Scientific Company).

Lycopene content: 4 g of tomato juice and sauce were precisely weighed into 250 mL brown bottles to exclude light and protect lycopene from degradation (Sadler et al., 1990). One hundred mL hexane:acetone:ethanol (2:1:1 v/v) was added to each vial, stoppered, and agitated for 10 min on a wrist action shaker (Burrel Corp., Pittsburg, PA). This was followed by the addition of 15 mL of water and further shaking for 5 min. The solution separated into distinct, aqueous polar (65 mL) and non-polar (50 mL) layers. The non-polar phase was removed and filtered with 0.45 μm nylon membrane filter (Fisher Scientific). Fifty μm of the filtered aliquot was subjected to HPLC analysis using an Exterra C18 column (Waters 600S) with acetonitrile:methanol (85:15, solvent A) and methanol:hexane (75:25 solvent B). The elution was started with 100% of solvent A and 0% of solvent B at time 0 and ended with 100% of solvent B, and 0% of solvent A, in a linear gradient during a period of ten minutes. The elution of β -carotene and lycopene was monitored at 475 nm. The lycopene used was 95% pure (Sigma Chemical Co.) and showed a retention time of 3.88 min.

Yield was calculated using the total amount of different harvests of tomatoes. Ripe tomatoes from the plot were harvested and weight determined. Statistical analyses were conducted using a SAS program.

Results

As in previous seasons, at early stages of growth, tomato plants in plots supplemented with high P showed an enhanced vigor and showed better filling of the plots. The plants provided with regular P fertilization, low levels of P supplementation, Hydrophos and Seniphos treatments were very similar. At later stages of growth, no differences could be noticed between any of the treatments.

In general, during the 2002 season, the yield was nearly double that obtained during the previous seasons of study. The yield of H9478 was nearly similar in all treatments (**Figure 6**) reaching 200 t/ha on the average. The yield was slightly lower in H9997 with about 150 t/ha on the average. The high P treatment enhanced the yield in H9997 by about 25 t/ha, which is not statistically significant. However, a similar increase has been observed in previous seasons in response to higher soil P supplementation.

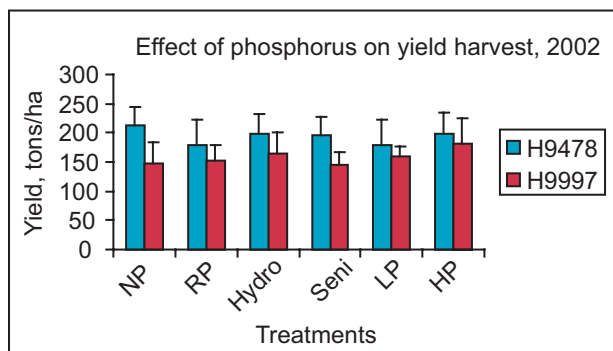


Figure 6. Effect of different P fertilization on the yield of H9478 and H9997 tomatoes.

In all treatments, the yield of H9478 was higher than that of H9997. Within cultivars, there was no significant difference among treatments.

Hydrophos and Seniphos were also applied at a lower dilution (higher concentration), which is usually recommended by Phosyn. Hydrophos application did not have any significant difference on the yield either from NP treatment or regular Hydrophos application (**Table 1**). However, Seniphos application gave an increase in the yield. The increase in yield with low dilution Seniphos application was observed in both H9478 and H9997, which is an interesting observation.

Various physico-chemical parameters of tomato juice were analyzed (**Table 2**) before heat sterilization. The P content of the tomato increased in response to P supplementation from 103 mg in no P to 108 mg in regular P treatment and above 110 mg in low and high soil P supplementation treatments. The Brix level, acidity, and non-soluble solids are very similar in all treatments. The ratio of red (a) to

yellow (b) color increased slightly (not significant) in response to Hydrophos and Seniphos treatment. The ash content also increased in low and high soil P supplementation treatments.

The above parameters were also analyzed in H9997 tomato juice preparation (**Table 3**). The results do not show any significant differences between treatments. The P content was higher under low and high soil P supplementation (LP, HP), as seen in the case with H9478.

Further analysis of tomato juice was conducted after sterilization (heating, removal of the skin). The stability parameters of the juice such as gross viscosity (GV), serum density (SD), and precipitate weight ratio (PWR) were analyzed. The results for such analyses conducted with H9478 are given in **Table 4**. None of the parameters analyzed showed any significant changes in response to P fertilization.

Table 1. Effect of Hydrophos and Seniphos application on the yield of H9478 and H9997 tomatoes.

Cultivar	Treatment	Yield, Tons/ha
H9478	Control (Regular P)	170 ± 40
	Hydrophos (Regular)	198 ± 35
	Hydrophos (high conc.)	207 ± 21
	Seniphos (Regular)	190 ± 30
	Seniphos (high conc.)	227 ± 47
H9997	Control (Regular P)	150 ± 25
	Hydrophos (Regular)	165 ± 30
	Hydrophos (high conc.)	178 ± 28
	Seniphos (Regular)	148 ± 20
	Seniphos (high conc.)	192 ± 23

Note: ± figures are standard deviation (SD)

Table 2. Physico-chemical parameters of H9478 unprocessed juice.

Treatments	P, mg/100g	Brix, °	a/b	Acidity,%	NSS, %	Ash, %
NP	103.60±15.66	5.35±0.19	1.69±0.31	0.37±0.03	5.77±0.23	1.05±0.23ab
RP	108.43±5.86	5.40±0.38	1.62±0.40	0.37±0.02	5.94±0.51	0.95±0.18b
Hydro	101.92±10.90	5.40±0.36	1.81±0.13	0.38±0.02	5.96±0.50	1.11±0.15ab
Seni	110.32±9.88	5.23±0.39	1.81±0.13	0.39±0.02	5.73±0.38	1.02±0.16ab
LP	114.94±27.82	5.10±0.62	1.72±0.24	0.38±0.04	5.68±0.42	1.24±0.27a
HP	113.47±16.08	5.08±0.45	1.76±0.07	0.39±0.03	5.75±0.69	1.24±0.24a

Table 3. Physico-chemical parameters of H9997 unprocessed juice.

Treatments	P, mg/100g	°Brix	a/b	Acidity,%	NSS, %	Ash, %
NP	90.99±5.59b	5.00±0.56	2.03±0.06	0.39±0.03	4.88±0.55	0.86±0.25
RP	97.08±7.47ab	4.83±0.36	2.10±0.00	0.36±0.04	5.05±0.48	0.89±0.16
HYDRO	92.25±4.36b	4.83±0.60	2.00±0.03	0.36±0.04	4.94±0.47	0.79±0.05
SENI	92.88±3.73b	4.73±0.26	1.98±0.01	0.39±0.02	4.88±0.21	0.85±0.15
LP	98.13±6.11ab	5.08±0.71	1.99±0.11	0.37±0.02	5.18±0.52	0.96±0.19
HP	101.71±4.95a	4.95±0.06	2.10±0.08	0.38±0.02	4.82±0.41	0.95±0.09

Table 4. Physico-chemical parameters of processed juice from H9478.

Treatments	°Brix	Lycopene, mg/100g	a/b	GV, mPa.s	SD, g/mL @ 24°C	PWR, %
NP	5.50±0.18	18.17±3.24	1.39±0.10	1150±87	1.0265±0.003ab	13.77±0.63
RP	5.63±0.48	17.98±1.53	1.40±0.04	1150±129	1.0288±0.003ab	14.40±0.71
Hydro	5.65±0.47	18.42±1.84	1.40±0.05	1200±163	1.0292±0.003a	14.18±1.30
SENI	5.35±0.44	16.70±2.42	1.36±0.10	1175±96	1.0265±0.003ab	14.11±1.41
LP	5.28±0.55	17.32±3.03	1.37±0.06	1075±189	1.0251±0.002b	13.47±0.39
HP	5.40±0.59	16.11±2.78	1.33±0.10	1175±171	1.0268±0.003ab	14.46±1.17

Similar studies were conducted with juice obtained from H9997 tomatoes. As in H9478, no major effect was observed as a result of P supplementation (**Table 5**). Further analyses were conducted with sauce preparation from H9478 and H9997 tomatoes. The sauce was concentrated to a Brix level of 9. The results are shown in **Tables 6 and 7**. The red color (a), lycopene levels, the red to yellow ratio (a/b), gross viscosity, serum density and precipitate weight ratio did not show any major changes in response to P supplementation. However, in H9997 tomato sauce

preparation, an increase in gross viscosity was observed in response to Hydrophos and Seniphos application (**Table 7**).

Discussion

The results from the 2002 season did not show major differences in response to P fertilization in most of the parameters analyzed. This could be due to several factors, including ideal growing conditions, the presence of high soil P, etc. Even under conditions when P was not supplied (NP), the yield and other

Table 5. Physico-chemical parameters of processed juice from H9997.

Treatments	°Brix	Lycopene, mg/100g	a/b	GV, mPa.s	SD, g/mL @ 24°C	Acidity, %
NP	5.13±0.47	17.39±1.51	1.53±0.09	1400±141	1.0412±0.003	0.39±0.03a
RP	4.90±0.42	16.80±3.00	1.51±0.12	1375±126	1.0351±0.003	0.36±0.04b
Hydro	5.00±0.62	17.90±1.12	1.54±0.07	1375±125	1.0324±0.002	0.36±0.04b
Seni	4.83±0.21	16.22±1.29	1.48±0.04	1400±469	1.0367±0.006	0.38±0.02ab
LP	4.50±1.89	17.53±3.10	1.51±0.11	1200±216	1.0382±0.006	0.37±0.02ab
HP	4.95±0.10	17.97±1.80	1.53±0.10	1250±100	1.0358±0.003	0.38±0.02ab

Table 6. Physico-chemical parameters of processed sauce with 9°Brix from H9478.

Treatments	a+	Lycopene, mg/100g	a/b	GV, mPa.s	SD, g/mL @ 24°C	PWR, %
NP	32.24±3.63	23.26±5.87	1.35±0.06	6600±712	1.0479±0.005	21.69±2.08
RP	33.07±2.61	22.13±3.07	1.27±0.19	6400±1030	1.0478±0.005	22.31±2.63
Hydro	33.75±1.35	23.04±3.96	1.28±0.18	6500±739	1.0446±0.002	21.49±2.68
Seni	34.07±1.11	23.29±4.29	1.16±0.13	7475±900	1.0455±0.003	21.89±3.79
LP	34.12±1.99	24.12±3.24	1.28±0.12	7075±1300	1.0451±0.005	21.89±2.30
HP	32.51±1.07	24.05±3.70	1.23±0.16	7475±1866	1.0469±0.003	23.11±2.69

Table 7. Physico-chemical parameters of processed sauce with 9°Brix from H9997.

Treatments	Acidity, %	Lycopene, mg/100g	pH	GV, mPa.s	SD, g/mL @ 24°C	PWR, %
NP	0.62±0.08	29.50±4.49ab	4.19±0.04	9950±1399	1.0412±0.003	22.93±0.52
RP	0.57±0.02	29.87±5.58b	4.25±0.02	11500±3185	1.0351±0.003	22.25±0.81
Hydro	0.62±0.08	32.32±5.19ab	4.26±0.04	12150±1350	1.0324±0.002	23.73±0.86
Seni	0.65±0.03	30.37±2.97ab	4.24±0.07	12200±849	1.0367±0.006	23.49±0.73
LP	0.58±0.05	33.96±7.46a	4.24±0.04	11500±3169	1.0382±0.006	23.92±1.17
HP	0.59±0.03	29.52±5.55ab	4.27±0.04	10500±1740	1.0358±0.003	22.81±1.44

physicochemical parameters were similar to those supplied with P. The higher yield observed in NP for H9478 could be an experimental variation and is not statistically significant. By comparison to the two previous seasons, the yield was more than doubled during the 2002 season. The growing conditions were not ideal during the 2000 and 2001 season, and yield increases were observed in response to P fertilization. Thus, it appears that effect of P application becomes pronounced mostly under stressful conditions, where the availability of extra P could result in added stress protection. In support of this hypothesis, we have observed higher levels of antioxidant enzyme activities in tomatoes from P supplemented plots. During 2002 season, we also studied the effects of P supplementation on a high lycopene tomato variety H9997. Though the lycopene levels in

the processed juice were similar between H9478 and H 9997, the lycopene levels appeared to be higher in the sauce preparation of H9997 tomatoes.

As observed in previous years, Hydrophos and Seniphos application appears to show positive results. In our earlier experiments, we have used a more diluted preparation of these formulations. Irrespective of the concentration differences, Hydrophos application tends to enhance the yield slightly. The effect is more pronounced with Seniphos, and we have observed these beneficial effects in the previous seasons as well. Improvements are noticeable in the quality of processed products as well.

Further analyses on vitamin C levels, antioxidant enzymes, flavor profiles, etc. are being conducted.

The Effect of Soil Nutrients on the Phytochemical Profile of Nutraceutical Crops

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Definitions and Examples of Functional Foods and Nutraceuticals

Functional foods are defined differently in Canada and the U.S.

Canada: “Foods that are similar in appearance to conventional food and are consumed as part of the usual diet. These foods have demonstrated physiological benefits, and/or reduce the risk of chronic disease beyond basic nutritional functions.”

U.S.: “Any food product with added ingredients or fortification to a functional level specifically for health or performance purposes.”

Functional food example: Tomatoes rich in lycopene, eggs enriched in omega three fatty acids

The definition of a nutraceutical differs somewhat. A nutraceutical is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. It is demonstrated to have a physiological benefit or provide protection against chronic disease.” It is now part of natural health products—medicinal ingredients including herbs, homeopathic preparations, traditional medicines, mineral or a trace element, a vitamin, an amino acid, an essential fatty acid or other botanical, animal, or microorganism derived substance.

Nutraceutical example: capsules containing bioflavonoids or gamma-linoleic acid.

Introduction

- Minor nutraceutical crops
- Deficit of information on optimal production practices that maximize levels of bio actives; many still collected in the wild
- Little consideration given to the impact of mineral nutrition on functionality of the crops

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- Great variability between plants; very limited breeding and selection work done
- Processes by which plants synthesize active compounds have not been well elucidated
- Bioactives are secondary metabolites (stress compounds) affected by genetic environmental and plant nutrition factors.

Purple coneflower (*Echinacea species*)

The species is native to North America. It has a long history of medicinal use. Its indications are for prevention and treatment of colds, flu, chronic infections of the upper respiratory tract, and other minority infections. The active compounds are uncertain, likely phenolic compounds (caffeic acid derivatives), alkylamides, and polysaccharides. Immuno stimulatory effect of echinacea has been documented *in vitro*, but clinical evidence from human trials is not conclusive.

Nitrogen (N) and phosphorus (P) appear to have little effect on echinacea yield (**Figure 1**). The effects on phenolic markers were more complex and inconsistent (**Table 1**).

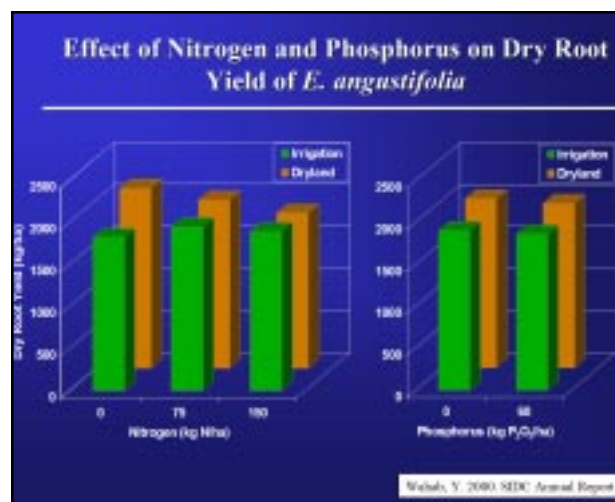


Figure 1. Fertilization with N and P had little effect on echinacea yield.

Table 1. Combined effect of dryland/irrigation, soil N and P levels, and plant density on phenolic markers of *E. angustifolia* root; 1999 harvest (Outlook, SK).

Trial	Fertility ¹	Echinacoside ² , %		Cynarin ² , %		Chlorogenic Acid ² , %	
		Dry	Irrig	Dry	Irrig	Dry	Irrig
<i>2-yr E. angustifolia</i> 15 cm in-row sp.							
	At						
	0N-0P	0.62	0.22	0.14	0.06	0.05	0.03
	0N-60P	0.93	0.25	0.21	0.08	0.06	0.04
	150N-0P	0.71	0.09	0.15	0.03	0.05	0.03
	150N-60P	0.65	0.13	0.15	0.03	0.05	0.03
<i>2-yr E. angustifolia</i> 30 cm in-row sp.							
	0N-0P	1.00	0.18	0.20	0.06	0.06	0.04
	0N-60P	0.84	0.22	0.16	0.08	0.04	0.04
	150N-0P	0.69	0.21	0.14	0.07	0.05	0.05
	150N-60P	0.69	0.15	0.10	0.08	0.03	0.03

¹Fertilization at 0 to 150 kg N/ha and 0 to 60 kg P₂O₅/ha

²All marker compounds are reported on dry weight basis; analyte expressed as a mean (n=2)

St. John's Wort (*Hypericum perforatum*)

The therapeutic indications for the use of St. John's Wort include mild to moderate depression, restlessness, anxiety, and irritability. Biomarkers considered to be related to its efficacy include hypericin, pseudohypericin, and flavonoids (rutin, hyperoside, quercitrin, quercetin).

A study in Outlook, Saskatchewan, found that a fertilizer supplying N and P increased hypericins slightly in St. John's Wort (**Table 2**). The increase appeared to vary among varieties, with 'Antos' showing the greatest response. Flavonoid concentration also tended to be higher with the fertilizer, though not significantly so (**Table 3**).

Table 2. Effect of soil fertility on Hypericins content of 3-year St. John's Wort flowering tops¹; 2001 harvest (Outlook, SK).

Variety	Fertility ²	Pseudohypericin, % w/w	Hypericin, % w/w	Total Hypericins ³ , % w/w
Anthos	0N-0P	0.07±0.01	0.03±0.00	0.10±0.01
	100N-100P	0.11±0.02	0.04±0.01	0.14±0.03
Elixir	0N-0P	0.08±0.01	0.08±0.00	0.15±0.01
	100N-100P	0.09±0.01	0.08±0.01	0.17±0.02
Standard	0N-0P	0.06±0.01	0.06±0.01	0.11±0.02
	100N-100P	0.07±0.01	0.05±0.01	0.11±0.01
Topas	0N-0P	0.08±0.01	0.08±0.00	0.15±0.01
	100N-100P	0.09±0.02	0.08±0.02	0.17±0.04

¹Three-year crop grown under irrigation, transplant 23/06/1999, harvest top half aerial parts 31/07/2001

²Fertilization at 0-100 kg N/ha and 0-100 kg P₂O₅/ha

³Hypericins content reported on "as is" basis; total hypericins refers to the sum of hypericin and pseudohypericin; analyte expressed as a mean±S.D. (n=2)

Table 3. Effect of soil fertility on flavonoid content of 3-year St. John's Wort flowering tops¹; 2001 harvest (Outlook, SK).

Variety	Fertility ²	Rutin, %	Hyperoside, %	Quercitrin, %	Quercetin, %	Total Flavonoids ³ , %
Anthos	0N-0P	0.51±0.07	0.58±0.06	0.06±0.00	0.01±0.00	1.16±0.13
	100N-100P	0.67±0.16	0.70±0.05	0.08±0.01	0.01±0.00	1.45±0.22
Elixir	0N-0P	0.45±0.01	0.84±0.07	0.08±0.01	0.01±0.00	1.38±0.09
	100N-100P	0.47±0.08	0.92±0.14	0.09±0.01	0.01±0.00	1.48±0.22
Standard	0N-0P	0.47±0.10	0.66±0.09	0.16±0.05	0.01±0.00	1.31±0.06
	100N-100P	0.45±0.05	0.66±0.05	0.17±0.06	0.01±0.00	1.29±0.10
Topas	0N-0P	0.42±0.04	0.82±0.03	0.08±0.01	0.01±0.00	1.34±0.07
	100N-100P	0.44±0.05	0.96±0.13	0.08±0.04	0.01±0.00	1.49±0.15

¹Three-year crop grown under irrigation, transplant 23/06/1999, harvest top half aerial parts 31/07/2001

²Fertilization at 0 to 100 kg N/ha and 0 to 100 kg P₂O₅/ha

³Flavonoid content reported on "as is" basis; total flavonoids refers to total flavonoids, which were calculated as a sum of rutin, hyperoside; analyte expressed as a mean±S.D. (n=2)

Table 4. Effect of level of fertilizer application on fresh herbage and essential oil yields for three peppermint cultivars over 2 years.

Fertilizer treatment kg/ha N-P ₂ O ₅ -K ₂ O	Herbage Yield, t/ha			Essential Oil Concentration, kg/t			Essential Oil Yield, kg/ha		
	No 1	Tundza	Zephir	No 1	Tundza	Zephir	No 1	Tundza	Zephir
0-0-0	6	9	9	12	9	13	71	82	112
150-0-0	7	11	12	13	10	12	90	107	150
300-0-110	9	14	14	11	10	15	96	136	207
530-180-240	10	15	16	13	12	14	130	179	226

German Chamomile (*Chamomile recutita* L.)

The effect of the growth environment on essential oil production has been contradictory and has not been quantified yet. For example, potassium(K)-rich soil has a positive impact on chamazulene content, but appears to have a negative impact on the essential oil yield.

Demand for N and K is high. It is lower for calcium and magnesium, and lowest for P. A higher rate of K accelerates flowering and increases biomass and production of flowerheads. The most intensive intake of mineral nutrients was recorded during flowering season, when the most increase of dry matter per day occurred.

Peppermint (*Mentha piperita* L.)

Peppermint is one of the most cultivated essential oil crops in the world. The U.S. is the largest world producer, with production mainly in Washington, Oregon, and Indiana.

Its indications are as a spasmolytic and sedative. It increases production of bile due to its essential oils and possibly flavonoids. It is usually fertilized

with 140 to 225 kg/ha of N, 110 kg/ha of P₂O₅, and 450 kg/ha of K₂O.

Balanced fertilization of peppermint with N, P, and K increased the yield of herbage and essential oil considerably, with concomitant slight increases in essential oil concentration (**Table 4**).

Summary

Plant production of bioactive compounds is most strongly influenced by genetics and the growing environment. The studies reported above indicate that soil fertility has an important role as well.

Acknowledgments

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Note: No references provided.

Functional Components in Citrus: Alteration by Mineral Elements

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Abstract

Grapefruit functional compounds such as carotenoids, flavanones, limonin 17- β -D glucopyranoside (LG), and vitamin C have been shown to have potential human health benefits including cancer and cardiovascular disease prevention. This study was conducted to determine different levels of potassium (K) on functional compounds. Field and laboratory studies were conducted at the University of Florida, Citrus Research and Education Center (CRES) in Lake Alfred, and Texas A&M University-Kingsville Citrus Center (TAMUK-CC) in Weslaco, respectively. 'Ray Ruby' grapefruits on Carrizo citrange rootstock were harvested from a nitrogen (N) and K rate effect study conducted in CREC and shipped to TAMUK-CC. Fifteen fruits each from three replications per treatment were stored. Reversed phase high performance liquid chromatography was used to quantify the functional compounds. The highest levels of N/K rate significantly decreased naringin concentration compared to that of fruits sampled from the tree which received no supplemental N or K. Although there were no significant differences in levels of beta carotene and lycopene due to different levels of K applied, total carotenoids levels were significantly higher in control fruits compared to that in the fruits from 186 kg K/ha treatment. Higher levels of K application decreased ascorbic acid while increased dehydroascorbic acid concentration as compared to those in the fruits from N/K unamended treatment. Total vitamin C concentration was not significantly influenced by different levels of K. Grapefruits sampled from the trees which received 93 kg K/ha had significantly lower LG concentrations compared to that of the fruits in the N/K unamended treatment. Since this study was conducted with variable rate of both N and K, further studies with variable rates of single elements are necessary to understand the effects of each element on the phytonutrient contents in the fruits.

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Introduction

Grapefruit (*Citrus paradisi* Macf.) contain various functional components including flavonones (naringin, narirutin), carotenoids, limonoids (limonin, nomilin, limonoid glucosides), folic acid, aurapatene, and vitamin C. The bitter compounds in grapefruit are naringin (7- β -neohesperidoside of naringenin), limonin, and nomilin. However, narirutin (naringenin 7- β -rutinoside) and limonoid glucosides are tasteless (Fong et al., 1990).

Certain functional components may not be considered essential by conventional measures, but are increasingly recognized for their beneficial effects on health promotion and disease prevention. Market research data from many sources showed that consumers in the U.S., Japan, and Western Europe have already accepted the concept of biogenic diets containing biologically active functional components and are willing to try diets to prevent chronic diseases (Shukla, 1992). In recent years, many epidemiological and case control studies have shown that citrus flavonoids have potential biological activities which include antioxidant, anticancer, antiviral, anti-inflammatory activities, effects on capillarity, cholesterol lowering ability, and an ability to inhibit human platelet aggregation (Benavente-Garcia et al., 1997; Guthrie et al., 2000; Middleton and Kandaswami, 1994). Bronner and Beecher (1995) reported that average intake of flavonones from citrus juices by Americans is estimated to be about 23 mg per day while the average daily intake of flavonoids is 1 g per day (Kuhnau, 1976).

The limonoids exist in citrus fruit primarily in the form of glucosides and are not bitter (Fong et al., 1990). Recent studies have shown that limonoids and their corresponding glucosides are inhibitors of chemical carcinogenesis in laboratory animals (Lam et al., 1994; Miller, et al., 1989, 2000) and in human breast cancer cells (Guthrie et al., 2000; Tian et al., 2001) as well as reduce cholesterol (Kurowska et al., 2000).

Vitamin C is an essential nutrient for humans and citrus fruit as well as their processed products are the most commonly consumed sources (Kefford, 1973). Investigators have postulated a role of vitamin C in the prevention of cancer, heart disease, and in the augmentation of immune function such as in the prevention of common cold (Hemila, 1992). It is clearly established that the pigment lycopene is most

sensitive to low levels of K. Evidences have shown increased lycopene concentration in tomato with increasing K concentration (Trudel and Ozbun, 1971), and higher levels of carotene in cucumber leaves supplied with higher K (Lamarani et al., 1996).

There is considerable interest in understanding the relationship between the management of crop production inputs and the crop product quality including the components that contribute to human health benefits. These relationships are extremely important in an effort to cater to the needs of rapidly increasing health conscience consumers. Studies have begun to evaluate the effects of different fertilization rates on fruit production and fruit quality of grapefruits. This study was conducted to understand whether functional components of grapefruit are influenced by different levels of N and K.

Materials and Methods

Field experiment. The fruits used in this study were from a large field experiment to evaluate the long term effects of application of variable rates of N or K on fruit production and fruit quality in sandy soils in Central Florida. 'Ray Ruby' grapefruit trees on Carrizo citrange rootstock were used. The plot unit comprised five trees within a row chosen in alternate rows. The N and K sources used in this experiment were granular ammonium nitrate and muriate of potassium. A commercial dry granular fertilizer spreader calibrated to apply different per hectare rates was used. Most of the fertilizer was delivered under the canopy within the wetting area under the tree micro sprinklers. Fruits were sampled from middle three trees to minimize the border effects. The treatments included: 112, 168, 224, and 280 kg N/ha rates as well as no supplemental N application treatment. The N:K₂O ratio in the fertilizer was 1:1. Therefore, with the above rates of N, K rates also varied at 93, 140, 186, and 233 kg K/ha.

Functional component analysis. Fifteen fruits were sampled per plot from the above five fertilizer rate treatments with three replications. These fruits were used for analysis of lycopene, beta carotene, naringin, narirutin, vitamin C, ascorbic acid, dehydroascorbic acid, and LG concentrations.

Standards. Standard naringin, ascorbic acid and dehydroascorbic acid were purchased from Sigma Chemical Co. St. Louis, Missouri. Limonin 17-β-D-glucopyranoside was supplied by Shin Hasegawa of the USDA-ARS, Albany, CA. Narirutin was supplied by John Manthey, USDA-ARS, Winter Haven, FL.

Sample preparation. Fruits (commercial size 40) were washed, and flavedo and albedo were removed with a potato peeler. The remaining parts (juice sacs and pulp) were blended for 3 min and filtered with qualitative 415 filter paper. Filtered samples were collected in 50 mL tube and stored in -80°C until analyzed for limonoids, flavonones, and vitamin C.

Chromatographic conditions. A high performance liquid chromatography (HPLC) consisted of a Perkin-Elmer binary LC pump 250, an LC-600

autosampler, a UV/VIS Spectrometric detector LC295, and PE 1020 Integrator program was used for limonoid and flavonones. The same HPLC system with an HP 3394 integrator was used for measuring total vitamin C.

Carotenoids. Sample preparation procedures described by Sadler et al. (1990) and Rouseff et al., (1992) were used. Approximately 12 g of pulp sample was weighed in a 125-mL brown Erlenmeyer flask. A 100 mL solvent with hexane: ethanol: acetone (50:25:25) was added to the flask and agitated for 10 min on a magnetic stirrer at 8 speed, 15 mL water was added followed by another 5 min agitation. Solvent layers were allowed to separate into distinct polar and non polar layers. The top hexane layer of 2 mL was taken and dried with liquid N and heated at 30°C for 5 min. Samples were dissolved in 100 μL tetrahydrofuran (THF), diluted to 2 mL with methanol : THF (75:25), and filtered through 0.45 μL filter prior to injection on to the HPLC system.

Flavonoid analysis. Samples were analyzed for flavonone content by reverse phase HPLC with some modifications of the Berhow (2000) method. An aliquot of juice sample was diluted with dimethylsulfoxide (1:1), centrifuged and filtered through 0.45-μm nylon filters (Alltech Associates, Deerfield, IL.) and 20 ml of this solution was injected into HPLC system. The same HPLC system as explained in the LG section was used and a Adsorbosil C-18 column (250 X 4.6μm, 5mm) was eluted with a linear gradient starting with 10% acetonitrile, 5 mM phosphoric acid and ending at 26% acetonitrile in acetonitrile 5 mM phosphoric acid for 36 min. The narirutin and naringin peak were detected at 280 nm with retention times of 25 and 27 min, respectively. The flavonones were identified by confirmation of their respective spectra, authentic standard and retention times.

Limonoid analysis. Juice sample prepared as described in sample preparation section was filtered through qualitative P8 filter paper. The LG was analyzed with little modification from the previous procedure (Ozaki et al., 1991). A 10 mL of juice was passed through a C-18 Sep-Pak (Waters Associates, Milford, MA.), LG was retained on the column, rinsed with water, eluted with methanol and determined by HPLC using a Waters Spherisorb ODS2 C-18 column (250 X 4.6 μm, 5mm) along with a guard column. One mL of juice was diluted with 3 mL of 80% ethanol. The mobile phase consisted of linear gradient starting with 10% acetonitrile, 3 mM phosphoric acid and ending at 26% acetonitrile in 3 mM phosphoric acid for 60 min. The flow rate was 1 mL/ min. Detector was set to 210 nm and LG peak was identified at 21 min.

Vitamin C. Modified procedure of Zapata and Dufour, (1992) was used to separate dehydroascorbic acid, ascorbic acid. Twenty μL of the solution was injected into HPLC system. A Waters Bondapak C-18 column (30 X 0.4 cm) with a guard column was used and the flow rate was 1.8 mL/min. Mobile phase

was methanol:water (5:95 v/v) with 0.01 M ammonium phosphoric acid. Dehydroascorbic acid and ascorbic acid were detected at 348 and 261 nm, respectively.

External standards were used to quantify the compounds. Peak areas were normalized to the external standard and the standard curve was fitted by linear regression (peak area vs. concentration in $\mu\text{g/g}$). Total flavonone concentration was calculated by combining naringin and narirutin concentration. The long term variability of LG and both flavonones in the laboratory was low (coefficient of variation <4% and 6%, respectively).

Statistical analysis. Data were analyzed as a completely randomized design using GLM procedures (SAS Institute Inc., Cary, NC). Fisher protected LSD was employed for all mean separation analysis.

Results and Discussion

Potassium rates in the range of 0 to 233 kg/ha/year did not significantly influence lycopene and beta carotenoids. However, concentrations of total carotenoids were significantly greater in the fruits from no supplemental K treatment as compared to that of the fruits from trees which received 186 kg K/ha/year (**Figure 1**). Lycopene concentrations increased with increasing rate of K in Tomato (Trudel and Ozbun, 1971), and in cucumber leaves (Lamarani et al., 1996).

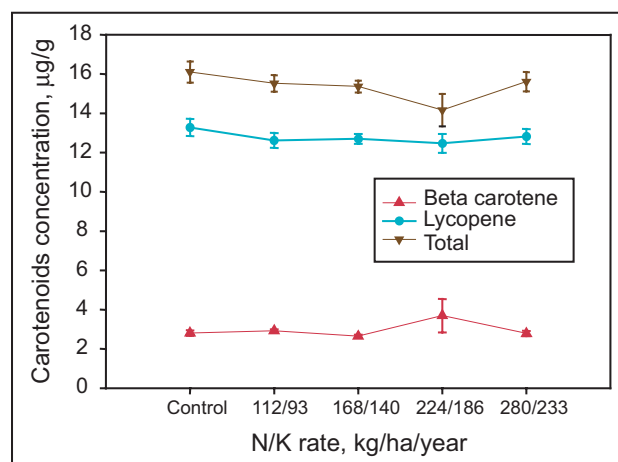


Figure 1. Effect of K and N on beta carotene, lycopene, and total carotenoids.

In general, naringin decreased with an increase in K rates (**Figure 2**). Fruits from the highest K rate treatment 233 kg/ha/year had the lowest naringin concentration. Indeed, naringin, narirutin, and total flavonone concentrations were high in the fruits of the trees which received no fertilization.

It is evident that although total vitamin C concentrations were not significantly affected by K treatments, K applied at 93 kg/ha/year significantly increased dehydroascorbic acid and decreased ascorbic acid and LC concentrations in the fruits compared to those in the fruits from unfertilized trees (**Figures 3 and 4**). This agrees with the previous con-

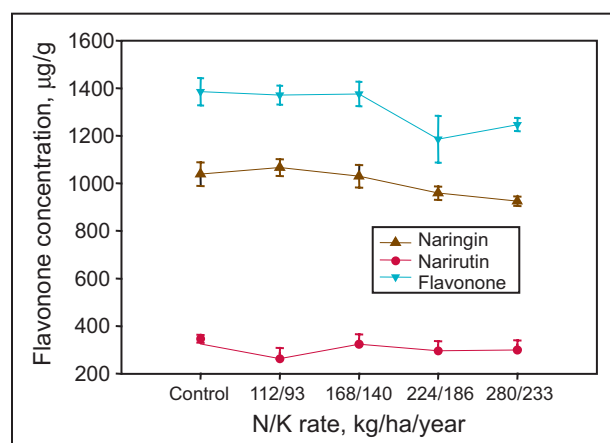


Figure 2. Effect of K and N effect on naringin, narirutin, and total flavonone concentrations.

clusion that optimum supplies of K increases the vitamin C content of citrus fruit (Hearn, 1993; Smith, 1966; Jones et al., 1973; Nagy, 1980). Because carbohydrate metabolism is strongly influenced by K availability, optimum K nutrition of citrus trees has a beneficial effect on the vitamin C content of citrus fruits (Embleton and Jones, 1966; Smith and Rasmussen, 1960).

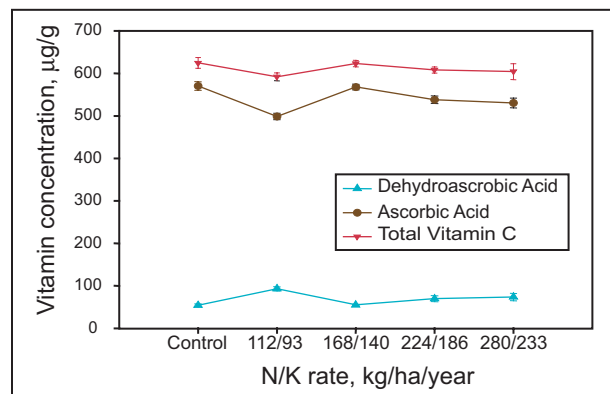


Figure 3. Effect of K and N on dehydroascorbic acid, ascorbic acid and total vitamin C concentrations.

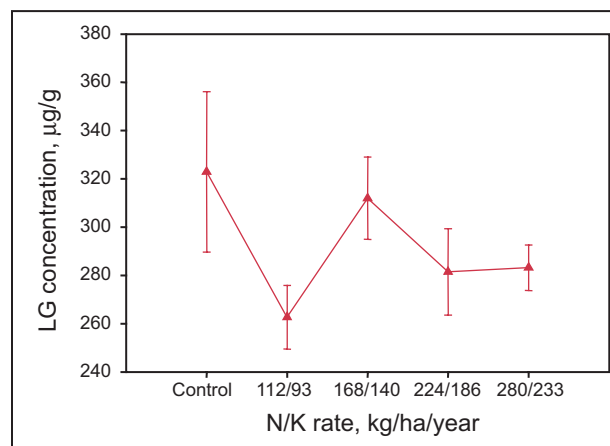


Figure 4. Effect of K and N on limonin 17-b-D glucopyranoside concentrations.

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Potassium Fertilization Effects on Isoflavone Concentrations in Soybean

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Abstract

Soybean isoflavone concentrations vary widely, but the contribution of soil fertility and nutrient management to this variability is unknown. Field experiments from 1998 to 2001 on soils with low to high exchangeable potassium (K) concentrations evaluated K fertilizer application and placement effects on isoflavone concentrations and composition of soybean in various tillage and row width systems. Soybean seed yield and concentrations of daidzein, genistein, glycitein, leaf K, and seed K were measured. Significant increases in daidzein, genistein, and total isoflavone in soybean seed were observed with deep-banded K or surface-broadcast K fertilizer on low and medium K soils. Positive effects of K fertilization on isoflavones were less frequent on

medium- to high-testing K soils. Both individual and total isoflavones were often positively correlated with seed yield and concentrations of trifoliolate leaf K and seed K. Appropriate K management could be an effective approach to increase isoflavone concentrations for soybeans produced on low- to medium-K soils.

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Reference

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