THE EFFECT OF ENVIRONMENT AND MANAGEMENT ON YIELD AND NO_3-N CONCENTRATIONS IN ORGANICALLY MANAGED LEAFY GREENS

By

HALY LURY INGLE

A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTERS OF SCIENCE IN SOIL SCIENCE

WASHINGTON STATE UNIVERSITY
Department of Crop and Soil Sciences

May 2010

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of HALY LURY INGLE find it satisfactory and recommend that it be accepted.

Richard T. Koenig, Ph.D., Chair

Teresa Cerny-Koenig, Ph.D.

Carol A. Miles, Ph.D.

John P. Reganold, Ph.D.

ACKNOWLEDGMENT

I am grateful for the funding provided by the Washington State University Center for Sustainable Agriculture and Natural Resources, and the Glen D. Franklin Endowed Graduate Research Assistantship in the Department of Crop and Soil Sciences. I am incredibly grateful to my committee for their continual support and guidance: Dr. Teresa Cerny-Koenig, Dr. Carol Miles, and Dr. John Reganold. This project was also assisted by the technical support and guidance provided by Brad Jaeckel, Kelsi Alcott, Ron Bolton, and John Rumph.

THE EFFECT OF ENVIRONMENT AND MANAGEMENT ON YIELD AND NO_3-N CONCENTRATIONS IN ORGANICALLY MANAGED LEAFY GREENS

Abstract

by Haly Lury Ingle, M.S.
Washington State University
May 2010

Chair: Richard T. Koenig

In leafy green crops, plant tissue nitrate concentration (TNC) is influenced by environment and management factors. Although evidence is inconclusive, excessive dietary nitrate has been implicated in digestive tract cancers and the European Commission (EC) has set limits on TNC in leafy greens. A series of field studies evaluated the effect of environment and management on yield and TNC in organic leafy greens. To compare the effect of photoperiod on TNC, a study was conducted in Pullman, WA, and Fairbanks, AK. Two lettuce (*Lactuca sativa*) and 2 spinach (*Spinacia oleracea)* cultivars were sampled and analyzed for TNC over 3, 24-hour periods. Light intensity and duration were measured for all sampling periods. There was no evidence that photoperiod influenced TNC in field growing conditions. A second study evaluated the effect of planting and harvest dates on yield and TNC in Asian greens grown in winter. A commercial Asian greens mix was direct seeded in a hoophouse on 3 different dates. Initial and subsequent regrowth was harvested, and each crop type evaluated for TNC and yield. TNC for the same crop type was significantly different between planting dates with no clear trends. Some crop types yielded better in early harvest dates while others did not recover from repeat harvesting. All TNC was well below EC regulations. In a third study, 2 lettuce and 2 spinach cultivars were seeded into plots fertilized with dry fish meal (8-3-0) applied at 168 kg N ha^{-1}. An unfertilized control was used for comparison. Crops were grown in 2008 and 2009. At harvest, plants were separated into 3 whorls (outer, inner, center), petioles, and leaf blades. In 2008, there were significant 2-way interactions between fertility and cultivar, cultivar and plant part, and fertility and plant part. Within the fertilized treatment, lettuce had higher fresh weight TNC than spinach. Petioles had higher TNC than leaf blades. In 2009, there was a significant 3-way interaction. There were complex interactions between experimental variables, suggesting an opportunity to select cultivars and/or fertility regimes to reduce TNC.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Dedication

This thesis is dedicated to my advisor, Dr. Rich Koenig, whose support and guidance were invaluable to my development as a student, scientist, and person.

Chapter 1

The Effect of Planting Date, Photoperiod, Repeat Harvest and Location on Yield and NO_3-N Concentration in Organically Managed Leafy Greens

Justification

Tissue nitrate concentration (TNC) in leafy green crops has been shown to respond to a wide variety of environmental and management factors including light intensity (Steingrover et al., 1986; Behr and Wiebe, 1992), light duration (Steingrover et al., 1986; Pavlou et al., 2007), temperature (Cantliffe, 1972c), fertilizer source and application method (Barker et al., 1971), cultivar (Ott, 2007) and part of the plant sampled (Maynard et al., 1976; Ott et al., 2008). Crops like spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), Swiss chard (*Beta vulgaris* var. *cicla*), and radish (*Raphanus sativus*) are considered nitrophilic, and TNC can be in excess of plant growth requirements (Vogtmann et al., 1984).

Although nitrate itself is not toxic to humans, ingestion of nitrate is potentially harmful because nitrate is reduced to nitrite in the digestive system. Nitrite has been implicated in methaemoglobinaemia in infants. There is also concern that excess nitrates in the diet may cause digestive tract cancers (Santamaria, 2006; Powlson et al., 2008). Vegetables are often the main source of nitrates in the human diet (Santamaria et al., 1999). The European Commission (EC) has set limits on acceptable TNC in leafy greens, depending on crop type, growing season, and method of cultivation (Anon., 2005). The U.S. has not set limits on TNC.

New research shows that nitrates may not be exclusively harmful. Some of the proposed effects of nitrate in the body are host defense, vasodilatation, and possibly anti-inflammatory effects (Lundberg et al., 2004). Nitrate and its reduced forms also play important roles in cell activity including several signaling pathways (Bryan et al., 2005). Much is unknown about the role of nitrate in the human body and more research is needed in this area.

More research is needed to understand the influence of environment and management factors on TNC in leafy greens, and how best to minimize any risk of excess nitrate on human health. This research continues the work done by Ott (2007) in winter production of lettuce and Asian greens as well as research evaluating TNC in varying plant parts of lettuce and spinach. We attempted to improve winter production by using a cut-and-come-again system to maximize space efficiency and yield potential. We continued Ott's (2007) plant part and fertilizer effect research. In addition, lettuce and spinach crops were grown in Pullman, WA and Fairbanks, AK to compare the effect of photoperiod on TNC in lettuce and spinach.

Literature Review

A profile of leafy greens crops

Lettuce and spinach are major leafy green crops grown in the United States. Head lettuce ranks first in production and crop value for all vegetable crops grown in the United States. Major production areas for leafy greens are California and Arizona, but lettuce and spinach are grown in most states (USDA, 2008). The average person consumes more than 11 kilograms of lettuce per year (Reader, 2003).

Lettuce is grown on 7% of acres used for vegetable production in the U.S. (USDA, 2009). Other crops such as spinach, kale (*Brassica oleracea*) and mustard greens (*Brassica juncea*) contribute another combined 1.5% of the acres (USDA, 2009). These crops are mostly grown on small farms, with over 70% of lettuce operations under 1 acre, and over 60% of spinach operations under 1 acre (USDA, 2009).

In Washington State, lettuce is produced on 260 acres with leaf lettuce as the dominant type, followed by head and romaine. Lettuce production for all types has decreased since 2002 (USDA, 2009). Kale was produced on 57 acres, and mustard greens on 3 acres, down from 34 acres in 2002. Spinach was produced on 215 acres (USDA, 2009). Despite the low acreage numbers, there is potential for these crops to be grown during winter in Washington State with appropriate growing practices (Ott, 2007).

Growing practices

Leafy greens consist of many species and cultivars and are adapted to most areas (Reader, 2003; Anon. 2009) so both summer and winter production are possible. Both lettuce and spinach are cool-season leafy greens; lettuce grows best between 12 to 18^{o}C and can be produced in either a greenhouse or in the field. Lettuce can withstand freezing temperatures (Jackson et al., 1996; LeStrange et al., 1996) and has been shown to survive temperatures as low as -2^{o}C (Mansour and Raab, 1996). High temperatures will cause bolting in both crops (Jackson et al., 1996; LeStrange et al., 1996).

Asian greens like kale, arugula (*Eruca sativa*) and mustard greens are winter hardy and could also be suitable crops for the Palouse region. In Pullman, WA, Asian greens, including pak choy (*Brassica rapa chinensis*), tatsoi (*Brassica rapa narinosa)*), and komatsuna (*Brassica rapa perviridis group*) were consistently higher yielding than lettuce or spinach during a hoophouse winter study (Ott, 2007).

If nitrogen (N) fertilizer is applied in excess of crop needs, there is potential to increase TNC within the plant (Barker, 1975; Maynard et al., 1976). USDA certified organic sources of fertilizer include compost (variable), fish meal (10-1.3-0), blood meal (12-0-0) and manures (variable) (Gaskell and Smith, 2007; USDA, 2010).

Several extension publications recommend staggering planting dates for a longer harvest of leafy greens (Miles et al., 2005; Barr, 2007). In the cut-and-come-again system of harvesting leaf lettuce, whole plants are cut several centimeters above the soil surface. The plants are allowed to regrow from the crown, and the subsequent regrowth can be harvested again (Reader, 2003; Barr, 2007; Anon. 2009).

Hoophouse production

Hoophouses can extend the growing season and increase profit for growers of leafy green crops. Hoophouses have been shown to extend the growing season for leaf and Romaine lettuce in Alaska (Rader and Karlsson, 2006). The use of hoophouses for peppers (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*) extended the growing season by 2 weeks in Saskatchewan, Canada, and generated higher gross returns compared to field row cover (Waterer, 2003). Hoophouses have also been shown to increase yield and fruit quality in strawberries (*Fragaria ananassa*) grown in Kansas (Kadir et al., 2006). Lettuce has been grown successfully in eastern Washington through the winter using hoophouses (Ott, 2007).

Leafy greens as a source of nitrates in the human diet

Vegetables are a large source of nitrates in the human diet (Maynard et al., 1976; Vogtmann et al., 1984), and leafy greens, in particular, can accumulate excess nitrates (Santamaria et al., 1999). In the 1980s, the main source of nitrates in the human diet in the

United Kingdom came from vegetables (Stopes et al., 1988). Plants like spinach, lettuce, Swiss chard, and radishes are considered nitrophilic (Vogtmann et al., 1984) where TNC in the plant can exceed the amount required for growth (Grindlay, 1997).

In Italy, the largest source of nitrates was from lettuce, Swiss chard and beets, which equaled almost half of the average total intake of nitrates (Santamaria et al., 1999). Excess nitrates in the diet have been implicated in methaemoglobinaemia and digestive tract cancers (Powlson et al., 2008; Santamaria, 2006). The EC (Anon., 2005) has set limits on acceptable TNC in leafy greens, depending on crop, harvest time, and method of cultivation. The U.S. has no limits in place at this time.

Environmental factors influencing nitrate concentrations in leafy greens

TNC in leafy greens has been shown to vary within species and cultivars (Barker et al., 1971; Maynard et al., 1976). Environmental changes including light intensity (Steingrover et al., 1986; Behr and Wiebe, 1992; Amr and Hadidi, 2001), photoperiod (Maynard et al., 1976), and temperature (Cantliffe, 1972c) have been shown to affect TNC in leafy greens. However, not all research has observed consistent, measurable differences in TNC due to these environmental factors (Weightman et al., 2006). For example, Weightman et al. (2006) found no consistent pattern in TNC when sampling field grown lettuce at 3-hour intervals over a 24-hour period.

Light intensity plays a major role in TNC for some crops. An increase from 200 to 800 $\mu mol\ m^{-2}\ s^{-1}$ decreased TNC in spinach by over 50% (Proietti et al., 2004). Cantliffe (1972a) reported spinach grown under light levels of approximately 120 $\mu mol\ m^{-2}\ s^{-1}$ had more than twice the TNC compared with spinach grown under approximately 320 $\mu mol\ m^{-2}\ s^{-1}$. Chen and

Ries (1969) reported similar results in oat seedlings. These studies indicate that as light intensity increased, TNC decreased.

This change in TNC due to changes in light intensity often follows a diurnal pattern, where TNC follows the light intensity pattern. TNC increases during the dark period and decreases during light periods in plants such as corn (*Zea mays* L.) and wheat (*Triticum aestivum*) (Hageman et al., 1961; Harper and Paulsen, 1968). Spinach plants grown at light intensities less than 250 µmol m^{-2} s^{-1} exhibited a diurnal rhythm with TNC more than doubled following the dark period (Steingrover et al., 1986).

Studies have shown that a shorter photoperiod increased TNC in crops such as leafy greens (Steingrover et al., 1986; Pavlou et al., 2007) and beets (Cantliffe, 1972b). Light activates the enzyme nitrate reductase (NR), which reduces nitrate to nitrite. This nitrite is then converted to ammonium. In the absence of light, NR is not activated and nitrate becomes concentrated in the cells (Taiz and Zeiger, 2006). Additionally, nitrate can be an osmotic solute within cell vacuoles. During low light conditions, or at night, soluble carbohydrates are transported to the cytoplasm to be metabolized (Steingrover et al., 1986). In these situations, nitrate can act as an osmotic replacement for the carbohydrates (Steingrover et al., 1986).

Nitrogen fertilizer source can also contribute to TNC. In addition to environmental influences, N fertilizer applied in excess of crop needs has been shown to potentially increase nitrates within the plant (Barker et al., 1971; Barker, 1975; Maynard et al., 1976). In wheat, increased nitrate concentration in nutrient media increased TNC in blades and roots (Harper and Paulsen, 1968). When comparing compost and soluble fertilizer, Stopes et al. (1989) found that soluble fertilizer significantly increased TNC in fresh tissue when compared to compost. TNC in lettuce has been shown to nearly double when using synthetic fertilizers compared to composted manure, and yields from compost treatments were significantly decreased only in the first crop season (Pavlou et al., 2007). Soil N levels influence TNC, so compost may limit N availability and thus limit yields (Ott et al., 2008).

Nitrate variation within plants

TNC has been shown to have unequal distribution within plants. In leafy greens, TNC is highest in the petioles, with lower amounts in the roots, leaves, fruits and floral parts in descending order (Maynard et al., 1976). Spinach plants exhibited a diurnal rhythm with the most significant fluctuation observed in the leaf blades compared to petioles, where TNC more than doubled following the dark period (Steingrover et al., 1986). Outer leaves and petioles tended to have higher TNC than inner leaves and leaf blades; however, crop type and cultivar played a significant role in nitrate accumulation and distribution within plants (Ott, 2007).

The purpose of these studies is to evaluate organically managed leafy greens to determine how planting date, photoperiod, sequential harvest and location affect yield and TNC.

These studies were designed to evaluate these specific objectives:

- The influence of photoperiod on TNC in field-grown lettuce and spinach.
- The effect of cut-and-come-again winter production on TNC and yield in leaf lettuce and Asian greens.
- The variation of TNC in lettuce and spinach among the following plant parts: center whorl, inner whorl, outer whorl, petioles, and leaf blades.
- The effect of cultivar and fertilizer application on TNC in summer grown lettuce and spinach.

Literature Cited

Amr, A. and N. Hadidi. 2001. Effect of cultivar and harvest date of nitrate (NO_3) and nitrite (NO_2) content of selected vegetables grown under open field and greenhouse conditions in Jordan. J. Food Composition Analysis 14:59-67.

Anon. 2005. Commission Regulation (EC) No. 1822/2005 of 8th November 2005, amending Regulation (EC) No. 466/2001 as regards nitrate in certain vegetables. Official Journal of the European Communities L293/11:9 November 2005. 10 Jan. 2010 <http://europa.eu.int>.

Anon. 2009. Try several kinds of lettuce. Univ. of Illinois Extension Gardener's Corner: 2(4). 18 Mar. 2010. <http://urbanext.illinois.edu/gardenerscorner/issue_02/summer_05_06.html>.

Barker, A.V., N.H. Peck, and G.E. MacDonald. 1971. Nitrate accumulation in vegetables. I. Spinach grown in upland soils. Agron. J. 63:126-129.

Barker, A.V. 1975. Organic vs. inorganic nutrition and horticultural crop quality. HortScience 10:50-53.

Barr, C. 2007. Don't forget the salad greens. Colorado State Univ. Ext. 18 Mar. 2010. <http://www.ext.colostate.edu/Pubs/columngw/gr070519.html>.

Behr, U., and H.-J. Wiebe. 1992. Relation between photosynthesis and nitrate content of lettuce cultivars. Sci. Hort. 49:175-179.

Bryan, N.S., B.O. Fernandez, S.M. Bauer, M.F. Garcia-Saura, A.B. Milsom, T. Rassaf, R.E. Maloney, A. Bharti, J. Rodriquez, and M. Feelisch. 2005. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nature Chem. Biol. 1:290-297.

Cantliffe, D.J. 1972a. Nitrate accumulation in spinach grown under different light intensities. J. Amer. Soc. Hort. Sci. 97:152-154.

Cantliffe, D.J. 1972b. Nitrate accumulation in vegetable crops as affected by photoperiod and light duration. J. Amer. Soc. Hort. Sci. 97:414-418.

Cantliffe, D.J. 1972c. Nitrate accumulation in spinach grown at different temperatures. J. Amer. Soc. Hort. Sci. 97:674-676.

Chen, T.M. and S.K. Ries. 1969. Effect of light and temperature on nitrate uptake and nitrate reductase activity in rye and oat seedlings. Can. J. Bot. 47:341-343.

Gaskell, M. and R. Smith. 2007. Nitrogen sources for organic vegetable crops. HortTechnology 17:431-441.

Grindlay, D.J.C. 1997. Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. J. Agr. Sci. 128:377-3986.

Hageman, R.H., D. Flesher and A. Gitter. 1961. Diurnal variation and other light effects influencing the activity of nitrate reductase and nitrogen metabolism in corn. Crop Sci. 1:201-204.

Harper, J.E. and G.M. Paulsen. 1968. Influence of intensity, quality, and duration of light on nitrogen reduction and assimilation in wheat. Crop Sci. 8:537-539.

Jackson, L., K. Mayberry, F. Laemmlen, S. Koike, K. Schulbach, and W. Chaney. 1996. Leaf lettuce production in California. Univ. of California, Davis. Veg. Res. Info. Ctr: Veg. Prod. Ser. Pub. 7216.

Kadir, S., E. Carey, and S. Ennahli. 2006. Influence of high tunnel and field conditions on strawberry growth and development. HortScience 41:329-335.

LeStrange, M., S. Koike, J. Valencia, and W. Chaney. 1996. Spinach production in California. Univ. of California, Davis. Veg. Res. Info. Ctr: Veg. Prod. Ser. Pub. 7212.

Mansour, N.S. and C.A. Raab. April 1996. Grow your own lettuce, spinach and Swiss chard. Oregon State Univ. Ext. Serv. EC1268.

Maynard, D.N., A.V. Barker, P.L. Minotti, and N.H. Peck. 1976. Nitrate accumulation in vegetables. Adv. Agron. 28:71-118.

Miles, C., K. Kolker, and G. Becker. 2005. Winter lettuce variety trail. Washington State Univ. Ext. 18 Mar. 2010. < http://agsyst.wsu.edu/WinterLettuceReport05.pdf>.

Ott, K.A. 2007. Impacts of winter growing conditions on yield and nitrate accumulation in organically produced leafy greens. MS thesis, Washington State Univ., Pullman, WA.

Ott, K.A., R. Koenig, and C. Miles. 2008. Influence of plant part on nitrate concentration in lettuce and spinach. Int. J. Veg. Sci. 14:351-361.

Pavlou, G.C., C.D. Ehaliotis, and V.A. Kavvadias. 2007. Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. Sci. Hort. 111:319-325.

Powlson, D.S., T.M. Addiscott, N. Benjamin, K.G. Cassman, T.M. de Kok, H. van Grinsven, J.-L. L'hirondel, A.A. Avery, and C. van Kessel. 2008. When does nitrate become a risk for humans? J. Environ. Qual. 37:291-295.

Proietti, S., S. Moscatello, A. Leccese, G. Colla, and A. Batistelli. 2004. The effect of growing spinach (*Spinacia oleracea* L.) at two light intensities on the amounts of oxalate, ascorbate and nitrate in their leaves. J. Hort. Sci. Biotechnol. 79:606-609.

Rader, H.B., and M.G. Karlsson. 2006. Northern field production of leaf and Romaine lettuce using a high tunnel. HortTechnology 16:649-654.

Reader, L. 2003. Lettuce for the cool season. Univ. of Arizona Coop. Ext. Coop. Ext. Maricopa County J. 18 Mar. 2010. <http://ag.arizona.edu/maricopa/garden/html/pubs/1203/landscaping.html>.

Santamaria, P. 2006. Review: nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agr. 86:10-17.

Santamaria, P., A. Elia, F. Serio, and E. Todaro. 1999. A survey of nitrate and oxalate content in fresh vegetables. J. Sci. Food. Agr. 79:1882-1888.

Stopes, C., L. Woodward, G. Forde, and H. Vogtmann. 1989. Effects of composted FYM and a compounds fertiliser on yield and nitrate accumulation in three summer lettuce cultivars grown in an organic system. Ag. Eco. Environ. 27:555-559.

Taiz, L. and E. Zeiger. 2006. Plant Physiology, 4th ed. Sinauer Associates, Inc.: Sunderland, MA.

U.S. Department of Agriculture. 2008. Vegetables 2007 Summary. U.S. Department of Agriculture National Agricultural Statistics Service. Vg 1-2 (08). 18 Mar. 2010 <http://usda.mannlib.cornell.edu/usda/nass/VegeSumm//2000s/2008/VegeSumm-01-25-2008.pdf>.

U.S. Department of Agriculture - National Agricultural Statistics Service. 2009. 2007 Census of Agriculture: United States, Summary and State Data. Vol 1, Geographic Area Series, Part 51. USDA-NASS, Washington, DC. 18 Mar. 2010 <http://www.agcensus.usda.gov/Publications/2007/Full_Report/index.asp>.

U.S. Department of Agriculture. 2010. CFR Regulatory Text, 7 CFR Part 205, Subpart A — Definitions. § 205.2 Terms defined. 21 Mar. 2010. <http://www.ams.usda.gov/nop/NOP/standards/DefineReg.html> .

Vogtmann, H., A.T. Temperli, U. Künsch, M. Eichenberger and P. Ott. 1984. Accumulation of nitrates in leafy vegetables grown under contrasting agricultural systems. Biol. Agr. Hort. 2:51-68.

Waterer, D. 2003. Yields and economics of high tunnels for production of warm-season vegetable crops. HortTechnology 13:339-343.

Weightman, R.M., C. Dyer, J. Buxton, and D.S. Farrington. 2006. Effect of light level, time of harvest and position within field on the variability of tissue nitrate concentration in commercial crops of lettuce (*Lactuca sativa*) and endive (*Cichorium endiva*). Food Add. Contam. 23:462-469.

Chapter 2

The Effect of Photoperiod on NO_3-N Concentration in Field-grown Leafy Greens at Two Latitudes

Abstract

Potential negative health effects of nitrates in the diet has generated much interest in plant tissue nitrate concentration (TNC). In response, the European Commission (EC) set limits on TNC in lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*); the U.S. has no limits. Plant TNC has been shown to fluctuate with light intensity and photoperiod in leafy greens grown in controlled environments. Late-day harvests have been recommended as a means of controlling TNC, though few field studies have been done to support that recommendation. This study investigated the effect of photoperiod on TNC in field-grown lettuce and spinach at 2 latitudes (46 and 64^{o}N). On each of 3 separate harvest dates at each location, whole plants were sampled every 2 hours for 24-hours. Photoperiod did not influence TNC at either site or for either crop type. It is likely that other factors were masking the influence of photoperiod on TNC in field-grown leafy greens.

Introduction

Light intensity and photoperiod influence TNC (Maynard et al., 1976; Steingrover et al., 1986; Behr and Wiebe, 1992; Amr and Hadidi, 2001). These effects have been documented in spinach (Steingrover et al., 1986), lettuce (Behr and Wiebe, 1992), oat seedlings (*Avena sativa*) (Chen and Ries 1969), corn (*Zea mays* L.) (Hageman et al., 1961), and wheat (*Triticum* *aestivum*) (Harper and Paulsen, 1968). However, each of these studies was conducted in a growth chamber or greenhouse.

There is considerable interest in TNC in food crops because of its potential impacts on human health. Nitrate itself has low toxicity, but in the human digestive system it is reduced to nitrite (Walker, 1990). Excess nitrates in the diet have been implicated in methaemoglobinaemia and digestive tract cancers (Santamaria, 2006; Powlson et al., 2008). The EC has set limits on acceptable concentrations of nitrates in leafy greens, depending on crop, harvest time, and method of cultivation (Anon., 2005). The U.S. has no such regulations.

New research shows that, at least in adults, nitrates may not be as harmful as previously thought. Some proposed beneficial effects of nitrate in the body are host defense, vasodilatation, and possibly an anti-inflammatory (Lundberg et al., 2004). Nitrate and its reduced forms also play important roles in animal cell activity including several signaling pathways (Bryan et al., 2005).

Regardless of its effect, vegetables are a large source of nitrates in the human diet (Maynard et al., 1976; Vogtmann et al., 1984), and leafy greens, in particular, can accumulate nitrates in excess of plant need (Santamaria et al., 1999). Plants like spinach, lettuce, Swiss chard (*Beta vulgaris* var. *cicla*), and radish (*Raphanus sativus*) are considered nitrophilic (Vogtmann et al., 1984) where nitrogen (N) accumulation can exceed the amount required for maximum growth (Grindlay, 1997).

One significant factor in nitrate concentration in leafy vegetables is fertilizer supply and N availability (Maynard et al., 1976; Guadagnin et al., 2005). When comparing conventional and organic production systems, vegetables grown in the absence of synthetic fertilizer had lower TNC than those grown with synthetic fertilizer (Woese et al., 1997; Guadagnin et al., 2005).

Light intensity also plays a major role in tissue nitrate accumulation for some crops. Chen and Ries (1969) reported an inverse relationship between light intensity and TNC in oat seedlings: as light intensity increased TNC decreased. Cantliffe (1972a) reported spinach grown under light levels of approximately 120 µmol m^{-2} s^{-1} had more than twice the TNC compared to those grown under approximately 320 µmol m^{-2} s^{-1}. Additionally, spinach TNC was reduced by over 50% when light intensity was increased from 200 to 800 µmol m^{-2} s^{-1} (Proietti et al., 2004). Nitrate accumulated after a dark period or in short-day treatments in tobacco (*Nicotiana tabacum*) (Matt et al., 1998).

The change in TNC due to changes in light intensity follows a diurnal pattern where TNC decreases the light intensity increases. Light regulates nitrate reductase (NR) enzyme activity through the addition of carbon dioxide (CO_2) produced by photosynthesis, which activates the enzyme (Kaiser and Huber, 2001). TNC increased during the dark period and decreased during light periods in corn and wheat (Hageman et al., 1961; Harper and Paulsen, 1968). Spinach plants grown in a controlled environment at 145 µmol m^{-2} s^{-1} exhibited a diurnal rhythm when sampled every 2 hours for 24-hours, with the most significant fluctuation observed in the leaf blades. In this study, TNC more than doubled following the dark period (Steingrover et al., 1986). To lower TNC in leafy greens, it has been recommended that growers implement late day harvests (Cantliffe, 1972a; Maynard et al., 1976; Steingrover et al., 1982)

Field studies on the effects of photoperiod on TNC are rare. In contrast to greenhouse studies, Weightman et al. (2006) found no consistent pattern when sampling field-grown lettuce at 3-hour intervals from 07:00 to 22:00. TNC was significantly lower at 7:00 and 13:00, but did not show true diurnal variation (Weightman et al., 2006). The objective of our study was to determine the influence of photoperiod on TNC in field-grown lettuce and spinach at 2 different latitudes.

Materials and Methods

Sites and preparation

Studies were conducted at 2 sites using lettuce and spinach. One study was conducted at the Washington State University (WSU) Tukey Organic Research Farm (46.43^{o}N, 117.10^{o}W) in a Palouse silt loam (Fine-silty, mixed, superactive, mesic pachic ultic haploxeroll). The other study was conducted at the University of Alaska Fairbanks (UAF) Experiment Farm (64.86^{o}N 147.68^{o}W) in a Tanana mucky silt loam (Coarse-loamy, mixed, superactive, subgelic typic aquiturbels). Studies at both sites involved a randomized complete block design with 4 replications. Plot sizes at WSU were 1.2 m wide by 7.6 m long (9.12 m^2) with 5 rows of plants. Plot sizes at UAF were 0.6 m wide by 6.1 m long (3.66 m^2) with 3 rows of plants.

Lettuce (‘Cracoviensis’) and spinach (‘Giant Winter’) were selected based on TNC data from previous research conducted at the WSU site (Ott et. al., 2008). The cultivars of these crop types were selected based on an apparent greater sensitivity to changes in the environment and more variable TNC in this earlier work.

At WSU, the plot area was prepared using a tractor-driven spader, and compost was applied at an approximate rate of 56 kg N $ha^{-1}$and incorporated with the spader. At UAF, a bed shaper-cultivator created raised beds. No compost was applied. Fishmeal was applied at WSU (8-3-0; Peaceful Valley Farm Supply Inc., Grass Valley, CA) and at UAF (9-4.5-0; Pro-Pell-It!, Marion Ag. Services, Inc., Aurora, Oregon) at 168 kg N ha^{-1}. The fishmeal was broadcast by hand and incorporated with a rake to a depth of 5 cm several days before seeding or transplanting at both sites.

Soil samples were taken with a 2-cm diameter probe at depths of 0 to 15 cm, 15 to 30 cm, and 30 to 60 cm after compost application at WSU, but before planting or fishmeal application at both sites. Both sites were sampled again after harvest. Separate samples were taken from each replication, with 2 soil cores combined for each replication. Samples for both sites were sent to a commercial soil testing lab and analyzed for available soil moisture, pH, organic matter, NO_3-N, NH_4-N, S, P, and K.

At WSU, both cultivars were direct seeded on May 13, 2009 at 20 cm within-row spacing, and rows spaced 20 cm apart. After emergence, plants were thinned to 1 per space. At UAF, plant spacing was 20 cm within the row and 25 cm between rows. Lettuce was planted in a greenhouse on May 16, 2009 and transplanted into the field on June 9; spinach was direct seeded on June 9, 2009.

At WSU, 1 line of drip tape irrigated each row. Plots were irrigated twice a week, with approximately 2.5 cm of water applied at each event. At UAF, 2 drip lines were used for 3 rows of plants and plots were irrigated 5 times a week. Weeds were controlled by hand.

Tissue sampling

Tissue samples were collected every 2 hours during 3 separate, 24-hour periods at each location. Light intensity was measured every 15 minutes using a PAR sensor and recorded with a data logger (Hobo Micro Station S-LIA-M003, H21-002; Onset, Bourne, MA).

Harvest dates occurred on June 12, June 18, and June 24, 2009 at WSU. Two plants were taken per plot at each harvest. Harvests always commenced at 12:00 pm and ended at 12:00 pm the following day. At UAF, harvest dates were July 2, July 7, and July 10. Due to limitations on plant number, only 1 plant was taken per plot at each harvest period at UAF.

Plants were cut approximately 3 cm below the soil surface, placed in labeled plastic bags, and transported from the field to the lab within 1 hour. Plants were trimmed of roots and damaged leaves. If needed, plants were rinsed with deionized water and dried with paper towels. Plant fresh weight was recorded. Samples were placed in paper bags and dried at $80^{o}C$ for at least 48 hours. Dry weights were recorded.

Tissue sample processing

Oven-dry plant tissue was finely ground using a mortar and pestle. To extract NO_3-N, 0.4 g of ground tissue was transferred to a 50 ml vial, to which 40 ml of 0.025 M $Al_2(SO_4)_3$ extracting solution was added. If 0.4 g was not available, then the maximum amount was added and the weight recorded. Vials were capped and shaken for 30 minutes. The solution was then filtered into glass tubes through a Whatman #2 paper. The extract was poured into clean 50 ml vials and refrigerated until analysis.

Nitrate concentration was measured using an Orion 720A plus TM Benchtop meter and ion selective nitrate electrode (ISE) (Thermo Electron Corporation, Waltham, MA). The meter was calibrated using 3 NO_3-N standards with concentrations of 1.4, 14 and 140 mg NO_3-N L^{-1}. Approximately 30 ml of room temperature tissue extract was poured into a beaker with a magnetic stir bar. The extract was stirred at a constant rate. The electrode was placed directly in the solution. The meter was checked for accuracy by reading the midrange standard every 6 samples, and recalibrated as needed.

Whole-plant TNC on a dry and fresh weight basis was calculated and plotted as a function of time for each harvest at each site. Results are presented on a fresh weight basis only to facilitate comparison with other studies reported in the literature. Data were analyzed using the PROC MIXED procedure in SAS (SAS Inst., Cary, N.C.) at a significance level of 0.05.

Results

There was no diurnal pattern in tissue NO_3-N concentration during any harvest at either site (Figure 1). There was a 3-way interaction between site, harvest date, and crop type on TNC (p-value < 0.0001). The data were analyzed separately by site and crop type within a site to determine if there were any statistical differences among harvest dates (Figure 2). At WSU, harvest date 1 had the highest TNC for both crop types. There was no statistical difference between harvest dates 2 or 3 for lettuce 'Cracoviensis.' For spinach 'Giant Winter,' TNC for harvest date 3 was significantly higher than harvest date 2. At UAF, lettuce 'Cracoviensis' had the highest TNC during harvest date 2. There was no statistical difference between harvest dates 1 and 3. Spinach 'Giant Winter' had higher TNC at harvest dates 1 and 2; however, harvest date 2 was not significantly different than harvest date 3. The main effect of harvest date was not significant ($p = 0.07$).

The mean TNC at WSU was 214 mg NO_3-N kg^{-1} fresh weight with a range of 15.6 to 587 mg NO_3-N kg^{-1}. At UAF, the mean TNC was 649 mg NO_3-N kg^{-1}, with a range of 9.9 and 2635 mg NO_3-N kg^{-1}. Soil nitrate level in the top 60 cm was not significantly different between sites or time of sampling (Table 1). This was due largely to high variability among the replications.

Table 1. Soil analysis for study sites and sampling times.

Location		Depth (cm)	NO_3-N (kg ha^{-1})	NH_4-N (kg ha^{-1})	pH	Organic matter (%)
WSU	Pre- fertilization	0-30	14.3	16.6	6.67	3.60
		30-60	10.1			
	Study completion	0-30	77.3	34.5	6.25	3.75
		30-60	40.4			
UAF	Pre-fertilization	0-30	24.7	13.5	7.53	5.57
		30-60	42.6			
	Study completion	0-30	70.3	8.1	6.93	5.86
		30-60	42.6			

Discussion

Our results do not support the recommendation of late day harvest to lower TNC in leafy greens (Cantliffe, 1972a; Maynard et al., 1976; Steingrover et al., 1982). Weightman et al. (2006) found results similar to ours in field-grown lettuce; TNC did not follow diurnal variation when sampled at 3-hour intervals from 07:00 to 22:00.

Part of the reason why no diurnal pattern in TNC was measured could be that the differences in maximum PAR between the 2 sites was not as great as expected, and photoperiods were long. The average harvest date maximum PAR for WSU was 1936 µmol m^{-2} s^{-1}, and for UAF was 1482 µmol m^{-2} s^{-1}. However, WSU had approximately 8 hours of 0 µmol m^{-2} s^{-1} PAR, while UAF had approximately 2 hours of 0 µmol m^{-2} s^{-1} PAR (Figure 1), although at UAF there was always sufficient light for visibility at sampling times. Therefore, photoperiods for WSU and UAF were approximately 16 and 22 hours respectively. Steingrover's research (1986) did show a diurnal pattern in TNC in spinach with a 10 hour photoperiod in a controlled environment, which is considerably shorter than photoperiods observed here.

Cantliffe (1972b) found beets (*Beta vulgaris*) increased in TNC as N fertilizer rates increased. Additionally, when comparing photoperiods of 8, 12, 16, and 20 hours, Cantliffe (1972b) found that fertilization rates greater than 224 kg N ha^{-1} had greater differences among photoperiods, and longer photoperiods had lower TNC compared with the shorter photoperiods. Fertilizer from organic sources, like the fishmeal used in this study, has a lower N availability than inorganic N (Gaskell and Smith, 2007) and could have contributed to this trend. It may be possible that, because both sites had lower N availability and TNC than other published studies, diurnal patterns in TNC were not observed between sites.

In this study, TNC did not approach the maximum set by the EC (Anon., 2005). For fresh lettuce and spinach grown in summer under field conditions, maximum permissible nitrate levels are 2500 mg NO_3-N kg^{-1} of fresh weight for both crop types. On average, neither site in this study produced TNC that approached this maximum. The highest TNC observed was 2635 mg NO_3-N kg^{-1} of fresh weight, which occurred in 1 replication at UAF in lettuce 'Cracoviensis' during harvest date 3. However, the average TNC for lettuce 'Cracoviensis' during harvest date 3 was 548 mg NO_3-N kg^{-1} of fresh weight, which is well below EC limits.

Conclusions

Our results suggest that TNC of field-grown leafy greens may not vary diurnally when N availability and TNC is relatively low, as in organic production systems, and light intensity is high, as in the field in summer. Field conditions are more variable than those in controlled environments and many other factors may vary. It is possible that one or more of these factors is masking the effect of photoperiod in field grown leafy greens. More research is needed to fully understand how TNC is influenced by photoperiod in field conditions.

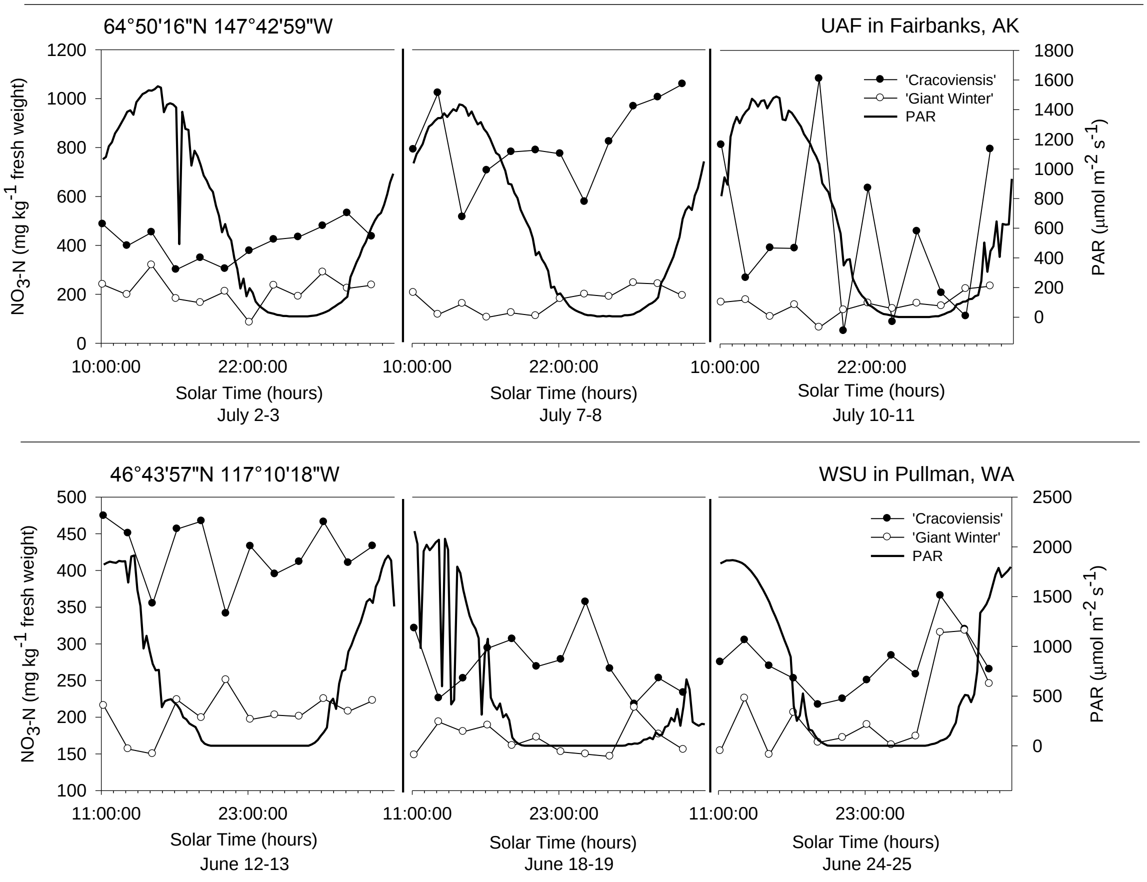

Figure 1. Changes in PAR and TNC in leafy greens sampled over 3, 24-hour periods at 2 different latitudes.

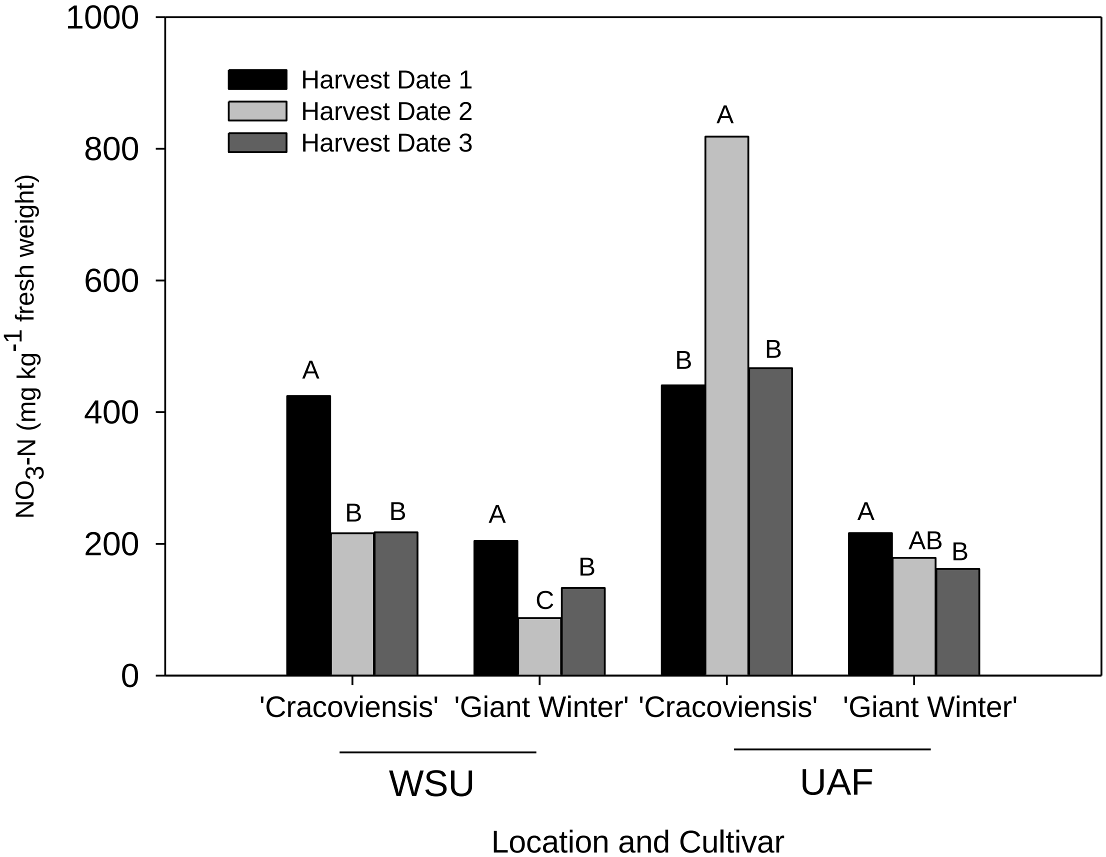

Figure 2. The effect of harvest date on TNC within a cultivar and location. Bars capped with the same letter within a cultivar and location are not significantly different ($p < 0.05$).

Literature Cited

Amr, A. and N. Hadidi. 2001. Effect of cultivar and harvest date of nitrate (NO_3) and nitrite (NO_2) content of selected vegetables grown under open field and greenhouse conditions in Jordan. J. Food Composition Analysis 14:59-67.

Anon. 2005. Commission Regulation (EC) No. 1822/2005 of 8th November 2005, amending Regulation (EC) No. 466/2001 as regards nitrate in certain vegetables. Official J. of the European Communities L293/11:9 November 2005. 10 Jan. 2010 <http://europa.eu.int>.

Behr, U., and H.-J. Wiebe. 1992. Relation between photosynthesis and nitrate content of lettuce cultivars. Sci. Hort. 49:175-179.

Bryan, N.S., B.O. Fernandez, S.M. Bauer, M.F. Garcia-Saura, A.B. Milsom, T. Rassaf, R.E. Maloney, A. Bharti, J. Rodriquez, and M. Feelisch. 2005. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nature Chem. Biol. 1:290-297.

Cantliffe, D.J. 1972a. Nitrate accumulation in spinach grown under different light intensities. J. Amer. Soc. Hort. Sci. 97:152-154.

Cantliffe, D.J. 1972b. Nitrate accumulation in vegetable crops as affected by photoperiod and light duration. J. Amer. Soc. Hort. Sci. 97:414-418.

Chen, T.M. and S.K. Ries. 1969. Effect of light and temperature on nitrate uptake and nitrate reductase activity in rye and oat seedlings. Can. J. Bot. 47:341-343.

Gaskell, M. and R. Smith. 2007. Nitrogen sources for organic vegetable crops. HortTechnology 17:431-441.

Grindlay, D.J.C. 1997. Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. J. Agr. Sci. 128:377-3986.

Guadagnin, S.G., S. Rath, and F.G.R. Reyes. 2005. Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. Food Add. Contam. 22:1203-1208.

Hageman, R.H., D. Flesher and A. Gitter. 1961. Diurnal variation and other light effects influencing the activity of nitrate reductase and nitrogen metabolism in corn. Crop Sci. 1:201-204.

Harper, J.E. and G.M. Paulsen. 1968. Influence of intensity, quality, and duration of light on nitrogen reduction and assimilation in wheat. Crop Sci. 8:537-539.

Kaiser, W.M. and S.C. Huber. 2001. Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. J. Exp. Bot. 52:1981-1989.

Matt, P., U. Schurr, D. Klein, A. Krapp, and M. Stitt. 1998. Growth of tobacco in short-day conditions leads to high starch, low sugars, altered diurnal changes in the *Nia* transcript and low nitrate reductase activity, and inhibition of amino acid synthesis. Planta 207:27-41.

Maynard, D.N., A.V. Barker, P.L. Minotti, and N.H. Peck. 1976. Nitrate accumulation in vegetables. Adv. Agron. 28:71-118.

Ott, K.A., R. Koenig, and C. Miles. 2008. Influence of plant part on nitrate concentration in lettuce and spinach. Int. J. Veg. Sci. 14:351-361.

Pavlou, G.C., C.D. Ehaliotis, V.A. Kavvadias. 2007. Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. Sci. Hort. 111:319-325.

Powlson, D.S., T.M. Addiscott, N. Benjamin, K.G. Cassman, T.M. de Kok, H. van Grinsven, J.-L. L'hirondel, A.A. Avery, and C. van Kessel. 2008. When does nitrate become a risk for humans? J. Environ. Qual. 37:291-295.

Proietti, S., S. Moscatello, A. Leccese, G. Colla, and A. Batistelli. 2004. The effect of growing spinach (*Spinacia oleracea* L.) at two light intensities on the amounts of oxalate, ascorbate and nitrate in their leaves. J. Hort. Sci. Biotechnol. 79:606-609.

Santamaria, P., A. Elia, F. Serio, and E. Todaro. 1999. A survey of nitrate and oxalate content in fresh vegetables. J. Sci. Food. Agr. 79:1882-1888.

Santamaria, P. 2006. Review: nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agr. 86:10-17.

Steingrover, E., R. Oosterhulis, and F. Wieringa. 1982. Effect of light treatment and nutrition on nitrate accumulation in spinach (*Spinacia oleracea* L.). Z. Pflanzenphysiol. Bd. 107. S. 97-102.

Steingrover, E., P. Ratering and J. Siesling. 1986. Daily change in uptake, reduction and storage of nitrate in spinach grown at low light intensity. Physiol. Plant. 66:550-556.

Vogtmann, H., A.T. Temperli, U. Künsch, M. Eichenberger and P. Ott. 1984. Accumulation of nitrates in leafy vegetables grown under contrasting agricultural systems. Biol. Agr. Hort. 2:51-68.

Walker, R. 1990. Nitrates, nitrites and N-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. Food Add. Contam. 7:717-768.

Weightman, R.M., C. Dyer, J. Buxton, and D.S. Farrington. 2006. Effect of light level, time of harvest and position within field on the variability of tissue nitrate concentration in commercial crops of lettuce (*Lactuca sativa*) and endive (*Cichorium endiva*). Food Add. Contam. 23:462-469.

Woese, K., D. Lange, C. Boess, and K. W. Bogl. 1997. A comparison of organically and conventionally grown food-results of a review of the relevant literature. J. Sci. Food Agr. 74:281-293.

Chapter 3

The Effect of Planting Date and Repeat Harvest on Yield and NO_3-N Concentration in Winter Grown Leafy Greens

Abstract

Excess nitrates in the diet have been implicated in methaemoglobinaemia and digestive tract cancers. Vegetables are a large source of nitrates in the human diet and leafy greens, in particular, can accumulate excess nitrates. The European Commission (EC) has regulations on maximum concentrations allowed. Tissue nitrate concentration (TNC) in leafy greens has been shown to respond to environmental changes and can vary within species and cultivar. Environmental factors including photoperiod, light intensity, and temperature have been shown to affect TNC in leafy greens. A commercial seed mix was evaluated for TNC and yield in a winter production system with repeat harvests. A commercial Asian greens mix was seeded in a hoophouse on 3 separate planting dates in the fall. Each planting date was harvested 3 times, and crop types analyzed separately for TNC and yield. Planting date, harvest date, and crop type all had significant effects on both TNC and yield. TNC within a crop type was significantly different between planting dates, and there was no clear trend followed by all crop types. TNC was well below EC regulations. There was a crop type by harvest date interaction, as not all crop types had similar yields in all harvest dates. It is possible to grow low TNC leafy greens in the winter without supplementary heat or light in hoophouses.

Introduction

Locally produced leafy greens provide communities with fresh vegetables and also support local growers. These benefits are encouraging growers in cold climates to use protected-growing techniques to extend their growing season (Schonbeck et al., 1991). However, some studies suggest that winter growing conditions, characterized by short photoperiods, can increase plant TNC (Maynard et al., 1976; Pavlou et al., 2007).

Excess nitrates in the diet have been implicated in methaemoglobinaemia and digestive tract cancers (Santamaria, 2006; Powlson et al., 2008). Nitrate itself is not toxic to humans, but when reduced to nitrite in the human digestive system, it becomes extremely harmful to human health (Walker, 1990).

Research is inconclusive on the role nitrate plays in human health. Lundberg et al. (2004) cites benefits such as host defense, and suggests researchers reconsider the idea that nitrate is only detrimental. New research also shows that, at least in adults, nitrates may not be as harmful as previously thought. Some of the proposed beneficial effects of nitrate in the body are host defense, vasodilatation, and possibly an anti-inflammatory (Lundberg et al., 2004). Nitrate and its reduced forms also play important roles in animal cell activity including several signaling pathways (Bryan et al., 2005).

Vegetables are a large source of nitrates in the human diet (Maynard et al., 1976; Vogtmann et al., 1984), and leafy greens, in particular, can accumulate excess nitrates (Santamaria et al., 1999). Plants like spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), Swiss chard (*Beta vulgaris* var. *cicla*), and radish (*Raphanus sativus*) are considered nitrophilic

(Vogtmann et al., 1984) where nitrogen (N) uptake can exceed the amount required for growth (Grindlay, 1997).

In Italy, the largest source of nitrates was lettuce, Swiss chard and beets (*Beta vulgaris*), which equaled almost half of the average total intake of nitrates (Santamaria et al., 1999). The EC has set limits on acceptable concentrations of nitrates in leafy greens, depending on crop type, harvest time, and method of cultivation (Anon., 2005). A study in New Zealand found that the combined amounts of nitrate and nitrite from food sources and drinking water would not exceed 20 percent of the accepted daily intake for an average consumer (Thomson et al., 2007).

TNC in leafy greens has been shown to respond to environmental changes and can vary within species and cultivar (Barker et al., 1971; Maynard et al., 1976). Environmental factors including photoperiod (Maynard et al., 1976), light intensity (Steingrover et al., 1986; Behr and Wiebe, 1992; Amr and Hadidi, 2001), and temperature (Cantliffe, 1972c) have been shown to affect TNC in leafy greens. For example, in a controlled-environment study, as temperature increased from 5^{o}C to 25^{o}C, TNC increased within plants (Cantliffe 1972c). However, from 25^{o}C to 30^{o}C, TNC decreased (Cantliffe 1972c).

Light intensity plays a major role in TNC for some crops. An increase from 200 to 800 µmol m^{-2} s^{-1} decreased TNC in spinach by over 50 percent (Proietti et al., 2004). Cantliffe (1972a) reported spinach grown under light levels of 120 µmol m^{-2} s^{-1} had more than twice the TNC compared with those grown under 320 µmol m^{-2} s^{-1}. Chen and Ries (1969) reported similar results in oat seedlings (*Avena sativa*), as light intensity increased TNC decreased.

Studies have shown a shorter photoperiod, as in winter growing conditions, increased TNC in some leafy green crops (Steingrover et al., 1986; Pavlou et al., 2007), and other crops such as beets (Cantliffe, 1972b). However, not all research has observed consistent, measurable differences in TNC due to these environmental factors (Weightman et al., 2006). For example, Weightman et al. (2006) found no consistent pattern when sampling field-grown lettuce at 3-hour intervals from 7:00 to 22:00.

TNC is regulated by nitrate reductase (NR) enzyme activity in plant tissue. Light regulates NR activity through the addition of carbon dioxide (CO_2) produced by photosynthesis, which activates the enzyme (Kaiser and Huber, 2001). Additionally, nitrate can be an osmotic solute within cell vacuoles. During low light conditions or at night soluble carbohydrates are transported to the cytoplasm to be metabolized. In these situations, nitrate can act as an osmotic replacement for the carbohydrates (Steingrover et al., 1986).

TNC has been shown to be species and even cultivar specific (Barker et al., 1971; Maynard et al., 1976). An extensive study evaluated TNC and total yield in 24 crop types and cultivars of winter-grown leafy greens, including Asian greens, spinach, and lettuce (Ott, 2007). Significant differences in TNC were found between crop types and cultivars, with the highest TNC and most variation in Asian greens. The highest TNC observed was 1269 mg NO_3-N kg^{-1} fresh weight in tatsoi (*Brassica narinosa*), well below EC limits for spinach and lettuce (Anon., 2005). The highest TNC in lettuce and spinach was 475 mg NO_3-N kg^{-1} fresh weight and 221 mg NO_3-N kg^{-1} fresh weight, respectively. In the second growing season, TNC was lower in Asian greens, but higher in lettuce and spinach cultivars. The effect of harvest date was not significant for most cultivars (Ott, 2007).

Age of the plants also has an effect on TNC. More mature kale (*Brassica oleracea acephala*) plants tended to have higher TNC, though the trend was not significant for all cultivars of kale in the study (Korus and Lisiewska, 2009).

Hoophouses, or high tunnels, can extend the growing season and increase profit for growers of leafy green crops. Although in the United States, the use of hoophouses is a fairly recent development, hoophouses are very popular in China, Spain, Japan, and other countries world-wide (Lamont, 2009). Row covers, or low tunnels, are also used to help extend the growing season and provide protection for crops (Lamont et al., 2003).

Lettuce can withstand freezing temperatures (Jackson et al., 1996; LeStrange et al., 1996) and has been shown to survive temperatures as low as $-1.5^{o}C$, but growth is slowed (Mansour and Raab, 1996). Asian greens such as kale, arugula (*Eruca sativa*) and mustard greens (*Brassica juncea*) are winter hardy and could also be a suitable crop for the Palouse region. Over the winter in Pullman, WA, Asian greens were consistently higher yielding than lettuce or spinach (Ott, 2007).

Hoophouses have been shown to extend the growing season in Alaska (Rader and Karlsson, 2006). The use of hoophouses for peppers (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*) extended the growing season by 2 weeks in Saskatchewan, Canada, and generated higher gross returns compared to field row cover (Waterer, 2003). Hoophouses have also been shown to increase yield and fruit quality in strawberries (*Fragaria ananassa*) grown in Kansas (Kadir et al., 2006). Several extension publications recommend staggering planting dates for a longer harvest (Miles et al., 2005; Barr, 2007). In addition, many salad greens can be harvested multiple times in a production system called cut-and-come-again to produce salad mix

(Reader, 2003; Barr, 2007; Anon., 2009). In repeat harvesting, where leaf lettuce plants are harvested by cutting the whole plant several centimeters above the soil surface, the plants will regrow from the crown and produce additional harvests without re-planting. The subsequent regrowth can be harvested several times before re-planting is required (Reader, 2003; Barr, 2007; Anon. 2009).

The purpose of this study was to evaluate organically managed leafy greens and determine how planting date and repeat harvest affect yield and TNC.

Materials and Methods

Site preparation and management

A loose leaf lettuce mix and an Asian greens mix were sown into an unlit and unheated hoophouse on 3 separate dates and harvested multiple times. The hoophouse used in this study was covered with 6-mil UV treated greenhouse plastic. This study was conducted during the winter in 2008-09 at the Washington State University Tukey Organic Research Farm in Pullman, Washington (46^{o}43’39” N, 117^{o}10’55” W) in a Palouse silt loam (Fine-silty, mixed, superactive, mesic pachic ultic haploxeroll).

The lettuce mix seed was purchased from Peaceful Valley Farm Supply, marketed as Lettuce Gourmet Salad Blend contained the following cultivars of lettuce: ‘Red Salad Bowl’ (leaf), ‘Green Salad Bowl’ (leaf), ‘Buttercrunch’ (head), ‘Paris Island Cos’ (romaine), and ‘Lolla Rossa’ (leaf). The Asian greens mix seed was purchased from Johnny’s Selected Seeds, marketed as Ovation Greens Mix included ‘Red Russian’ kale (*Brassica napus pabularia*), ‘Early Mizuna’ mizuna (*Brassica rapa japonica*), tatsoi (*Brassica rapa narinosa*), arugula

(*Eruca sativa*), and ‘Red Giant’ mustard (*Brassica juncea*). Not all crop types had identifiable cultivars.

The study was a randomized complete block, split plot with repeat measures design with planting date and harvest date as the main plot and crop type as the subplot. Repeat harvest was the repeated measure. The hoophouse was divided into 4 beds, each approximately 1 m wide and 12.8 m long. Individual beds were used as blocks. Plots were 2.13 m long by 1 m wide, or 2.28 m^2. Compost was applied to all plots 1 day before the first planting date, approximately 1 cm deep, and rotary-tilled.

The study included 3 planting dates: October 23, October 29, and November 5, 2009. Both the lettuce and Asian greens mix were direct seeded in 6 rows with 1 seed per 2.5 cm sown approximately 2 cm deep. Row covers draped over wire hoops were used to provide another layer of frost protection for both crops when temperatures were below freezing. The row cover was an Agribon Remay ™ fabric (AG-30 26 g m^{-3}; Peaceful Valley Farms and Garden Supply, Grass Valley, CA).

When plants developed the first true leaves, kelp emulsion (Liquid Kelp, Peaceful Valley Farm and Garden Supply, Grass Valley, CA) was applied foliarly every 3 weeks to be consistent with common farm practices. During the first kelp application, powdered fishmeal (8-3-0) (Fishmeal, Peaceful Valley Farm Supply Inc., Grass Valley, CA) was dissolved with the kelp emulsion.

Environmental data collection

Soil samples were taken with a 2-cm soil probe at depths of 0 to 15 cm, 15 to 30 cm, and 30 to 60 cm before and after compost application, and also after each harvest. Samples were sent to a commercial soil testing facility, and analyzed for available soil moisture, pH, organic matter, NO_3-N, NH_4-N, S, P, and K.

Temperature was monitored using 2, 4-channel dataloggers (Hobo Micro Station S-LIA-M003, H21-002; Onset, Bourne, MA). Outdoor and indoor air and soil temperatures were recorded every 30 minutes at 2 positions within and outside of the hoophouse. Air temperature was measured at 1 m above the soil surface. Soil temperature was measured 2 cm below the soil surface.

Light intensity was recorded approximately once a week starting in December using a hand-held quantum meter (Spectrum Technologies, Inc., Plainfield, IL). At each sample time, 4 light measurements were taken and an average was calculated for each of the following areas: outside the hoophouse, inside the hoophouse, and under the row cover. Measurements were taken in each corner of the hoophouse, approximately 1.5 m from each side wall.

Plant sampling and processing

A harvest area of 1 m by 3 rows was marked in each plot with wooden stakes. Harvest was done when plants reached marketable size, or when the largest plants were approximately 60 cm tall. Individual crop types had different growth rates so plants were at a variety of growth stages the time of harvest. All plants were clipped with scissors approximately 4 cm from the soil surface. Each harvest was done between 13:00 and 14:00.

At each harvest, plants were placed in plastic bags to minimize water loss during transportation from the field to the lab. In the lab, total fresh weight was measured. Unmarketable leaves and roots were removed. Crop types were then separated from one another and total fresh weight of each crop type in each plot was recorded. Samples were placed in labeled paper bags and dried at 80^{o}C for at least 48 hours. Dry weights were recorded.

Oven-dry plant tissue was finely ground using a mortar and pestle. To extract NO_3-N, 0.4 g of ground tissue was transferred to a 50 ml vial, to which 40 ml of 0.025 M $Al_2(SO_4)_3$ extracting solution was added. If 0.4 g was not available, then the maximum amount was added and the weight recorded. Vials were capped and shaken for 30 minutes. The solution was then filtered into glass tubes through a Whatman #2 paper. The extract was poured into clean 50 ml vials and refrigerated until analysis.

Nitrate concentration was measured using an Orion 720A plus TM Benchtop Ion Selective Nitrate Electrode (ISE) (Thermo Electron Corporation, Waltham, MA). The meter was calibrated using 3 NO_3-N standards with concentrations of 1.4, 14 and 140 mg NO_3-N L^{-1}. Approximately 30 ml of room temperature tissue extract was poured into a beaker with a magnetic stir bar. The electrode was placed directly in the solution. The meter was checked for accuracy by reading the midrange standard every 6 samples, and recalibrated as needed.

Whole-plant TNC on a dry and fresh weight basis were calculated and plotted as a function of time for each harvest at each site. Results are presented on a fresh weight basis only to facilitate comparison with other studies reported in the literature. Data were analyzed using the PROC MIXED procedure in SAS (SAS Inst., Cary, N.C.) at a significance level of 0.05.

Results

The lettuce mix was completely eaten by rodents, and so was removed from the analysis. Additionally, ‘Red Russian’ kale was also completely eaten by rodents, and was also removed from the analysis. The remaining 4 crop types from the Asian greens mix were analyzed for yield and TNC on a fresh weight basis.

There was a significant planting date by harvest date interaction for TNC. The second harvest date consistently had a higher TNC (Figure 1). However, for planting date 2 there was no difference between harvest date 1 and harvest date 2. For planting date 3, there was no significant difference between harvest date 2 and harvest date 3.

There was a crop type by planting date interaction for TNC (Figure 2). Arugula and ‘Early Mizuna’ mizuna had higher TNC than ‘Red Giant’ mustard and tatsoi for planting date 1. ‘Early Mizuna’ mizuna was not significantly different from any crop type, and arugula had the highest TNC for planting date 2. Arugula had the highest TNC for planting date 3 as well. There seemed to be a general downward trend of TNC among planting dates, with ‘Early Mizuna’ mizuna having the most dramatic decline. Arugula always had relatively high TNC, though not always statistically different from ‘Early Mizuna’ mizuna.

When analyzing fresh weight yield, there was a significant interaction between planting date and harvest date (Figure 3). During planting dates 2 and 3, there was a trend of increasing yield from harvest dates 1 to 3, although increases were not always statistically different. Planting date 2 was not statistically different between harvest dates 1 and 3. Planting date 1 had higher yield for harvest dates 2 and 3, while planting date 3 had higher yield for harvest date 3.

There was also a harvest date by crop type interaction for yield (Figure 4). Arugula yielded highest during harvest dates 2 and 3. There was no significant difference between harvest dates for ‘Early Mizuna’ mizuna. ‘Red Giant’ mustard had higher yield for harvest date

3. Tatsoi had the highest yield during harvest dates 1 and 2; however, there was no significant difference in yield between harvest date 2 and 3.

There was a significant decrease in soil nitrate levels after compost application and before the first harvest (Table 1). There was no difference in soil (N) for planting dates or harvest dates. PAR was reduced, relative to full sunlight outside, within the hoophouse by 24 percent, and reduced another 49 percent under the row cover. On average throughout the growing season, PAR reduction from outside the hoophouse to under the row cover was 63 percent (data not shown).

Temperature was averaged by week for overall average and high and low temperatures for soil and air both inside and outside the hoophouse (Figures 5, 6, and 7). Soil temperatures were generally warmer inside the hoophouse than outside (Table 2). Air temperatures were generally warmer inside the hoophouse than outside.

Harvesting began in February. Earlier planting dates were harvested in less days than later planting dates (Table 3). Planting date 1 was ready for harvest in 110 days, while planting date 3 was ready for harvest in 125 days. After harvest date 1, the number of days to harvest date 2 was between 27 and 38 days depending on planting date, and the time to harvest date 3 was 20 to 22 days. The decrease in days to harvest with later harvests was most likely due to the increase in air and soil temperature.

Table 1. Soil analysis in the top 60 cm for sampling times.

		Sampling Date	NO_3-N (kg ha^{-1})	NH_4-N (kg ha^{-1})	pH	Organic matter (%)	P (mg kg^{-1})	K (mg kg^{-1})	SO_4-S (mg kg^{-1})
	Pre-compost	10/22	162	20	7.7	4.8	66	760	41.8
	Post-compost	11/15	142	29	7.6	4.2	65	1112	45.8
Harvest 1	Planting 1	2/10	17	19	7.7	4.2	56	732	13.3
	Planting 2	2/24	39	26	7.9	4.9	71	885	17.3
	Planting 3	3/10	22	19	7.7	4.8	55	790	10.0
Harvest 2	Planting 1	3/20	31	21	7.9	4.9	57	778	12.0
	Planting 2	3/25	17	26	7.6	4.8	72	823	13.3
	Planting 3	4/6	21	35	7.7	4.9	68	836	13.3
Harvest 3	Planting 1	4/9	31	38	7.4	4.6	44	885	11.0
	Planting 2	4/16	33	38	7.4	4.8	60	801	13.3
	Planting 3	4/28	29	52	7.3	4.9	87	821	18.3

Table 2. Overall average, average high and average low temperatures for the study period in winter 2008-2009.

	Average temperature (C)	Average high temperature (C)	Average low temperature (C)
Outside air	4.0	14.3	-5.1
Inside air	6.6	25.1	-4.3
Outside soil	4.9	8.5	2.6
Inside soil	7.8	13.3	4.3

Table 3. Harvest dates within planting dates for winter 2008-2009. Days to harvest refer to the number of days between the planting date and harvest date 1, or between repeat harvests.

Planting	Planting date	Days to harvest	Harvest 1	Days to harvest	Harvest 2	Days to harvest	Harvest 3
1	10-23	110	2-10	38	3-20	20	4-9
2	10-29	118	2-24	29	3-25	22	4-16
3	11-5	125	3-10	27	4-6	22	4-28

Discussion

Although there are no regulations on TNC in Asian greens, the concentrations observed in this study were well below the limits set by the EC for spinach and lettuce for the same growing conditions (Anon., 2005). The limit set for lettuce under these growing conditions is 4500 mg NO_3-N kg^{-1}, and for spinach is 3000 mg NO_3-N kg^{-1} (Anon., 2005). The highest TNC occurred in 'Early Mizuna' mizuna (1667 mg NO_3-N kg^{-1}). This is consistent with Gent (2002), who also did not find that leafy greens accumulated high TNC when grown in a hoophouse, and suggested that cool temperatures may restrict N uptake. Pavlou et al. (2007) found that while TNC increased in late fall compared with summer growing conditions, they also found that TNC did not exceed EC regulations. Average TNC for winter growing conditions was less than 500 mg NO_3-N kg^{-1}.

It has been reported that short photoperiods, such as those present during winter growing conditions in northern latitudes, significantly increase TNC in leafy greens (Cantliffe, 1972b; Steingrover et al., 1986). Their conclusions led them to caution growers against growing greens in low light conditions. Our results do not support this concern, at least under the conditions which these plants were grown.

One explanation for low TNC could be the low soil nitrate levels. It is possible that the compost immobilized N in the soil. Soil nitrate levels were all below 40 kg NO_3-N ha^{-1} at the time of harvest. This could be because soil temperatures were between 6.2^{o}C and 14.5^{o}C during harvest dates, with the warmer soils during later harvest days (data not shown). Cassman and Munns (1980) found low soil temperature decreased nitrification, although soil moisture was also an important factor. However, Clark et al. (2009) found that soils will still mineralize N from organic sources at temperatures above -2^{o}C. Because soil nitrate levels appeared fairly consistent, it is possible that mineralization rates were moderating plant N uptake rates.

It appears the best way to control TNC is through planting date and crop type selection. The planting date by harvest date interaction suggests that harvest date is not a good predictor of TNC. Depending on the planting date, multiple harvest dates may decrease TNC in later harvests or it may not (Figure 1). The highest TNC was during planting date 1 and harvest date 2, which also corresponded to the highest air temperature recorded on a harvest date, but not the highest soil temperature. At the time of harvest, the air temperature inside the hoophouse was 34^{o}C, which was 5^{o}C higher than any other harvest day (data not shown). Cantliffe (1972c) reported that TNC decreased with temperature over 25^{o}C. It is possible that the high temperatures decreased NR activity, thus increasing nitrate stored in plant tissue.

A harvest date by crop type interaction found that not all crop types responded the same to repeated harvesting. Arugula and 'Red Giant' mustard increased in yield in later harvests. However, 'Early Mizuna' mizuna and tatsoi did not yield as well in later harvest dates, and tatsoi appeared to decrease in yield with each harvest. More trials should be done to identify crop type and cultivars that tolerate repeated harvesting. It would be helpful to identify crops that are more cold tolerant to try to decrease the time from planting to harvesting.

Certain crop types and cultivars may yield higher later in the winter growing season due to the increasing temperatures. Ott (2007) found higher yields in later harvest dates in Asian greens grown through the winter in a hoophouse, although these results were for single harvests. A napa cabbage (*Brassica rapa* subsp. *pekinensis* 'China Express') and a pak choy (*Brassica* *rapa* subsp. *pekinensis* ‘Shanghi’) were found to be particularly high yielding (Ott, 2007), and should be evaluated in this repeat harvest system.

Conclusions

It is possible to grow low TNC leafy greens during the winter in a hoophouse without supplemental heat or light. Several crop types were high yielding in a repeat harvest system. Our results suggest that, by using a hoophouse, it is possible to grow leafy greens year round in eastern Washington. By repeat harvesting instead of re-seeding, it may be possible to increase profits. In spite of some production difficulties, we may have identified another source of income for local growers and the community may have access to fresh, locally grown greens during the winter months. Additional studies are necessary to identify the best adapted crop types and cultivars for these growing conditions and management style.

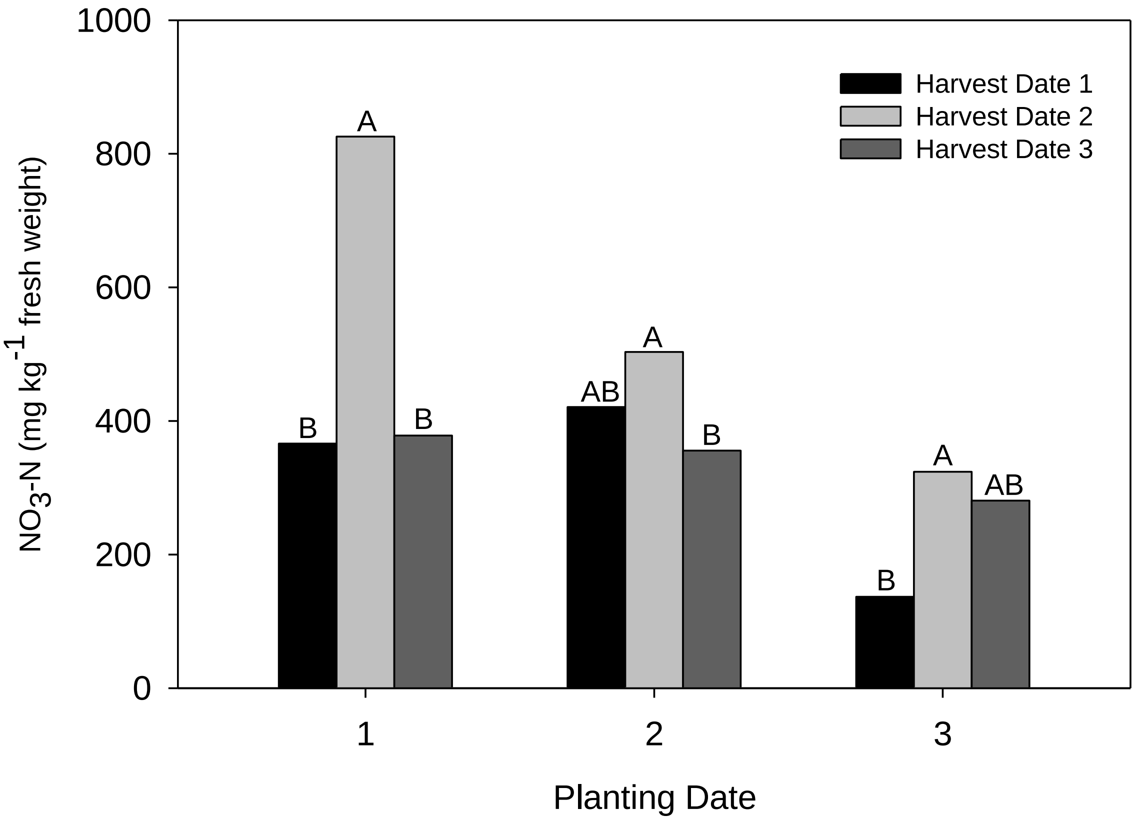

Figure 1. The effect of harvest date on TNC for 3 separate planting dates. Within a planting date, bars capped with the same letter are not significantly different ($p < 0.05$).

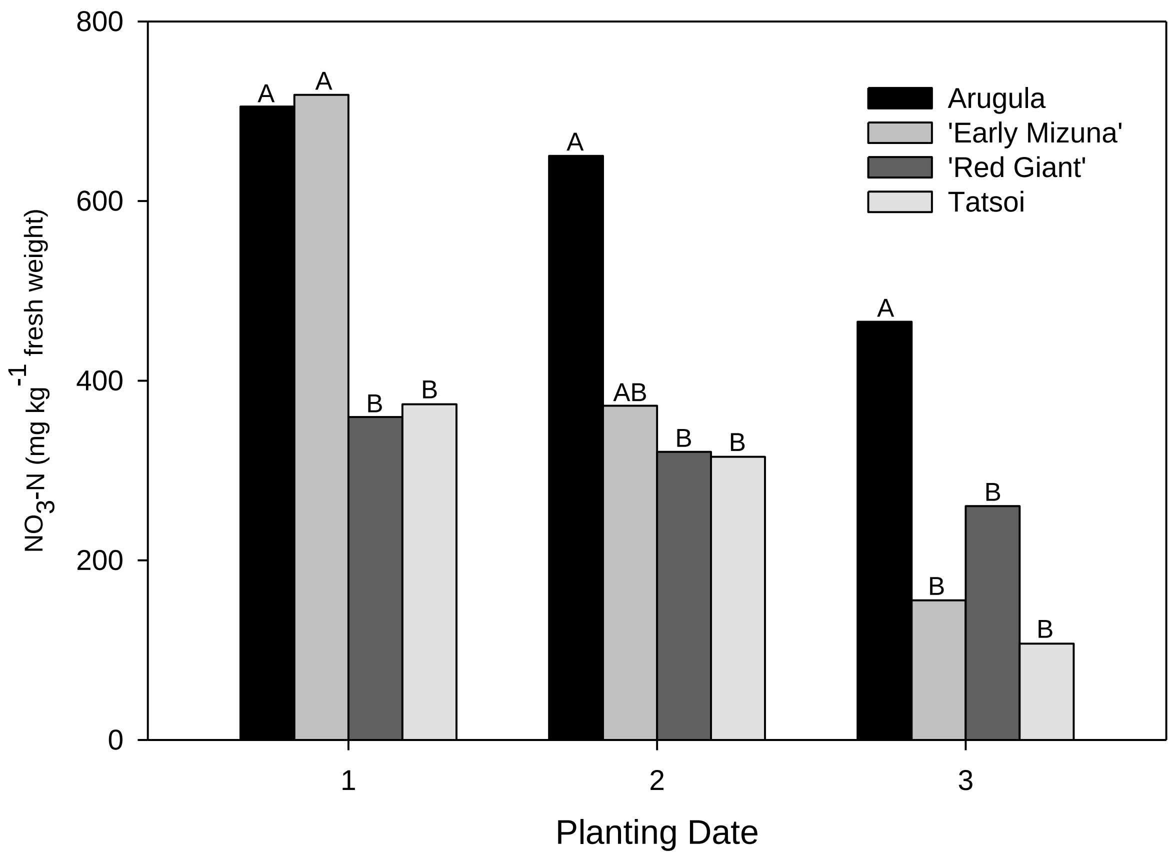

Figure 2. The effect of crop type on TNC for 3 separate planting dates. Within a planting date, bars capped with the same letter are not significantly different ($p < 0.05$).

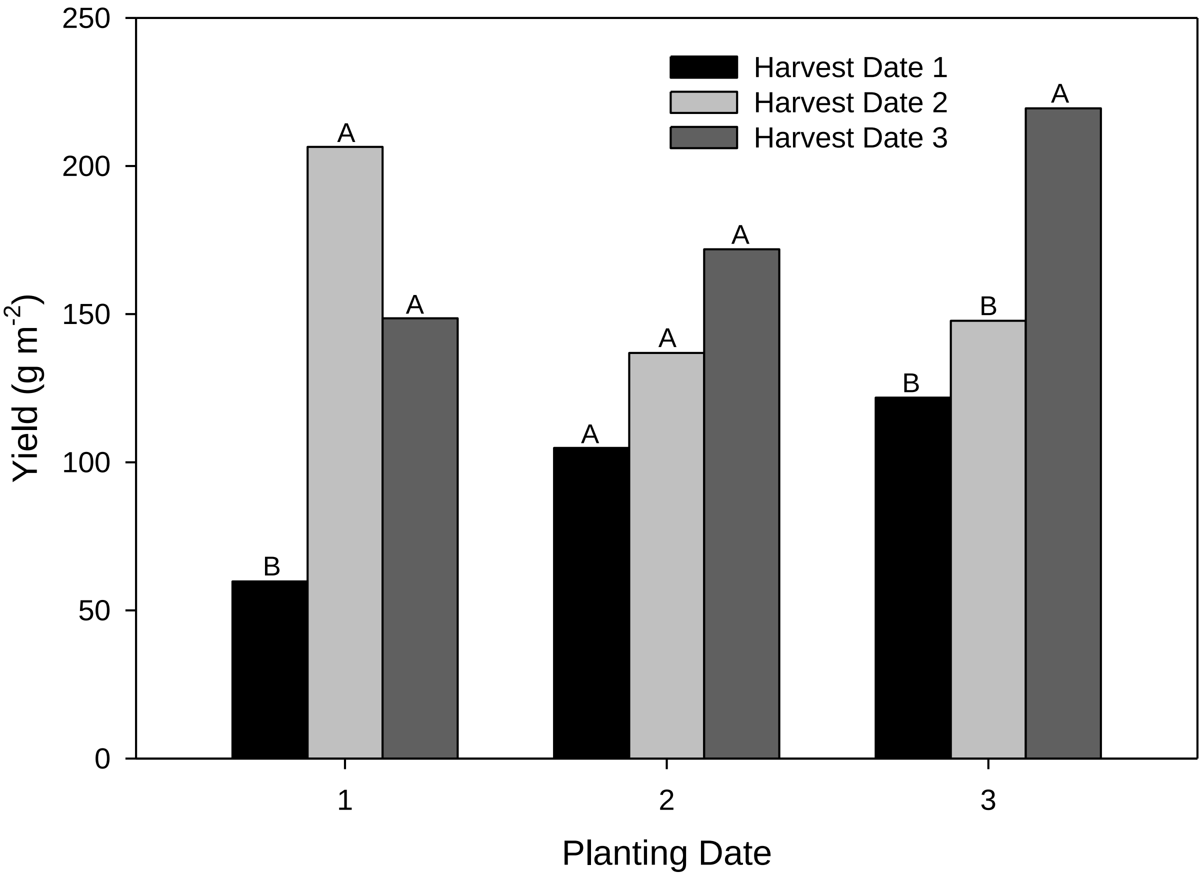

Figure 3. The effect of harvest date on yield for 3 planting dates. Within a planting date, bars capped with the same letter are not significantly different ($p < 0.05$).

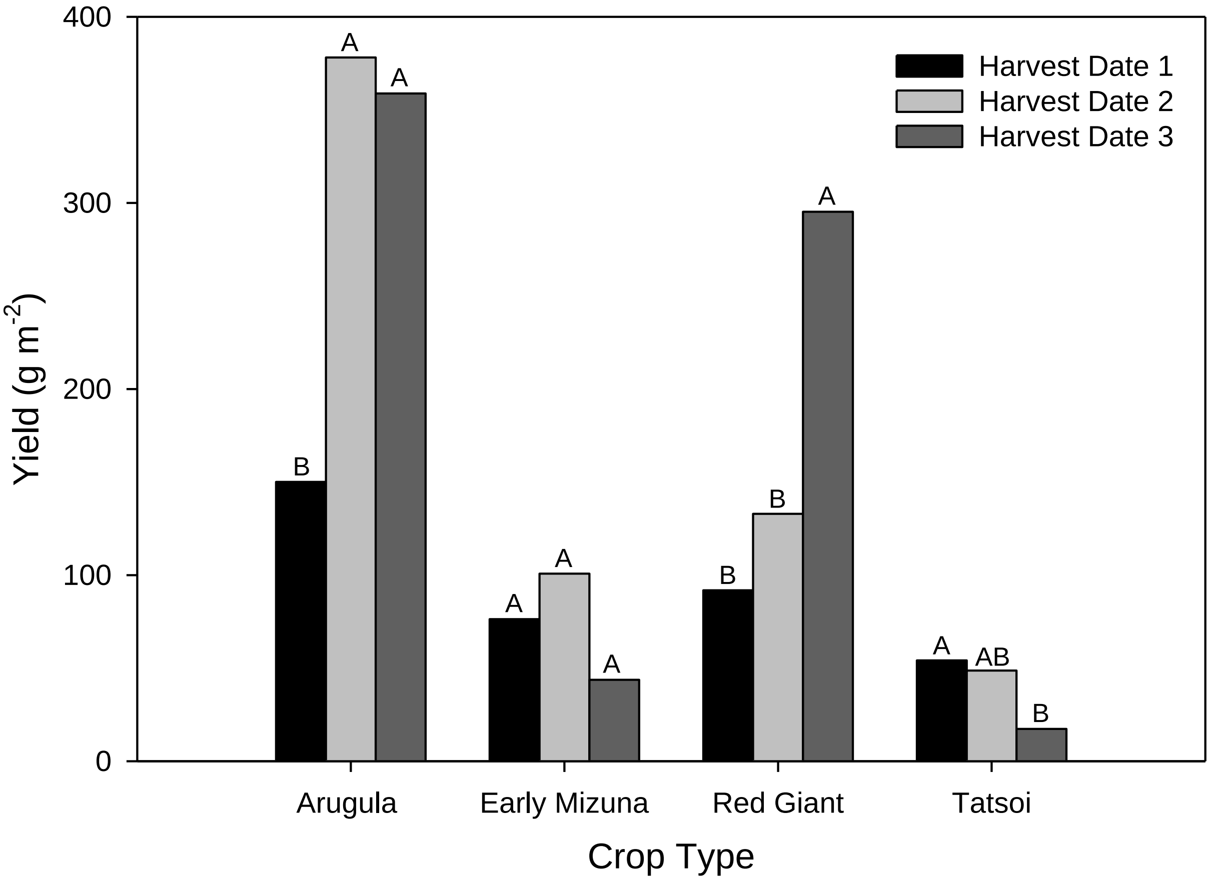

Figure 4. The effect of harvest date on yield for 4 crop types. Within a crop type, bars capped with the same letter are not significantly different ($p < 0.05$).

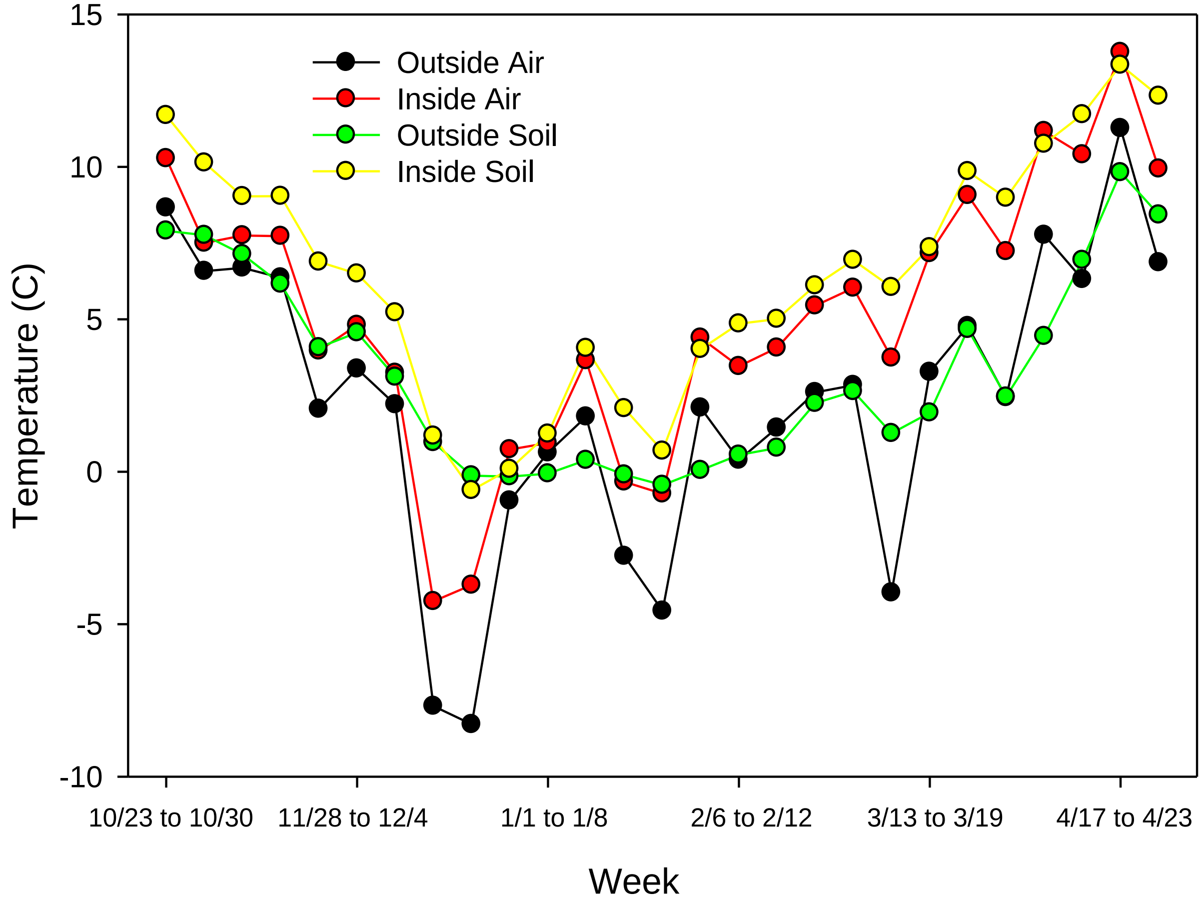

Figure 5. Average temperatures by week during the study period.

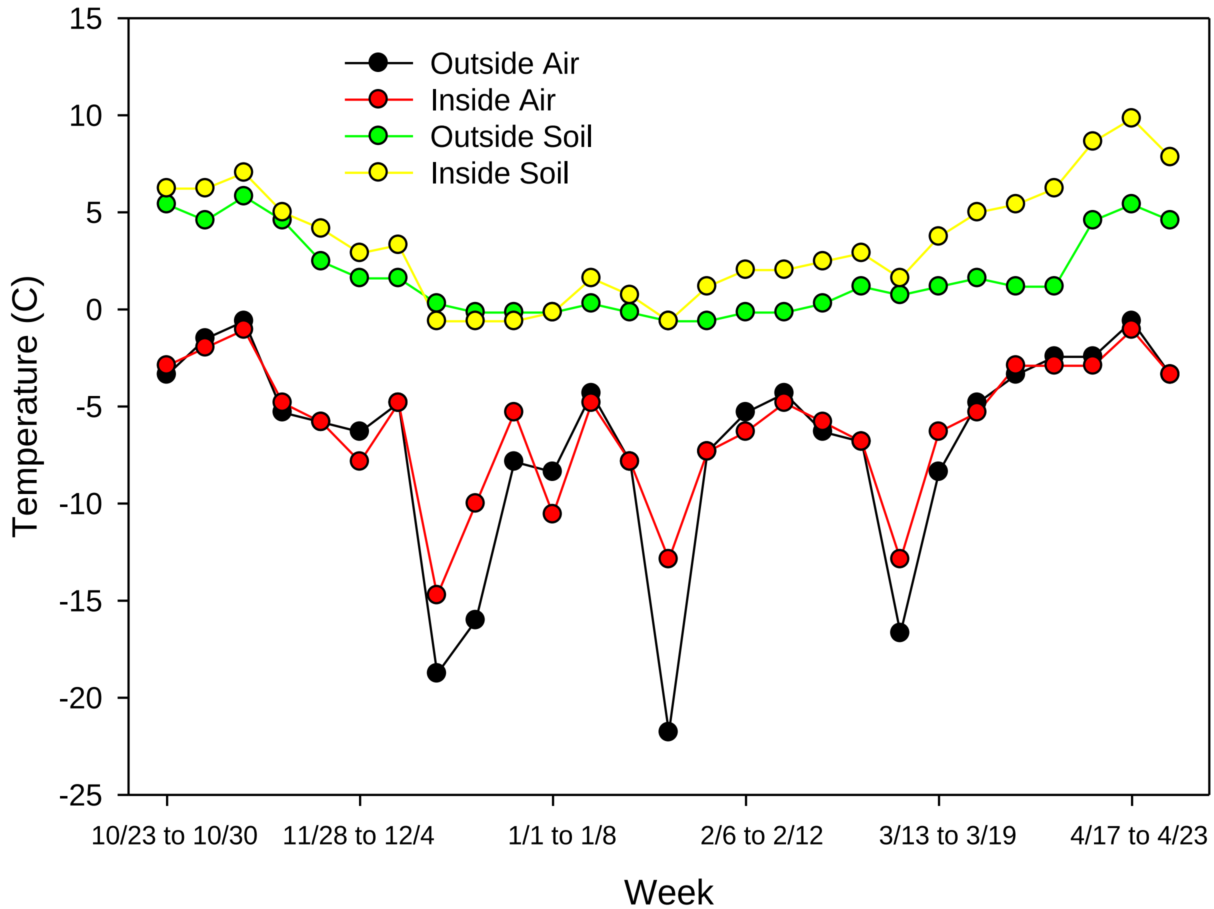

Figure 6. Average low temperature by week for the study period.

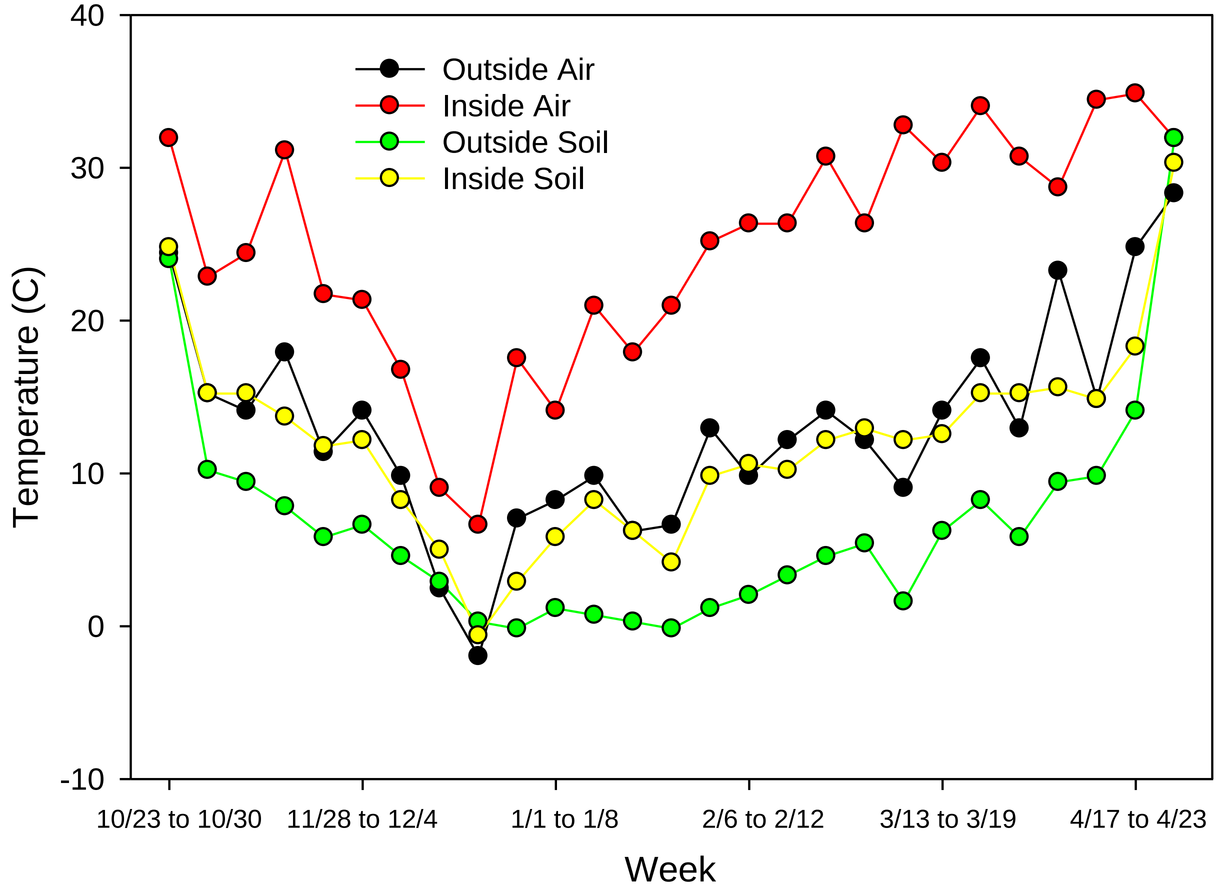

Figure 7. Average high temperature by week for the study period.

Literature Cited

Amr, A. and N. Hadidi. 2001. Effect of cultivar and harvest date of nitrate (NO_3) and nitrite (NO_2) content of selected vegetables grown under open field and greenhouse conditions in Jordan. J. Food Composition Analysis 14:59-67.

Anon. 2005. Commission Regulation (EC) No. 1822/2005 of 8th November 2005, amending Regulation (EC) No. 466/2001 as regards nitrate in certain vegetables. Official Journal of the European Communities L293/11:9 November 2005. 10 Jan. 2010 <http://europa.eu.int>.

Anon. 2009. Try several kinds of lettuce. Univ. of Illinois Extension Gardener's Corner: 2(4). 18 Mar. 2010. <http://urbanext.illinois.edu/gardenerscorner/issue_02/summer_05_06.html>.

Barker, A.V., N.H. Peck, and G.E. MacDonald. 1971. Nitrate accumulation in vegetables. I. Spinach grown in upland soils. Agron. J. 63:126-129.

Barr, C. 2007. Don't forget the salad greens. Colorado State Univ. Ext. 18 Mar. 2010. <http://www.ext.colostate.edu/Pubs/columngw/gr070519.html>.

Behr, U. and H.-J. Wiebe. 1992. Relation between photosynthesis and nitrate content of lettuce cultivars. Sci. Hort. 49:175-179.

Bryan, N.S., B.O. Fernandez, S.M. Bauer, M.F. Garcia-Saura, A.B. Milsom, T. Rassaf, R.E. Maloney, A. Bharti, J. Rodriquez, and M. Feelisch. 2005. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nature Chem. Biol. 1:290-297.

Cantliffe, D.J. 1972a. Nitrate accumulation in spinach grown under different light intensities. J. Amer. Soc. Hort. Sci. 97:152-154.

Cantliffe, D.J. 1972b. Nitrate accumulation in vegetable crops as affected by photoperiod and light duration. J. Amer. Soc. Hort. Sci. 97:414-418.

Cantliffe, D.J. 1972c. Nitrate accumulation in spinach grown at different temperatures. J. Amer. Soc. Hort. Sci. 97:674-676.

Cassman, K.G. and D.N. Munns. 1980. Nitrogen mineralization as affected by soil moisture, temperature, and depth. Soil Sci. Soc. Am. J. 44:1233-1237.

Chen, T.M. and S.K. Ries. 1969. Effect of light and temperature on nitrate uptake and nitrate reductase activity in rye and oat seedlings. Can. J. Bot. 47:341-343.

Clark, K., M.H. Chantigny, D.A. Angers, P. Rochette, and L.-E. Parent. 2009. Nitrogen transformations in cold and frozen agricultural soils following organic amendments. Soil Biol. Biochem. 41:348-356.

Gent, M.P.N. 2002. Growth and composition of salad greens as affected by organic compared to nitrate fertilizer and by environment in high tunnels. J. Plant Nutr. 25:981-998.

Grindlay, D.J.C. 1997. Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. J. Agr. Sci. 128:377-3986.

Jackson, L., K. Mayberry, F. Laemmlen, S. Koike, K. Schulbach, and W. Chaney. 1996. Leaf lettuce production in California. Univ. of California, Davis. Veg. Res. Info. Ctr: Veg. Prod. Ser. Pub. 7216.

Kadir, S., E. Carey, and S. Ennahli. 2006. Influence of high tunnel and field conditions on strawberry growth and development. HortScience 41:329-335.

Kaiser, W.M. and S.C. Huber. 2001. Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. J. Exp. Bot. 52:1981-1989.

Korus, A. and Z. Lisiewska. 2009. Effect of cultivar and harvest date of kale (*Brassica oleracea* L. Var. *Acephala*) on content of nitrogen compounds. Polish J. of Environ. Stud. 18: 235-241.

Lamont, W.J., M.D. Orzolek, E.J., Holcomb, K. Demchak, E. Burkhart, L. White, and B. Dye. 2003. Production system for horticultural crops grown in the Penn State high tunnel. HortTechnology 13:358-362.

Lamont, W.J. 2009. Overview of the use of high tunnels worldwide. HortTechnology 19:25-29.

LeStrange, M., S. Koike, J. Valencia, and W. Chaney. 1996. Spinach production in California. Univ. of California, Davis. Veg. Res. Info. Ctr: Veg. Prod. Ser. Pub. 7212.

Lundberg, J.O., E. Weitzberg, J.A. Cole, and N. Bengamin. 2004. Nitrate, bacteria and human health. Nat. Rev. Microbiol. 2:593-602.

Mansour, N.S., and C.A. Raab. April 1996. Grow your own lettuce, spinach and Swiss chard. Oregon State Univ. Ext. Serv. EC1268.

Maynard, D.N., A.V. Barker, P.L. Minotti, and N.H. Peck. 1976. Nitrate accumulation in vegetables. Adv. Agron. 28:71-118.

Miles, C., K. Kolker, and G. Becker. 2005. Winter lettuce variety trail. Washington State Univ. Ext. 18 Mar. 2010. < http://agsyst.wsu.edu/WinterLettuceReport05.pdf>.

Ott, K.A. 2007. Impacts of winter growing conditions on yield and nitrate accumulation in organically produced leafy greens. MS thesis, Washington State Univ., Pullman, WA.

Pavlou, G.C., C.D. Ehaliotis, V.A. Kavvadias. 2007. Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. Sci. Hort. 111:319-325.

Powlson, D.S., T.M. Addiscott, N. Benjamin, K.G. Cassman, T.M. de Kok, H. van Grinsven, J.-L. L'hirondel, A.A. Avery, and C. van Kessel. 2008. When does nitrate become a risk for humans? J. Environ. Qual. 37:291-295.

Proietti, S., S. Moscatello, A. Leccese, G. Colla, and A. Batistelli. 2004. The effect of growing spinach (*Spinacia oleracea* L.) at two light intensities on the amounts of oxalate, ascorbate and nitrate in their leaves. J. Hort. Sci. Biotechnol. 79:606-609.

Rader, H.B. and M.G. Karlsson. 2006. Northern field production of leaf and Romaine lettuce using a high tunnel. HortTechnology 16:649-654.

Reader, L. 2003. Lettuce for the cool season. Univ. of Arizona Coop. Ext. Coop. Ext. Maricopa County J. 18 Mar. 2010. <http://ag.arizona.edu/maricopa/garden/html/pubs/1203/landscaping.html>.

Santamaria, P., A. Elia, F. Serio, and E. Todaro. 1999. A survey of nitrate and oxalate content in fresh vegetables. J. Sci. Food. Agr. 79:1882-1888.

Santamaria, P. 2006. Review: nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agr. 86:10-17.

Schonbeck, M.W., R. Rivera, J. O'Brien, S. Ebinger, and R.E. DeGregorio. 1991. Variety selection and cultural methods for lowering nitrate levels in winter greenhouse lettuce and endive. J. Sustain. Ag. 2:49-75.

Thomson, B.M., C.J. Nokes, and P.J. Cressey. 2007. Intake and risk assessment of nitrate and nitrite from New Zealand foods and drinking water. Food Add. Contam. 24:113-121.

Vogtmann, H., A.T. Temperli, U. Künsch, M. Eichenberger and P. Ott. 1984. Accumulation of nitrates in leafy vegetables grown under contrasting agricultural systems. Biol. Agr. Hort. 2:51-68.

Waterer, D. 2003. Yields and economics of high tunnels for production of warm-season vegetable crops. HortTechnology 13:339-343.

Weightman, R.M., C. Dyer, J. Buxton, and D.S. Farrington. 2006. Effect of light level, time of harvest and position within field on the variability of tissue nitrate concentration in commercial crops of lettuce (*Lactuca sativa*) and endive (*Cichorium endiva*). Food Add. Contam. 23:462-469.

Chapter 4

The Effect of Cultivar, Fertility, and Plant Part on NO_3-N Concentration in Leafy Greens

Abstract

Lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) are important crops grown in the U.S. However, both have the potential to accumulate nitrates above what is required for maximum growth. These leafy greens are the main source of nitrates in the human diet. There are possible links between leafy greens with high plant tissue nitrate concentration (TNC) in the human diet and digestive cancers, among other health problems. Understanding the factors that influence TNC in plants is critical to minimize a potential risk. This study assessed how cultivar, fertility, and plant part affected TNC. Two lettuce cultivars and 2 spinach cultivars were grown in 2 different fertility treatments. Plants were dissected and analyzed by plant part for TNC. In 2008, there were significant interactions between cultivar and plant part, fertility and cultivar, and fertility and plant part. In 2009, there was a 3-way interaction. In general, our results suggest petioles have higher TNC than other plant parts, and ‘Brown Golding’ lettuce had higher TNC than other cultivars. Our results suggest it may be possible to control TNC through selecting crop types and cultivars that are characterized by low TNC and plant part selection.

Introduction

Lettuce and spinach are major leafy green crops grown in the United States. Head lettuce ranks first in production and crop value for all vegetable crops grown in the U. S. Major production areas for leafy greens are California and Arizona, but lettuce and spinach are grown in most states (USDA, 2008).

Both lettuce and spinach are cool-season leafy greens; lettuce grows best between 13 to 18ºC and can be grown either in the greenhouse or in field. Lettuce can withstand freezing temperatures (Jackson et al., 1996; LeStrange et al., 1996) and has been shown to survive temperatures as low as -2^{o}C, but growth is slowed (Mansour and Raab, 1996).

Vegetables are a large source of nitrates in the human diet (Maynard et al., 1976; Vogtmann et al., 1984), and leafy greens, like lettuce and spinach, can accumulate excess nitrates (Santamaria et al., 1999). Lettuce and spinach are considered nitrophilic (Vogtmann et al. 1984), where nitrogen (N) uptake can exceed the amount required for maximum growth (Grindlay, 1997).

Excess nitrates in the diet have been implicated in human methaemoglobinaemia and digestive tract cancers (Santamaria, 2006; Powlson et al., 2008). The European Commission (EC) has set limits on acceptable concentrations of nitrates in leafy greens, depending on crop, harvest time, and method of cultivation (Anon., 2005). No such regulations currently exist in the U.S.

New research shows that, at least in adults, nitrates may not be as harmful as previously thought. Some of the proposed beneficial effects of nitrate in the body are host defense, vasodilatation, and possibly anti-inflammatory (Lundberg et al., 2004). Nitrate and its reduced forms also play important roles in animal cell activity including several signaling pathways (Bryan et al., 2005).

TNC in leafy greens has been shown to respond to environmental changes and can vary with species and cultivar (Barker et al., 1971; Maynard et al., 1976). Environmental factors including short photoperiods (Maynard et al., 1976), low light intensity (Steingrover et al., 1986;

Behr and Wiebe, 1992; Amr and Hadidi, 2001), and temperature (Cantliffe, 1972) have been shown to affect TNC in leafy greens.

In addition to environmental influences, N fertilizer applied in excess of crop needs has been shown to potentially increase TNC (Barker et al., 1971; Barker, 1975; Maynard et al., 1976). In wheat (*Triticum aestivum*), increased nitrate concentration in nutrient media increased TNC in leaf blades and roots (Harper and Paulsen, 1968). Stopes et al. (1989) found that soluble N fertilizer significantly increased TNC when compared with compost. When comparing vegetables produced with different synthetic and organic fertilizer sources, vegetables grown in the absence of synthetic fertilizer had lower TNC than those grown with synthetic fertilizer (Woese et al., 1997; Guadagnin et al., 2005). TNC in lettuce has been shown to nearly double when using synthetic fertilizers compared to composted manure, and yield from compost treatments were significantly decreased only in the first crop season (Pavlou et al., 2007).

TNC has been shown to vary among species and among cultivars of the same species (Cantliffe, 1973; Maynard et al., 1976; Behr and Wiebe, 1992; Santamaria, 2006). However, in another study, TNC in 2 cultivars of spinach grown with organic fertilizer were not significantly different (Amr and Hadidi, 2001). In a similar study, there was no difference in TNC among 3 lettuce cultivars grown with both synthetic fertilizer and compost (Stopes et al., 1989).

Previous research has found cultivars within a single species to have variable TNC (Cantliffe, 1973; Maynard and Barker, 1974; Blom-Zandstra, 1989; Ott et al., 2008). Barker et al. (1974) evaluated 18 cultivars of spinach, and found significant differences in TNC that seemed to be correlated to leaf type. Olday et al. (1976) also found that when spinach cultivars were grown at different fertility levels, there was larger variability at high fertility levels.

TNC has been shown to have unequal distribution within plants. In leafy greens, TNC is highest in the petioles, with lower amounts in the roots, leaves, fruits and floral parts in that descending order (Maynard et al., 1976; Santamaria et al., 1999). Muramoto (1999) found petioles had higher TNC than leaf blades in spinach. Outer leaves and petioles tended to accumulate more nitrates than inner leaves and leaf blades; however, crop type and cultivar plays a significant role in nitrate accumulation (Ott et al., 2008). This research is a direct extension from previous research conducted by Ott et al. (2008).

The purpose of this study was to determine how fertility, crop type and cultivar, and plant part affect TNC in organically managed leafy greens.

Materials and Methods

Site preparation

This study was conducted at the Washington State University (WSU) Tukey Organic Research Farm (46^{o}43'39" N, 117^{o}10'55" W). The soil type is a Palouse silt loam (Fine-silty, mixed, superactive, mesic pachic ultic haploxeroll).

This study used a randomized complete block, split plot design with 4 replications. The 2 fertility treatments were: 1) fishmeal (8-3-0) applied at 168 kg N ha^{-1} and 2) a non-supplemented control. Both fertility treatments received a uniform application of compost. Two lettuce ('Cracoviensis' and 'Brown Golding') and 2 spinach ('Tyee' and 'Giant Winter') cultivars were used for this study. Crop types and cultivars were selected based on TNC data from previous research conducted previously at the WSU Organic Farm (Ott et al., 2008). In 2008, each plot was 1.2 m by 1.5 m long (1.86 m^2) with 4 rows spaced 30.5 cm apart. In 2009, plots were 1.2 m by 1.2 m long (1.44 m^2) with 5 rows spaced 25.4 cm apart.

In both years, plots were prepared using a tractor-driven spader, and compost was applied at an approximate rate of 56 kg N $ha^{-1}$and incorporated with a spader. In the fishmeal treatment, fishmeal was surface applied by hand and raked in at 5 cm several days before seeding.

Soil samples were taken with a 2-cm diameter soil probe at depths of 0 to 15 cm, 15 to 30 cm, and 30 to 60 cm before compost application. Separate samples were taken for each replication, with 2 soil cores bulked for each. Samples were sent to a commercial soil testing laboratory and analyzed for available soil moisture, pH, organic matter, NO_3-N, NH_4-N, S, P, and K.

Crops were hand seeded in all plots 3 days after the fishmeal application on May 13, 2008, and 2 days after the fishmeal application on May 13, 2009. Plants were hand thinned after emergence to 1 plant per spacing in both years. One line of drip tape irrigated each row in all plots with water being supplied as needed. Plots were irrigated about twice a week, with approximately 2.54 cm of water applied at each application. Weeds were controlled by hand removal.

Plant sampling

Plants were harvested whole when marketable size was reached. Five uniform plants were selected randomly from each plot. Plants were cut approximately 3 cm below the soil surface and placed in labeled plastic bags by plot. Plants were placed in a cooler for transportation from the field to the lab to minimize water loss. In the lab, plants were trimmed of roots and unmarketable leaves. If needed, plants were rinsed with deionized water and dried with paper towels. Fresh weight was recorded for each plant.

Three plants were randomly selected for whorl analysis. Whorls were designated as the outer 2 to 4 leaves and inner 2 to 4 leaves. The center was defined as all remaining leaves. Using a razor blade, each plant was separated into the outer whorl, inner whorl, and center. The remaining 2 plants were used for a petiole and leaf blade comparison. A razor blade was used to separate petioles from leaf blades of all the leaves and samples were bulked for each plot. Fresh weights of petioles and leaf blades were recorded for each plot. Each plant part was weighed, placed in labeled paper bags, and dried at 80^{o}C for at least 48 hours. Dry weights were recorded for each plot.

Tissue sample processing

Oven-dry plant tissue was finely ground using a mortar and pestle. To extract NO_3-N, 0.4 g of ground tissue was transferred to a 50 ml vial, to which 40 ml of 0.025 M $Al_2(SO_4)_3$ extracting solution was added. If 0.4 g was not available, then the maximum amount was added and the weight recorded. Vials were capped and shaken for 30 minutes. The solution was then filtered into glass filter tubes through a Whatman #2 filter paper. The extract was poured into clean 50 ml vials and refrigerated until analysis.

Nitrate concentration was measured using an Orion 720A plus TM Benchtop Ion Selective Nitrate Electrode (ISE) (Thermo Electron Corporation, Waltham, MA). The meter was calibrated using 3 NO_3-N standards at 1.4, 14 and 140 mg NO_3-N L^{-1}. Approximately 30 ml of room temperature tissue extract was poured into a beaker with a magnetic stir bar. The electrode was placed directly in the solution. The meter was checked for accuracy every 6 samples and recalibrated as needed.

TNC was calculated on a dry and fresh weight basis and plotted by treatment. Results are presented on a fresh weight basis only to facilitate comparison with other studies reported in the literature. Data were analyzed using the PROC MIXED procedure in SAS (SAS Inst., Cary, N.C.) at a significance level of 0.05. Main plots were cultivar and fertility, with plant part treated as a split plot.

Results

There was a significant 4-way interaction between year, fertility, cultivar and plant part. Therefore, the data were separated by year and re-analyzed (Table 1). In 2008, there were significant interactions between cultivar and plant part (Figure 1), fertility and cultivar (Figure 2), and fertility and plant part (Figure 3).

Although plant parts followed the general trend of decreased TNC from outer whorls to the center whorl, with high TNC found in petioles and low TNC found in leaf blades, the statistical separation among plant parts varied with cultivar (Figure 1). In spinach cultivars 'Tyee' and 'Giant Winter' there was no statistical difference among whorls, although petioles were significantly higher in TNC than leaf blades. 'Cracoviensis' lettuce was similar to both spinach cultivars. 'Brown Golding' lettuce was the only cultivar to have significant separation of whorls, with the center whorl being significantly lower in TNC than the outer and inner whorls. In both lettuce cultivars, TNC in petioles was also significantly higher than in leaf blades.

Fertility did not affect all cultivars the same way. Although it appeared that the addition of fishmeal increased TNC for all cultivars, 'Cracoviensis' lettuce was the only cultivar to experience a significant increase in TNC (Figure 2). In the compost only treatment, there was no difference in TNC between spinach cultivars and ‘Cracoviensis’; however, with the addition of fishmeal, ‘Cracoviensis’ lettuce was not significantly different from ‘Brown Golding’ lettuce.

Fertility also affected differences in TNC among plant parts. When grown with only compost, outer whorls and petioles had significantly higher TNC, while inner and center whorls and leaf blades had the lowest TNC (Figure 3). With the addition of fishmeal, petioles had significantly higher TNC and center whorls and leaf blades had the lowest TNC.

In 2009, there was a significant 3-way interaction between fertility, cultivar and plant part (Table 1). The data were partitioned by fertility treatment and analyzed for cultivar and plant part interactions (Figure 4). There was a significant cultivar by plant part interaction for both fertility levels. It appears that plant parts within cultivars did not follow the trend of TNC as closely as they did in 2008.

Soil analysis for soil N was similar for both years except nitrate (Table 2). In 2008, there was 107 kg NO_3-N ha^{-1} in the top 60 cm. In 2009, there was 34 kg NO_3-N ha^{-1} in the top 60 cm.

Table 1. ANOVA tables for fresh weight TNC for 2008 and 2009.

Effect	DF	2008 p-value	2009 p-value
Cultivar	3	<.0001	<.0001
Fertility	1	<.0001	<.0001
Cultivar*Fertility	3	0.0059	0.0163
Part	4	<.0001	<.0001
Cultivar*Part	12	<.0001	0.0018
Fertility*Part	4	<.0001	<.0001
Cultivar*Fertility*Part	12	0.0741	0.0006

Table 2. Preplant soil analysis in the top 60 cm for summer 2008 and 2009.

	Depth	NO_3-N (kg ha^{-1})	NH_4-N (kg ha^{-1})	pH	Organic matter (%)	P (mg kg^{-1})	K (mg kg^{-1})
2008	0-15	39	14	6	3	13	254
	15-30	33					
	30-60	35					
2009	0-15	14	17	7	4	26	369
	15-30	10					
	30-60	10					

Discussion

Although not always consistent, increased fertility generally increased TNC (Figures 2, 3, 4) similar to Ott et al. (2008). Fertility has previously been shown to affect TNC in lettuce and spinach (Barker 1975; Maynard et al., 1976; Stopes et al., 1989; Vogtmann et al., 1984; Muramoto, 1999), so this was expected. Also consistent with Ott et al. (2008) was the tendency for highest TNC to be found in petioles. Although not always statistically different, it was generally found that plant parts had decreasing TNC from outer whorls to center whorls, with lowest TNC in the leaf blades and highest in the petioles (Figures 1, 3, 4).

Genetic differences may determine much of the variability in TNC within and among leafy greens. Previous research has found that cultivars within a single species of both lettuce and spinach vary in TNC (Cantliffe, 1973; Maynard and Barker, 1974; Blom-Zandstra, 1989; Ott et al., 2008). Barker et al. (1974) found smooth-type cultivars had consistently lower TNC than savoy-type cultivars. The semi-savoy cultivars, which are produced by crossing savoy and smooth cultivars, had either high or low TNC. It may be that low TNC is a genetic trait and is heritable (Barker et al., 1974).

Both spinach cultivars ‘Giant Winter’ and ‘Tyee’ are semi-savoy and had similar TNC. ‘Brown Golding’ is a cos type lettuce and ‘Cracoviensis’ is semi-savoy lettuce. The differences between the lettuce cultivars were much greater than the differences between the spinach cultivars. ‘Brown Golding’ lettuce always had higher TNC; in this study it is possible that genetic factors have more influence than environmental factors or management practices. Olday et al. (1976) grew 2 spinach cultivars at low and high fertility levels. At low fertility, the 2 cultivars had similar TNC, but at high fertility 1 cultivar had 3 times the TNC of the other. Similarly, in this study when comparing lettuce cultivars, ‘Cracoviensis’ increased TNC much more than ‘Brown Golding’ at higher fertility (Figure 2).

Ott et al. (2008) found that when 2 crop types with 2 cultivars each were grown without supplemental fertilizer, there was no difference among crop types or cultivars and only petioles were significantly higher in TNC than other plant parts. However, when 1 cultivar of each crop type was grown with fertilizer, a plant part by cultivar interaction was observed. This is similar to our results, which suggests that higher fertility leads to increased separation in TNC among plant parts as well as higher overall TNC (Figure 4).

It was suggested by Ott et al. (2008) that the partitioning of TNC among plant parts was not only a function of genetic variation, but also fertility. We also found that this may be the case, as the plant part by fertility interaction suggests that the level of available N influences the distribution of TNC among plant parts (Figure 3). At increased fertility levels, there were greater differences between plant parts in TNC. Petioles always had high TNC, which is consistent with previous research (Maynard et al., 1976; Breimer, 1982; Muramoto, 1999; Ott et al., 2008).

All cultivars and plant parts had TNC that were well below EC regulations. For fresh lettuce and spinach grown in summer under field conditions, maximum permissible nitrate levels are 2500 mg NO_3-N kg^{-1} of fresh weight for both crops (Anon., 2005). Fertilizers from organic sources, like the fishmeal used in this study, have lower N availability than inorganic N (Gaskell and Smith, 2007) and could have contributed to this trend. Preplant soil nitrate levels did not appear to affect TNC in this study.

It appears from these results that TNC can be controlled through crop type and cultivar selection. 'Brown Golding' lettuce may have higher overall TNC than the other cultivars, and lettuce cultivars seemed to have generally higher TNC than spinach cultivars, particularly when fertilized. These results agree with previous research (Cantliffe, 1973; Maynard et al., 1976; Muramoto, 1999). It may be possible to keep TNC low by altering management practices based on the cultivars selected.

Conclusions

TNC can be controlled by crop type and cultivar selection, and fertilizer management. Nitrate consumption can also be controlled by which parts of the plants are consumed. There were complex interactions between experimental variables, suggesting an opportunity to select cultivars and/or fertility regimes to reduce TNC. Overall, nitrate accumulation was not a problem in this study as TNC was well below EC regulations.

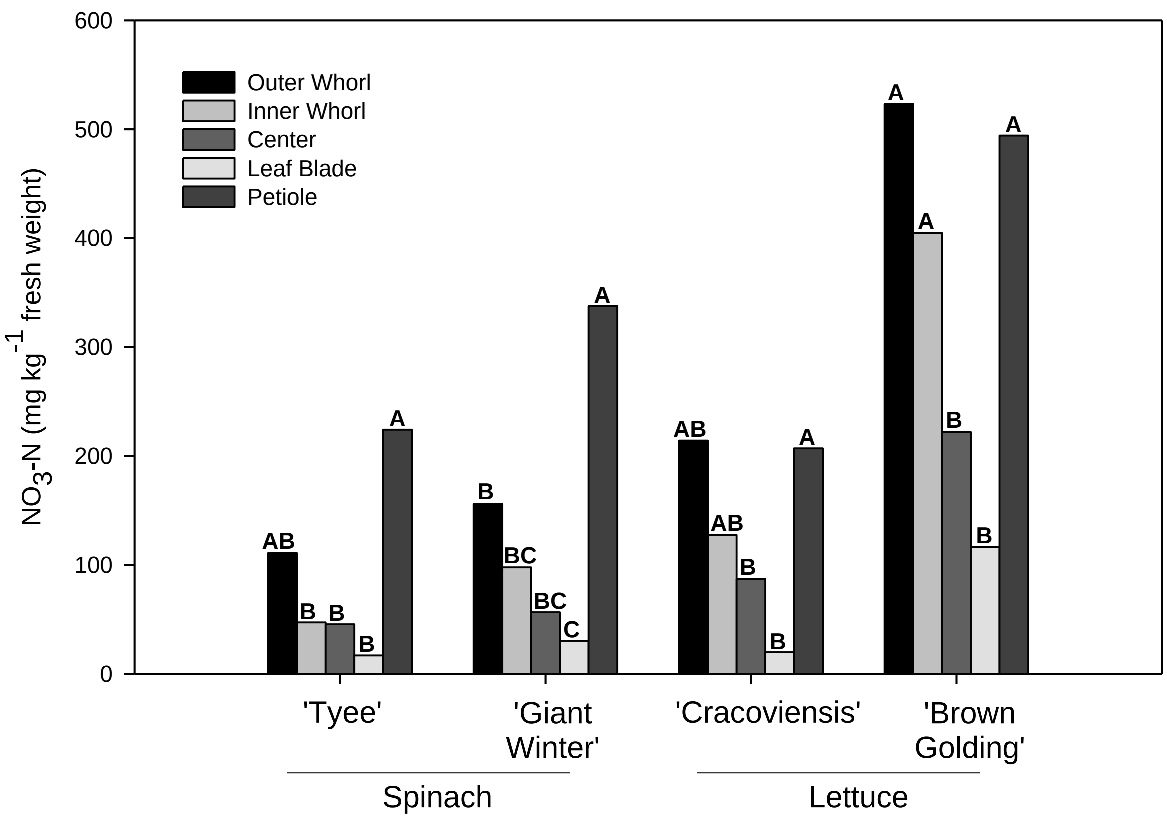

Figure 1. The effect of plant part and cultivar on TNC in 2008. Within a cultivar, plant part bars capped with the same letter not significantly different ($p < 0.05$).

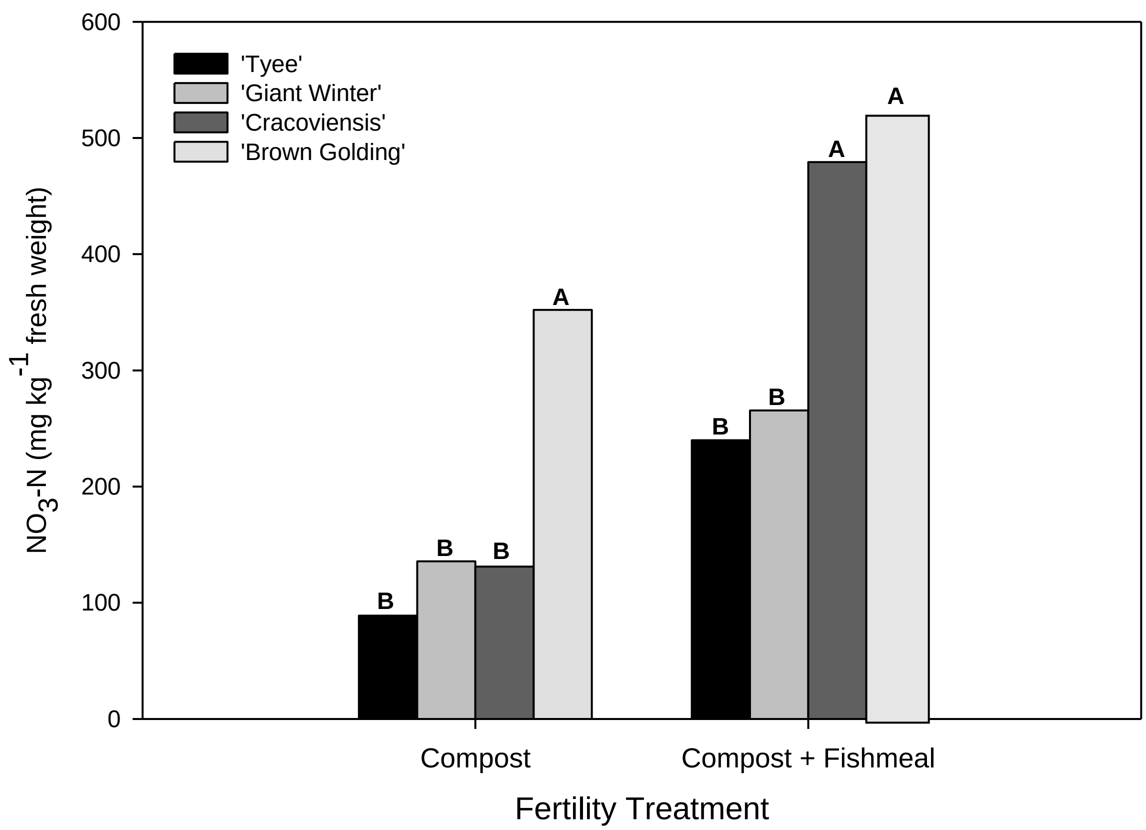

Figure 2. The effect of cultivar and fertility treatment on TNC in 2008. Within a fertility treatment, bars capped with the same letter not significantly different ($p < 0.05$).

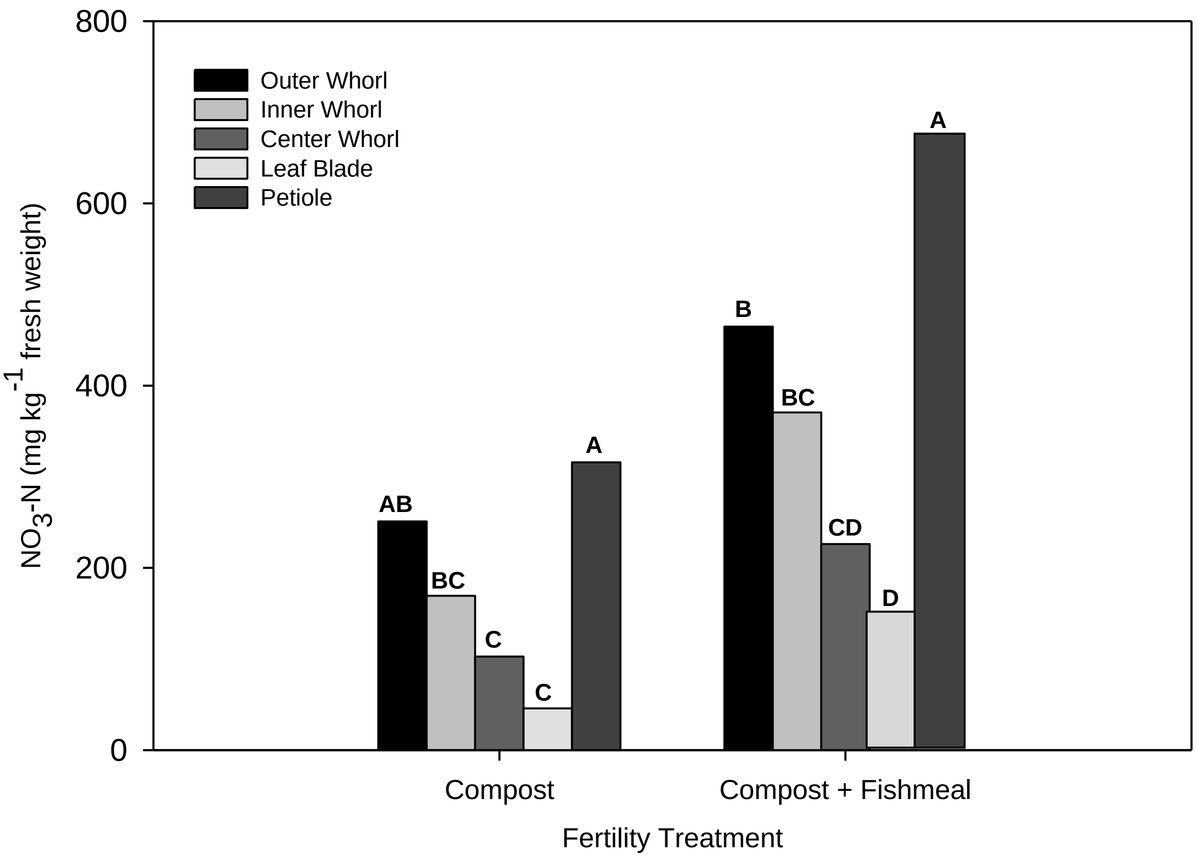

Figure 3. The effect of plant part and fertility treatment on TNC in 2008. Within a fertility treatment, bars capped with the same letter are not significantly different ($p < 0.05$).

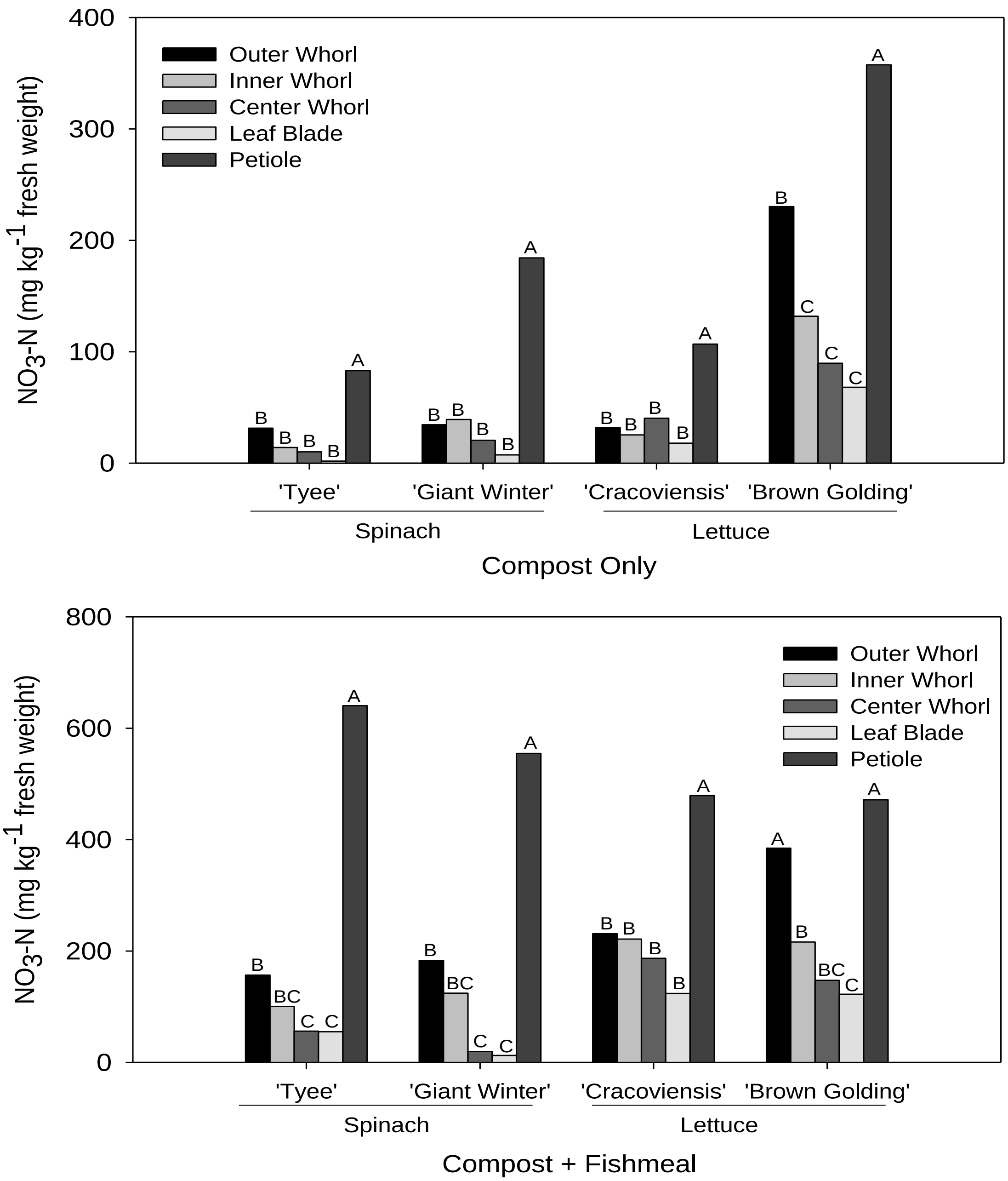

Figure 4. Analysis of the significant 3-way interaction between fertility, cultivar, and plant part in 2009 (p-value = 0.0495). Within a fertility treatment and cultivar, bars capped with the same letter are not significantly different ($p < 0.05$).

Literature Cited

Amr, A. and N. Hadidi. 2001. Effect of cultivar and harvest date of nitrate (NO_3) and nitrite (NO_2) content of selected vegetables grown under open field and greenhouse conditions in Jordan. J. Food Composition Analysis 14:59-67.

Barker, A.V., N.H. Peck, and G.E. MacDonald. 1971. Nitrate accumulation in vegetables. I. Spinach grown in upland soils. Agron. J. 63:126-129.

Barker, A.V., D.N. Maynard, and H.A. Mills. 1974. Variations in nitrate accumulation among spinach cultivars. J. Amer. Soc. Hort. Sci. 99:132-134.

Barker, A.V. 1975. Organic vs. inorganic nutrition and horticultural crop quality. HortScience 10:50-53.

Behr, U. and H.-J. Wiebe. 1992. Relation between photosynthesis and nitrate content of lettuce cultivars. Sci. Hort. 49:175-179.

Bryan, N.S., B.O. Fernandez, S.M. Bauer, M.F. Garcia-Saura, A.B. Milsom, T. Rassaf, R.E. Maloney, A. Bharti, J. Rodriquez, and M. Feelisch. 2005. Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. Nature Chem. Biol. 1:290-297.

Cantliffe, D.J. 1972. Nitrate accumulation in spinach grown at different temperatures. J. Amer. Soc. Hort. Sci. 97:674-676.

Cantliffe, D.J., 1973. Nitrate accumulation in spinach cultivars and plant introductions. Can. J. Plant Sci. 53:365-367.

Grindlay, D.J.C. 1997. Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. J. Agr. Sci. 128:377-3986.

Guadagnin, S.G., S. Rath, and F.G.R. Reyes. 2005. Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. Food Add. Contam. 22:1203-1208.

Harper, J.E. and G.M. Paulsen. 1968. Influence of intensity, quality, and duration of light on nitrogen reduction and assimilation in wheat. Crop Sci. 8:537-539.

Jackson, L., K. Mayberry, F. Laemmlen, S. Koike, K. Schulbach, and W. Chaney. 1996. Leaf lettuce production in California. Univ. of California, Davis. Veg. Res. Info. Ctr: Veg. Prod. Ser. Pub. 7216.

LeStrange, M., S. Koike, J. Valencia, and W. Chaney. 1996. Spinach production in California. Univ. of California, Davis. Veg. Res. Info. Ctr: Veg. Prod. Ser. Pub. 7212.

Mansour, N.S. and C.A. Raab. April 1996. Grow your own lettuce, spinach and Swiss chard. Oregon State Univ. Ext. Serv. EC1268.

Maynard, D.N. and A.V. Barker. 1974. Nitrate accumulation in spinach as influenced by leaf type. J. Amer. Soc. Hort. Sci. 99:135-138.

Maynard, D.N., A.V. Barker, P.L. Minotti, and N.H. Peck. 1976. Nitrate accumulation in vegetables. Adv. Agron. 28:71-118.

Muramoto, J. 1999. Comparison of nitrate content in leafy vegetables from organic and conventional farms in California. Ctr. Agroecol. Sustain. Food Sys. Univ. of California, Santa Cruz. 20 Mar. 2010. <http://envs.ucsc.edu/shennan/Joji/leafnitrate-1.pdf>.

Olday, F.C., A.V. Barker, and D.N. Maynard. 1976. A physiological basis for different patterns of nitrate accumulation in two spinach cultivars. J. Amer. Soc. Hort. Sci. 101:217-219.

Ott, K.A. 2007. Impacts of winter growing conditions on yield and nitrate accumulation in organically produced leafy greens. MS thesis, Washington State Univ., Pullman, WA.

Ott, K.A., R. Koenig, and C. Miles. 2008. Influence of plant part on nitrate concentration in lettuce and spinach. Int. J. Veg. Sci. 14:351-361.

Pavlou, G.C., C.D. Ehaliotis, V.A. Kavvadias. 2007. Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. Sci. Hort. 111:319-325.

Powlson, D.S., T.M. Addiscott, N. Benjamin, K.G. Cassman, T.M. de Kok, H. van Grinsven, J.-L. L'hirondel, A.A. Avery, and C. van Kessel. 2008. When does nitrate become a risk for humans? J. Environ. Qual. 37:291-295.

Santamaria, P. 2006. Review: nitrate in vegetables: toxicity, content, intake and EC regulation. J. Sci. Food Agr. 86:10-17.

Santamaria, P., A. Elia, F. Serio, and E. Todaro. 1999. A survey of nitrate and oxalate content in fresh vegetables. J. Sci. Food. Agr. 79:1882-1888.

Steingrover, E., P. Ratering and J. Siesling. 1986. Daily change in uptake, reduction and storage of nitrate in spinach grown at low light intensity. Physiol. Plant. 66:550-556.

Stopes, C., L. Woodward, G. Forde, and H. Vogtmann. 1989. Effects of composted FYM and a compounds fertiliser on yield and nitrate accumulation in three summer lettuce cultivars grown in an organic system. Ag. Eco. Environ. 27:555-559.

U.S. Department of Agriculture. 2008. Vegetables 2007 Summary. U.S. Department of Agriculture National Agricultural Statistics Service. Vg 1-2 (08). 18 Mar. 2010

<http://usda.mannlib.cornell.edu/usda/nass/VegeSumm//2000s/2008/VegeSumm-01-25-2008.pdf>.

Vogtmann, H., A.T. Temperli, U. Künsch, M. Eichenberger and P. Ott. 1984. Accumulation of nitrates in leafy vegetables grown under contrasting agricultural systems. Biol. Agr. Hort. 2:51-68.

Chapter 5

Summary, Conclusion, and Recommendations

The management practices and environmental factors we set out to investigate included photoperiod, planting and harvest date combinations in a cut-and-come-again winter production system, plant part, crop types and cultivars, and fertilize application rates. Three studies were designed to evaluate the effects of these factors and practices.

The only factor that did not appear to consistently influence tissue nitrate concentration (TNC) was photoperiod. Lettuce and spinach crops grown at 2 latitudes (46^oN and 64^oN) did not follow the expected pattern of decreasing TNC during the day and increasing TNC at night. It is possible that an additional factor that was not accounted for masked any photoperiod influence since field conditions are more variable that those in controlled environments. There were differences between the sites themselves, although a 3-way statistical interaction between site, crop type, and harvest date was observed. In general, crop types grown at 64^oN had higher TNC than the same crop type at the lower latitude. Additionally, lettuce generally had higher TNC that spinach.

We found it is possible to direct seed leafy greens into a hoophouse without supplementary heat or light over the winter season and produce a crop for harvest. Our results suggest that by using a hoophouse, it is possible to grow leafy greens year round in Eastern Washington. We evaluated an Asian greens mix for TNC and yield, and found that planting date, harvest date, and crop type influenced both. Several varieties were relatively high yielding in a repeat harvest system. However, not all crop types responded well to repeat harvesting, and there was evidence that, while crop types like arugula yielded higher with later harvests, tatsoi did not recover as well and yielded poorly with later harvests. By repeat harvesting instead of re-seeding, it may be possible to increase profits if the appropriate crop types are selected. In spite of some production difficulties, we may have identified another source of income for local growers and the community may have access to fresh, locally grown greens during the winter months. TNC was low in all crop types, suggesting that in spite of previous research, it is possible to grow low TNC leafy greens during the winter.

In a summer study, we suggest that TNC can be controlled by crop type and cultivar selection and removing petioles. However, there were complex interactions between these experimental factors that exclude any one factor from accurate TNC predictions. Trends indicate that 'Brown Golding' lettuce had higher TNC than the other lettuce cultivar or either spinach cultivar. However, there were many interacting factors, indicating that there is not a single factor that can accurately predict the occurrence of low TNC. Many factors should be taken into consideration when concerned about TNC.

Nitrate accumulation was not a problem in any study, as TNC was always below European Commission (EC) regulations (Anon., 2005). Although the U.S. does not have any such regulations on TNC in leafy greens, the EC regulations were used for comparison. It would be interesting to investigate if the low TNC observed in these studies was caused by the use of fertilizer from organic sources or the growing environment of the study site in Pullman, WA.

We conclude that through management practices like cultivar selection and fertility, TNC in leafy green crops can be managed. However, it also appears that environmental factors also play a role and are more difficult to anticipate or manage in unlit, unheated hoophouse systems, or in the field.

Future Recommendations

Based on these results, the most important area for further research would be identifying leafy green crop types that are high yielding in winter growing conditions, and that tolerate repeat harvesting. TNC should continue to be investigated, given that these results are not robust enough to conclude that TNC is always low in these conditions. Also, an economic analysis would be valuable for growers who are interested in this system.