

THESIS

ORGANIC FERTILIZER COMPARISON ON KALE (*Brassica* spp.) VARIETAL GROWTH
AND NUTRIENT CONTENT

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ABSTRACT

ORGANIC FERTILIZER COMPARISON ON KALE (*Brassica* spp.) VARIETAL GROWTH AND NUTRIENT CONTENT

Selecting supplemental N fertilizer for use on certified organic farms can be difficult and confusing. There are many options commercially available to farmers with similar N concentrations but widely different ingredients. Field experiments on three farms in Fort Collins, CO were conducted to evaluate the impact of a few commonly-used organic fertilizers on kale yield and nutrient concentrations. This study includes a fertilizer under development which is produced on-farm utilizing N-fixing cyanobacteria; this cyanobacterial bio-fertilizer may be a viable choice for farmers in the near future. The three fertilizer treatments (hydrolyzed fish, alfalfa meal and liquid cyanobacteria) were applied at rates calculated by subtracting soil nitrate-N concentration from a target 50 mg/kg. Cyanobacteria and hydrolyzed fish were applied in liquid form while alfalfa was incorporated dry into the soil pre-planting. Biweekly measurements of plant height and chlorophyll content were taken on three varieties of kale, Dinosaur, Red Russian, and Winterbor. Leaf weight, leaf area, N, Fe and Zn concentrations were measured during four monthly harvests. Organized in a split-plot experimental design, each farm had 3 treatment replications with subplots of different kale varieties. No significant effects were found on plant height, leaf weight, leaf area, N, Fe or Zn concentrations among fertilizer types. There were varietal differences in plant height, leaf area, and general performance as well as resistance to pest pressure. Residual N in each subplot was measured after this study and showed significant difference among varieties. Kale variety choice seems to have a much larger impact

on yield and nutrient concentrations than fertilizer choice, as long as fertilizers are applied at similar N rates.

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INTRODUCTION

Nitrogen (N), considered a limiting nutrient in all agricultural systems, is especially difficult to manage in certified organic vegetable crop production. Organic agriculture relies heavily on fertilizers and soil amendments from off-farm sources such as fish emulsion, blood meal, feather meal, alfalfa meal, compost, and animal manures (Gaskell et al., 2006) for soil fertility and crop nutrients. So common is the use of these supplemental fertilizers that the marketplace is now inundated with variations of these organic N options and there is little research comparing their true impacts on plant growth and quality (Hartz and Johnstone, 2006). The list of brands and small differences between N contents can be confusing to a grower. Examples of common organic fertilizers and corresponding fertilizer grades are given in Table 1.

There are 2 main classes of N fertilizers, solid and liquid. Solid fertilizers are often incorporated into the soil before planting, liquid is generally applied post planting and is frequently applied season-long through irrigation. All of these organic materials are rich in slow-releasing organic N and the rate of mineralization make it difficult to predict when planning to meet crop uptake needs. In a 2006 study by Hartz and Johnstone, fish powder, blood meal and feather meal were all found to have very high levels of organic N (93%-99% of total N was in organic form). These fertilizer types and their application methods may provide N at different rates because they rely on soil microbes to convert organic N into inorganic N forms such as ammonium (NH_4^+) and nitrate (NO_3^-) prior to plant uptake (Gaskell and Smith, 2007). This difficult to control factor leaves organic fertilizers less predictable in the field, and while there is an abundance of literature on manure and compost use in the field, little has been done

comparing supplemental N fertilizers. In this study, a few organic fertilizers commonly used in northern Colorado were compared for their impact on plant yield and nutrient content.

While all of the mentioned organic fertilizers are commercially available, many of them are produced off-farm and distributed widely throughout the United States. Fish emulsions are produced as a byproduct of the seafood industry and often traded internationally (Soares et al., 1973). The fish hydrolysate used in this study (Neptune's Harvest) is produced in Massachusetts and shipped to all states in a concentrated liquid form (it journeyed approx. 1,900 miles to the location of this study). Because these liquid fish fertilizers are affordable, relatively fast acting, and easy to apply, these characteristics encourage farmers to rely on outside sources for the additional N needed during the growing season and to pay the extra cost of shipping heavy liquids around the U.S. However, there are new fertilizer options being developed that allow farmers to produce N on-farm. This research includes one such fertilizer, cyanobacteria based, to compare with other off-farm sources.

Cyanobacteria (blue-green algae) was documented as a source of N fertilizer in rice paddies as early as 1973 (Roger, 1980) but was presumably present and feeding N to the paddies for centuries before. For this study the nitrogen-fixing cyanobacteria (*Anabaena cylindrica*) was cultured from local soils and inoculated into nutrient-supplemented raceways and as the cyanobacteria populations grew so too did the content of N in the water as the bacteria fixed the N₂ gas from the air and assimilated it into their cells (Barminski, 2014). This on-farm liquid cyanobacteria fertilizer (Lcyb) cuts out the costs and risks associated with relying on off-farm sources, and by using a biological process outside of the soil, the farmer has better control of application timing and N rates. A greenhouse study by Sukor (2013) found N availability in solid cyanobacteria to be 6% greater than compost but 9% less available than fish emulsion when

used on sandy soils. Sukor (2013) also found the use of Lcyb to increase lettuce yields by 58% when compared to composted manure. Work is currently being done to improve N fixation efficiency and N availability of cyanobacteria fertilizers.

Kale, a member of the *Brassica* family, is one of the most widely consumed vegetables in the United States along with others in the species *oleracea* (cauliflower, broccoli, cabbage) (Thomson et al., 2007). In 1993 Dangler and Wood found optimal yield increases in *Brassica oleracea* varieties at N rates of 112 kg/ha when grown in Norfolk-Orangeburg loamy sand. These rates produced yields of 9.9-14.9 t/ha depending on in-row spacing. These cruciferous vegetables are often high in iron (Fe) and zinc (Zn), micronutrients important in reducing oxidative stress which is highly correlated with chronic diseases (Tomey et al., 2007). These micronutrients also act as cofactors for antioxidant enzymes long known for their importance in human health. Zn plays an important role in blood coagulation and in protecting DNA from modifications, thus decreasing the risk of cancer (Messias et al, 2014). Zn has also been found to increase plant tolerance to adverse environments (Cakmak, 2008). While there are many studies focusing on Zn composition in cereal grains, there is a growing interest in leafy greens that can contain greater Zn concentrations and can provide a substantial dietary source of Zn (Broadley, 2010). Iron deficiency causes anemia and is the most common nutritional disorder worldwide, affecting more than 2 billion people across the globe (WHO, 2000). The accumulation of these micronutrients in edible plant tissue can vary based on the type of fertilizer fed to a plant (Cakmak, 2008). Nitrogen fertilizers have shown positive impacts on a plants ability to accumulate Fe and Zn; increased N rates can increase Fe and Zn concentration in leaf tissue (Aciksoz et al, 2011). Perhaps we can choose fertilizers that knowingly increase Fe and Zn

concentrations for health benefits. This study was conducted to evaluate these fertilizer effects on nutrient content.

The objective of this study was to compare the effects of different organic fertilizers on yield and nutrient content of three varieties of kale. Kale (*Brassica oleracea* & *Brassica napus*) was chosen for its long growing season (frost tolerance) and continuous need for a steady supply of N. The execution of this study included development of Lcyb fertilizer and application method, measurement of plant growth, and analysis of mineral nutrients in the plant tissue including N, Fe, and Zn.

MATERIALS AND METHODS

Sites

Field experiments were carried out at three different sites: the Colorado State University Horticulture Research Center (HRC), Spring Kite Farm (SK), and Native Hill farm (NH).

HRC (4300 E. County Road 50, Fort Collins, CO 80524) is part of Colorado State University and has 3.2 hectares of certified organic land with sandy clay soils which were classified in 1980 as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980). The site also includes 6 high tunnels, 9.1 m by 15.2 m, that are metal framed with insect screen covering and greenhouse plastic roll-up doors. The HRC experiment was carried out in 3 adjacent tunnels which are referred to in this study as high tunnel 4, 5, and 6 (replicates). Soil samples were collected to a depth of 30.5 cm from each tunnel on November 2nd, 2012 and analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University (Table 2).

Spring Kite Farm (3000 South Taft Hill Rd. Fort Collins, CO 80526) and Native Hill Farm (2100 County Road 54G Fort Collins, CO 80524) are both non-certified farms using organic practices in Fort Collins, CO with clay loam soils, SK of the Altvan-Satanta series and NH of the Caruso series (NRCS, 2014). Soil samples were collected from NH and SK research plots on May 21st, 2013 and all were analyzed by the Soil, Water, and Plant Testing Laboratory at Colorado State University (Table 2).

Kale Varieties

Organic Winterbor (F1 Hybrid), Red Russian, and Dinosaur (also known as Toscano or Lacinato) varieties of kale (*Brassica oleracea*) seeds were purchased from Johnny's Selected Seeds, Albany, ME in 2013.

Transplant Production

All seeds were started at W.D Holley Plant Environmental Research Center's (PERC) greenhouse facility on the CSU campus using Sunshine Organic potting soil and 128 cell flats. Dinosaur kale was seeded on April 26th, and Winterbor and Red Russian were seeded on May 3rd, 2013. All flats were placed on the misting benches and remained there until being transplanted on May 23rd, 2013 to the HRC and May 30th, 2013 at SK and NH.

Experimental Design

HRC: Each high tunnel was a replication containing whole plots of 3 fertilizer treatments and split plots of 3 kale varieties. Each split plot contained 18 plants, and each whole plot contained 3 varieties of 18 plants. The kale was planted in 2 rows per bed with an in-row spacing of 35 cm and a between row spacing of 30 cm.

SK & NH: Each farm was treated as a replicate and contained one whole plot of 3 fertilizer treatments and 1 control and split plots of 3 kale varieties. The control plot was irrigated with the other plots but was treated with no fertilizer during the growing season. Each split plot also contained 18 plants and each whole plot contained 3 varieties of 18 plants. The kale was planted in 2 rows per bed with an in-row spacing of 35 cm and a between row spacing of 30 cm.

Bed Preparation

HRC: Three 0.6 m x 12.2 m beds were hand cultivated 30.5 cm deep using a 50 cm wide broadfork in each of three high tunnels, nine beds in all. In each high tunnel the three beds represented replicates of a different treatment. Two lines of drip tape were stretched across each bed spaced 27.9 cm apart with a double header in each tunnel. The drip tape used was John Deere 15 mil, 20.3 cm spaced emitter, 2.5 Liters per minute per 30.5.

SK & NH: Bed preparation was performed by the farmers using cultivating tractor pulled tools. Each bed was approximately 0.6 m wide and 37 m long. Each farm ran two lines of drip tape (same drip tape used on all farms) down the length of the beds.

Fertilizer Treatments

The fertilizers compared in this study were alfalfa meal (2.5% N), Neptune's Harvest Hydrolyzed Fish Fertilizer (2-4-1), and liquid cyanobacteria (.0016% N). Fertilizers were applied at rates based on soil tests, and a target soil NO₃-N concentration of 50 mg/kg (100 lbs/acre) for a 15.2 cm depth. Samples of all fertilizers were sent to the Soil, Water, and Plant Testing Laboratory at CSU and analyzed for N, P, K, Ca, Mg, Fe, and Zn as most of these were the nutrients examined in leaf tissue (Table 3). Both the fish fertilizer and cyanobacteria were in liquid form, while the alfalfa meal was a dry powder and was incorporated into the soil before planting. It was applied by even dispersal over the alfalfa treatment rows by hand and incorporated into the top 15.2 cm using a hoe. The application quantity of dried alfalfa to each whole plot can be seen in Table 4. Liquid cyanobacteria was produced using methods described by Barminski, 2014.

Irrigation & Fertigation

Irrigation at HRC was applied three times weekly, adjusted for rainfall, and cut off the night before any liquid fertilizer treatments. Cyanobacteria were pumped directly from production raceways into the drip irrigation system using a sump pump. Fish fertilizer was mixed into watering troughs and applied directly into the drip irrigation using the same pump after being flushed with city water. Each treatment row had individual control valves allowing for uniform application to all treatment plots in all tunnels.

SPRING KITE FARM AND NATIVE HILL were irrigated on schedule with the farmers' other crops. Cyanobacteria and fish were applied by hand with watering cans. The cans were drained into the plot avoiding leaf contact at a rate that would allow for little run off.

Cultural Practices

Weeding was done by hand throughout the season. At HRC there were two applications of Hi-Yield Thuricide Concentrate (*Bacillus thuringiensis*) used to control cabbage worms. NH's site contained a threatening amount of flea beetles and an application of Captain Jack's Deadbug Brew Concentrate (Spinosad) was used once during the growing season.

Plant Height & SPAD

Weekly data was collected on plant height and SPAD (Special Products Analysis Division leaf chlorophyll measurement). SPAD measurements were collected using FieldScout

GreenIndex+, a commercially available app for the iPhone. It uses a calibrated photo board and Dark Green Color Index (DGCI) numbers from photographed pixels to estimate SPAD. Photos were taken weekly between 10am-2pm.

Harvests

There were 4 harvests during the growing season:

HRC: July 9th, August 13th, September 16, and October 14

SK & NH :July 23rd, August 20th, September 20th, and Oct 18th

During each harvest, 2 healthy, marketable, full sized leaves with petioles were removed from 10 predetermined, representative plants in the center of each subplot. Same day measurements were taken for weight, in grams, of all 20 leaves. The leaves were then measured for average leaf area using a LI-COR LI-3100 Area Meter. Ten leaves from each subplot were then dehydrated at the Agriculture Research Development & Education Center (ARDEC) in an oven set to 70 degrees Celsius for 72 hours. These dried samples were then moved to Colorado State University Greenhouses and ground in a plant grinder. Ground samples were sent to Ward Laboratories (Kearney, NE) and analyzed for N, Fe, and Zn concentration. Total N was measured using methods described in publication #A3769 from the University of Wisconsin, Recommended Methods of Manure Analysis (Combs et al, 2003). N, Fe and Zn was digested using Johnson and Ulrich's 'Analytical methods for use in plant analysis' (Johnson and Ulrich, 1959).

Post-Harvest

After the final harvest, all sub-plots were soil sampled to a 15.2 cm depth and dried for 2 weeks in a walk-in cooler at HRC to reduce NH₄ volatilization. Once dried, inorganic N was

extracted using 2 M KCL at a 1:10 ratio (2 g of soil in 20mL of 2M KCl). The samples were then filtered and sent to EcoCore Analytical Services at CSU and run through the Alpkem autoanalyzer for NH₄-N and NO₃-N content.

Statistical Analysis

All data was processed using SAS 9.3. Since the sites varied in growing environment (high tunnels vs open field) and Lcyb sources SK and NH were analyzed together, HRC was analyzed separate. All p-values were found using proc mixed and statistical differences within interactions were found using the slice statement.

RESULTS

Plant height

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Plant height showed no significant differences among fertilizer treatments ($P=.8807$) at the HRC. Significant differences were found among varieties ($P=.0019$) and among measurement dates ($P<.0001$). There was also a significant interaction between variety and harvest dates ($P<.0001$). This interaction could be explained by a difference in growth patterns of both the stems and leaves among varieties (Figure 1).

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There were no significant effects of treatment ($P=.7765$) or variety ($P=.2229$) on plant height. The trend for all varieties in all treatments was that of a gradual seasonal growth, increasing through the 3rd of October with a small drop off after seasonal freezing began. Kale plants at SK & NH were overall shorter than those at HRC (Figure 2).

SPAD index

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There was also no significant difference among fertilizer treatments in SPAD chlorophyll measurements ($P=.7635$). However, there was a significance among varieties ($P=.0018$), days after planting ($P<.0001$), and a significant interaction between these two variables ($P=.0083$) (Figure 3).

SPRING KITE FARM AND NATIVE HILL

At SK and NH, SPAD readings showed no statistical significance with regard to treatment ($P=.9673$) or variety ($P=.4495$). However, this field-grown kale (as opposed to the high tunnel kale at HRC) did reach higher SPAD values perhaps due to increased light intensity. Dark green Dinosaur kale was again above the other varieties, and Red Russian had the lowest SPAD readings. Both Red Russian and Winterbor experienced a steep decline in SPAD after day 98 not observable to the naked eye.

Leaf Weight

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Individual leaf weight showed no significant difference among fertilizer treatments ($P=.5860$). However, significant difference in leaf weight was found among varieties ($P=.0003$) and harvests ($P<.0001$). The harvest weights were significantly different at different growth stages at each harvest. Therefore the only significant effect of interest is within varieties (Figure 5). Dinosaur kale has a smaller, narrower leaf structure that probably accounts for the lower leaf weight of that variety.

SPRING KITE FARM AND NATIVE HILL

Leaf weight at SK and NH also showed no statistical significance among treatments ($P=.512$) but did have significant effects for variety ($P=.0092$) and harvest ($P=<.0001$). Dinosaur's leaf weight remains lower due to its narrow leaf morphology (Figure 6). Harvest 2 was the heaviest harvest with all varieties declining in subsequent harvest. Leaf weight of Red Russian was lower in Harvest 4 at SK and NH than at HRC, probably due to the protective nature of the high tunnels.

Leaf Area

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Leaf area only showed significant differences by variety ($P < .0001$). Treatment ($P = .4383$) and harvest date ($P = .1364$) were not significant. This is explained by leaf morphology (Figure 7). Red Russian and Dinosaur both have flat, easy to scan surfaces. Winterbor's frilled texture made it difficult to scan a 2D image of the total surface area, leading to lower leaf area measurements.

SPRING KITE FARM AND NATIVE HILL

Leaf area was statistically significant by harvest ($P < .0001$), variety ($P = .0006$), and variety by harvest ($P = .0108$). Harvest 2, as was seen in leaf weight, was significantly higher in leaf area than other harvest dates, while Red Russian and Winterbor were both much smaller at Harvest 4 (Figure 8). Total SK and NH leaf area was much lower than HRC leaf area. This was easily visible in the field and could be related to the insect screened high tunnels at HRC as the open field had visibly larger quantities of aphids and flea beetles. The tunnels also moderated day and night temperatures, lowered light intensity, and kept soil moisture higher than in open field conditions at SK and NH.

Leaf Nitrogen

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An interaction between kale variety and harvest date ($P = .0200$) was observed in leaf N concentration (Figure 9). There was no significant difference among fertilizers ($p = .7276$). Since all seedlings were started in the same soil and conditions, it takes a while for their new

environment to be reflected in tissue analysis, thus significant effects were not seen until Harvests 2 and 3, with Red Russian containing significantly higher N concentrations than the other varieties. These differences leveled out toward the end of the growing season but still showed significant differences among varieties.

SPRING KITE FARM AND NATIVE HILL

On the private farms, N content of the leaves was also found to be significantly different by variety ($P = .0069$), harvest ($P = <.0001$), and the interaction of the two ($P = .0069$), though it was not significant by treatment ($P = .4274$). Leaf N was not as uniform during the first harvest as was seen at HRC, and the general N content of kale at these research sites were lower (Figure 10). HRC's average N was 3.63% while SK and NH had an average of 3.22% N. The average initial soil $\text{NO}_3\text{-N}$ and organic matter concentrations were lower at SK and NH than at HRC which could explain this difference. Red Russian was not much greater in leaf N than the other varieties at these sites when compared to HRC.

Iron

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Iron and zinc were only analyzed on Dinosaur kale due to funding constraints. Therefore, no variety effects were detected. There was no significant difference in leaf Fe among fertilizer treatments ($P = .1412$). The only significant difference observed in Fe concentrations was among harvests ($P < .0001$). However, if treatments are analyzed by harvest, Lcyb has a significantly higher Fe concentration than alfalfa within harvest 1 ($p = .0007$). Interestingly, Fe concentration decreased dramatically over the growing season (Figure 11).

SPRING KITE FARM AND NATIVE HILL

There was no statistical significance among treatments in Fe content ($P=.2993$). There was only a significant difference among harvest dates ($P<.0001$). SK and NH showed the same steep decline in Fe content as was seen at HRC, but when compared to the control it is clear that this trend was related to plant growth patterns or other factors experienced by all variety and treatment group since control showed the same trend as the treatments (Figure 12).

Zinc

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There were also no significant effects among fertilizer treatments ($P=.8177$) or harvests ($P=.6812$) on leaf Zn (Figure 13).

SPRING KITE FARM & NATIVE HILL

Likewise there was no statistical significance among treatments ($P=.8909$) with regard to Zn concentration at SK and NH. Harvest was significant ($P=.0206$) with an increase in Zn concentration occurring after Harvest 2 and remained at that level for the final harvest. Zn content at SK & NH was generally lower than HRC (Figure 14).

Residual Soil N

HORTICULTURE RESEARCH CENTER, SK & NH

All plots were analyzed for residual soil $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$. There was no statistical significance among treatments with regard to $\text{NO}_3\text{-N}$ ($P=.7227$), but there was a significant difference in $\text{NO}_3\text{-N}$ levels among varieties at all farms ($P<.0001$). The Red Russian variety left

a larger residual level of $\text{NO}_3\text{-N}$ in the soil after a full season of growth (Figure 15). Residual $\text{NH}_4\text{-N}$ was not significantly affected by treatment ($P=.4856$) or variety ($P=.5154$) (Figure 16). Red Russian did not show the same trend in residual $\text{NH}_4\text{-N}$ as for $\text{NO}_3\text{-N}$ (Figure 16).

DISCUSSION

Plant height

Winterbor is an F1 hybrid which might explain its continued growth later in the season. At HRC however, Dinosaur continued to grow taller than the other varieties. The plant structure of Dinosaur, like Winterbor, is very upright with a strong vertical central stock but unlike Winterbor with its horizontal leaves, Dinosaur's leaves tend to grow vertically making them significantly taller appearing in the field. Red Russian, a low plant with extreme hydro-heading (branching at the base of the stem) is a lower plant which also received noticeable aphid damage mid-season which may have contributed to further reduced height. At SK and NH there were no treatment differences including the control. This could be due to the initial soil NO₃-N and OM concentrations from regular compost application practiced on all farms in the years prior to this study. This soil NO₃-N and OM (estimated to contribute 34 kg/ha N per percent) would supply all kale treatments with sufficient nutrients needed for healthy plant growth and make it difficult to see differences among fertilizer treatments.

SPAD

Nitrogen is an important element needed for photosynthesis in plant cells and is thus correlated to SPAD, a measurement of chlorophyll content in leaves. While the chlorophyll content tended to increase as the season progressed and the plant biomass increased, SPAD results show drastic varietal differences. A study performed in Brazil in 2008 using a chroma meter for non-destructive SPAD index readings on kale varieties found the results to be more useful when transformed. Using a Minolta CR-400 which measures color using L, C, and h^o the

transformed model that accounts for varietal difference is $h^o/(LxC)$ (Amarante, 2008).

Unfortunately their research was performed on common Brazilian varieties and not on those used in this study. The tool used in this study could perhaps be used in transformed models but is not useful as-is on varieties of kale that differ dramatically in leaf color. However, since all treatments were compared using each individual variety, i.e. comparing all treatments among the Dinosaur plots, then the results pertaining to treatment are still useful for comparing treatment effects. Thus the study found no difference among treatments but the difference found among varieties was unknown in usefulness or accuracy when measured with this tool. SPAD, however, does correspond with N concentration (Fig 3 and 9, Fig 4 and 10) perhaps suggesting that it is still useful for all varieties in measuring this correlation. Lastly, when looking at individual varieties at SK and NH SPAD decreased in Red Russian and Winterbor varieties at the end of the season (beginning 98 days after planting). This corresponds with August 28th, 2013 and could be a result of seasonal climate shifts as the days shorten and the weather cools. Similar results were not seen at HRC perhaps due to the climatic buffering effect of the insect screened high tunnels.

Leaf Weight

This measurement in kale is primarily useful for leaves that are sold by weight (fresh, processed and frozen) as opposed fresh market numbered bunches. The only significant difference in kale was found among varieties and was primarily due to leaf morphology. While the Dinosaur kale might sell well in numbered bunches, the heavier varieties, Winterbor and Red Russian, would be a better choice for growers interested in selling kale by weight.

Leaf Area

Supplemental N application has been shown to increase kale leaf area (Balcau 2012). Contrary to leaf weight, leaf area is useful for growers interested in selling kale by the bunch. If stems are counted into bunches a leaf with a higher leaf area will appear to be a larger bunch thus increasing consumer appeal. However, two dimensional leaf area measurements, as taken in this study, may not be most useful for some varieties of kale. Red Russian had the highest leaf area at all farms. Its leaves are relatively flat and large, yet it was most susceptible to insect damage throughout the season. Winterbor had the lowest leaf area on all farms yet it's frilled three dimensional texture made for fuller bunches that could not have been predicted on leaf area alone. While leaf area is useful in examining leaf growth within a variety, it proves less useful across varieties. An interesting trend among research sites occurred during the end of the season when Red Russian leaf area kept increasing at HRC but dramatically decreased at SK and NH. This could be a result of the covered high tunnels at HRC protecting the kale from harsh fall weather thus promoting increased plant growth.

Leaf Nitrogen

Sufficient ranges of leaf N tissue at harvest are reported to be 3-5% (Maynard and Hochmuth, 2007). At Harvest 2 and Harvest 4, Winterbor dropped below the sufficient range (<3%) at HRC in all treatment groups. Because it is an F1 hybrid and a vigorous feeder it may have needed more N to maintain sufficient levels even though it was receiving the same fertilizer treatments as the other varieties. Winterbor may also need less N, resulting in lower sufficiency levels. There is more research needed to conclude this. However, this clearly demonstrates a variety difference in N needs and uptake. Winterbor at SK and NH was deficient at Harvests 1, 3

and 4. This could be explained by the lower soil NO₃-N and OM levels prior to planting the kale.

Iron

Adequate Fe content in mature kale leaves ranges from 40-100 mg/kg (Maynard and Hochmuth, 2007). All kale at all sites fell within this sufficiency range. However, there was a steep decrease in Fe content in leaf tissue as the season progressed. Other metal cations can interact with Fe and compete for uptake in the plant (Havlin et al., 2014). Plants receiving proportionately higher amounts of NO₃-N than NH₄-N also uptake less Fe as the process of NO₃-N absorption increases the pH in the root zone and limits Fe availability (Havlin et al., 2014). As more fertilizer was cumulatively applied throughout the season it is possible that a buildup of metal cations were competing with Fe uptake however there was no Zn correlation found in this study. More research is needed to look at nitrogen ratios in the studied fertilizers and to analyzing soil Fe content throughout the season.

Zinc

Adequate Zn content in mature kale leaves ranges from 20-40 mg/kg (Maynard and Hochmuth, 2007). While all kale at all sites fell within this range, kale at SK and NH were closer to 20 mg/kg (Figure 13). Like Fe, Zn also competes for entry into the plant with other metal cations, especially Fe⁺² and Cu⁺² (Havlin et al., 2014). While the applied fertilizers all contained Zn it was at different concentrations, Cyano <0.1 mg/kg, Alfalfa meal 17.8 mg/kg and Fish 4.48 mg/kg and this difference in application could have impacted both Zn and Fe contents.

Residual Soil N

Residual soil NO₃-N was significantly higher in Red Russian than the other varieties in all three locations. The one unexplored difference among varieties is that of species; Red Russian is *Brassica napus* while both Dinosaur and Winterbor are *Brassica oleracea*. Not only are we comparing two different cultivars (Dinosaur and Winterbor) but a completely different species (Red Russian). Tiemens-Hulscher (2014) found that N uptake varied greatly among potato cultivars due to morphological traits bred into each cultivar. So it is possible that kale cultivars and species morphological traits impact N uptake. With further research, this could explain why residual soil NO₃-N was significantly higher in Red Russian plots. This information could also lead to organic variety choices based on plant nutrient need and uptake of N. As Tiemens-Hulscher (2014) points out, choosing cultivars based on N uptake can match available N in any system, especially organic.

CONCLUSION

There were no statistically significant effects measured with regard to fertilizer treatment. This demonstrates a possibility for fertilizer that are produced on-farm like liquid cyanobacteria to compete with organic off-farm inputs. Most significant differences were related to variety type and harvest dates, differences that would be expected. However, all varieties and harvest times showed no interaction with fertilizer treatments, perhaps showing a lack of preference for fertilizer types across varieties. The varieties did, however, demonstrate differences with respect to qualities that would interest farmers when making variety and cultivar selections. There was also a difference in residual $\text{NO}_3\text{-N}$ among varieties. This information could be used when choosing varieties based on available N and when planting in areas at high risk of leaching nitrates during winter months. Based on this study, we can conclude there was no evidence of a plant growth or nutrient concentration effect of the fertilizer treatments on these crop-soil combinations.

TABLES

Table 1. N-P-K concentrations of common organic fertilizer materials
(values from Maynard and Hochmuth, 2007 and product information from fertilizer companies).

Organic Fertilizers	Fertilizer Grade		
	N	P ₂ O ₅	K ₂ O
	-----%-----		
Alfalfa meal	2.5	1	2
Blood meal	13	2	1
Bat guano	10	4	2
Cottonseed meal	6	.4	1.5
Fish meal	10	6	2
Fish emulsion	5	1	1
Fish hydrolyzed	2	4	1
Feather meal	14-17	-	-

Table 2. Soil test results for all research sites.

Location	----paste----		%	-----AB-DTPA-----							Texture Estimate
	pH	EC		-----mg/kg-----							
		mmhos/ cm	OM	NO3-N	P	K	Zn	Fe	Mn	Cu	
Native Hill	7.4	0.6	3.6	8.8	34	302	2.6	15.8	3.6	3.9	Sandy Clay Loam
Spring Kite	7.6	0.5	4.9	14.6	20	472	3.4	15.1	2.8	4.0	Sandy Clay Loam
HRC Tunnel 4	7.3	0.5	1.5	10.1	28	419	4.4	10.7	6.7	3.6	Sandy Clay
HRC Tunnel 5	7.4	0.6	2.6	29.2	32	606	4.4	11.9	7.9	4.4	Sandy Clay
HRC Tunnel 6	7.4	0.6	2.1	9.4	39	562	5.4	9.8	7.5	4.7	Sandy Clay

Table 3. Fertilizer content analysis from field samples. (Lcyb= Liquid Cyanobacteria, Alfalfa=Alfalfa Meal, Neptunes= Neptunes Fish Hydrolysate)

Fertilizer	-----Total Digest-----						
	N %	P	K	Fe	Ca	Mg	Zn
	-----mg/kg-----						
Lcyb	0.189	5.78	<0.1	6.19	9.68	17.9	<0.1
Alfalfa	3.09	5141	55156	663	25421	11419	17.8
Neptunes	2.38	20780	5543	37.8	3906	720	4.38

Table 4. Alfalfa application rate at each site, calculated using soil sampled NO₃ levels

Location	Alfalfa applied (kg/ha)
Native Hill	92.4
Spring Kite	79.4
HRC Tunnel 4	89.5
HRC Tunnel 5	46.6
HRC Tunnel 6	91.0

FIGURES

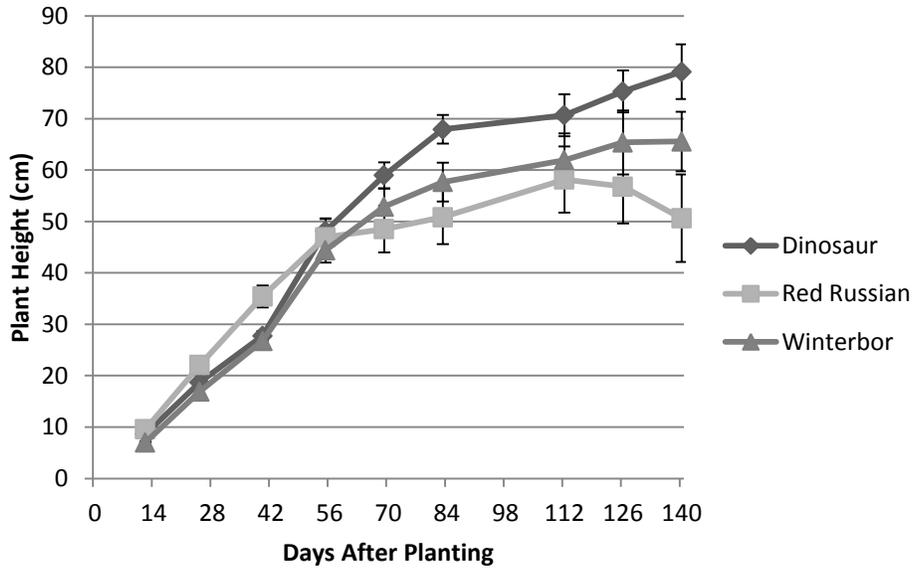


Figure 1. Plant height of three kale varieties throughout the growing season at the Horticulture Research Center in 2013. Bars represent the standard error of the mean for each sample group.

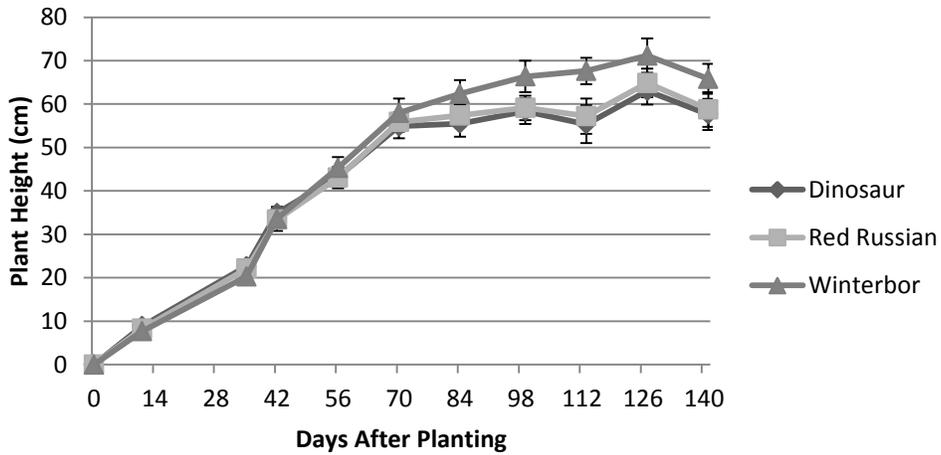


Figure 2. Plant height of three kale varieties throughout the growing season at Spring Kite and Native Hill Farms in 2013. Bars represent the standard error of the mean for each sample group.

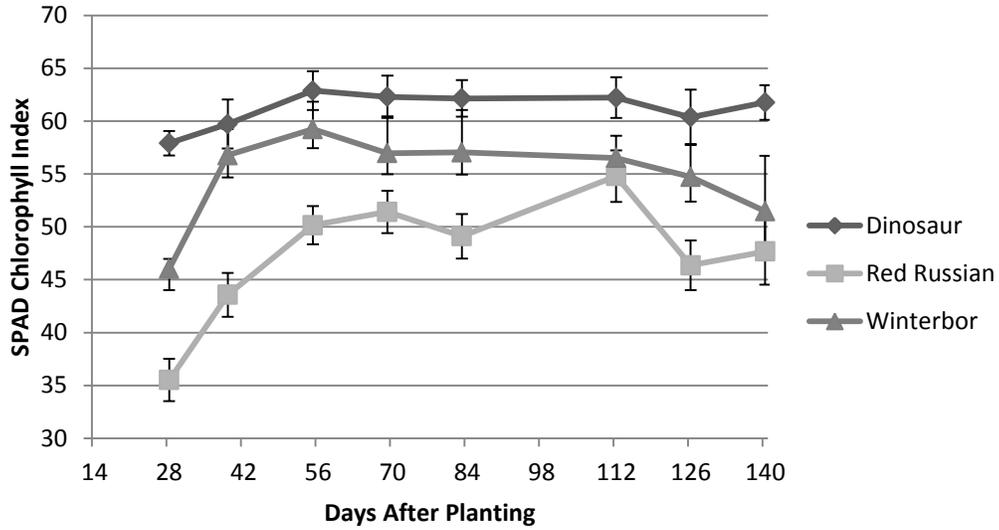


Figure 3. SPAD chlorophyll index for three varieties of kale throughout the growing season at the Horticulture Research Center in 2013. Bars represent the standard error of the mean for each sample group.

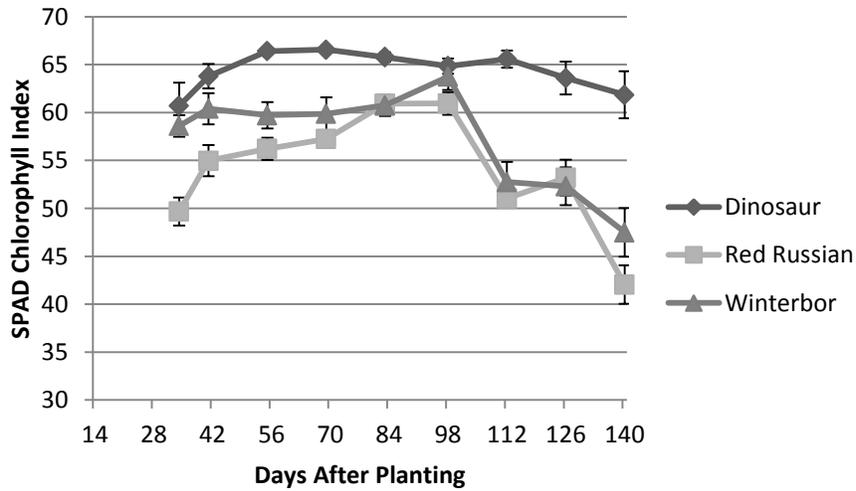


Figure 4. SPAD chlorophyll index for three varieties of kale throughout the growing season at Spring Kite and Native Hill farms in 2013. Bars represent the standard error of the mean for each sample group.

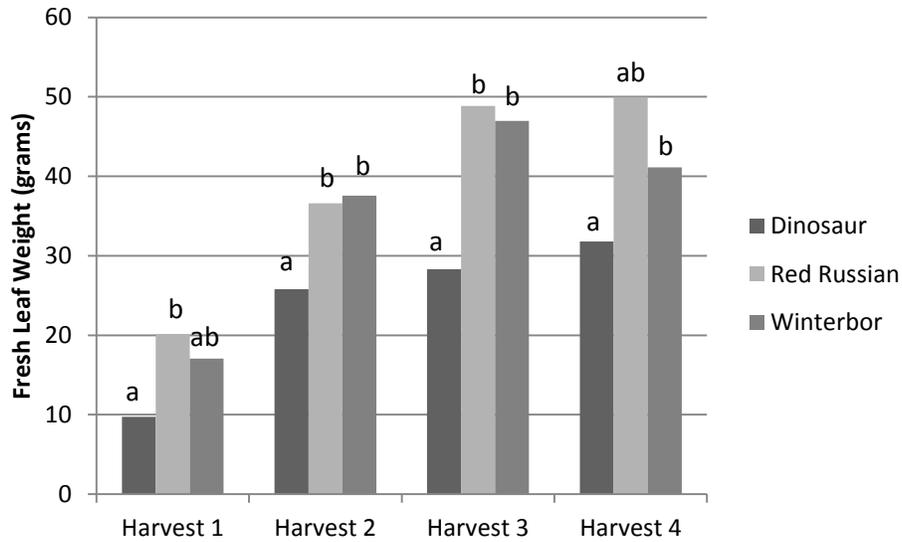


Figure 5. Individual leaf weight of three kale varieties at four different harvest times at the Horticulture Research Center in 2013. Varieties within harvest with a common letter are not significantly different. Significance in proc mixed ($p < 0.05$).

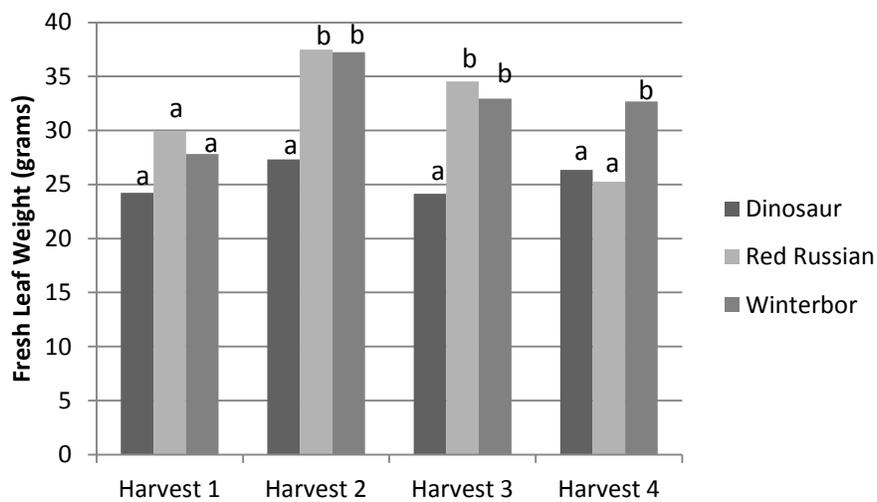


Figure 6. Individual leaf weight of three kale varieties at four different harvest time at Spring Kite and Native Hill farms in 2013. Varieties within harvest with a common letter are not significantly different. Significance in proc mixed ($p < 0.05$).

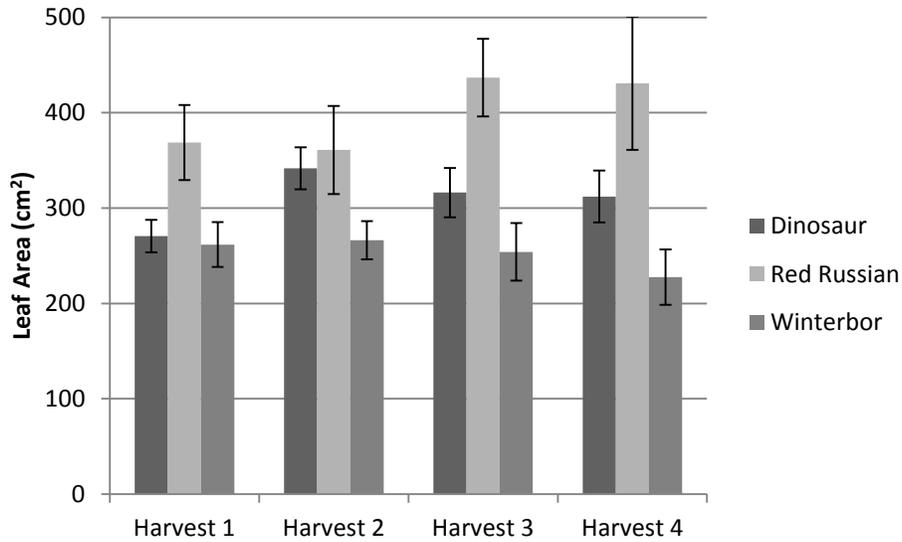


Figure 7. Individual leaf area of three different kale varieties at four harvest times at the Horticulture Research Center in 2013. Bars represent the standard error of the mean for each sample group.

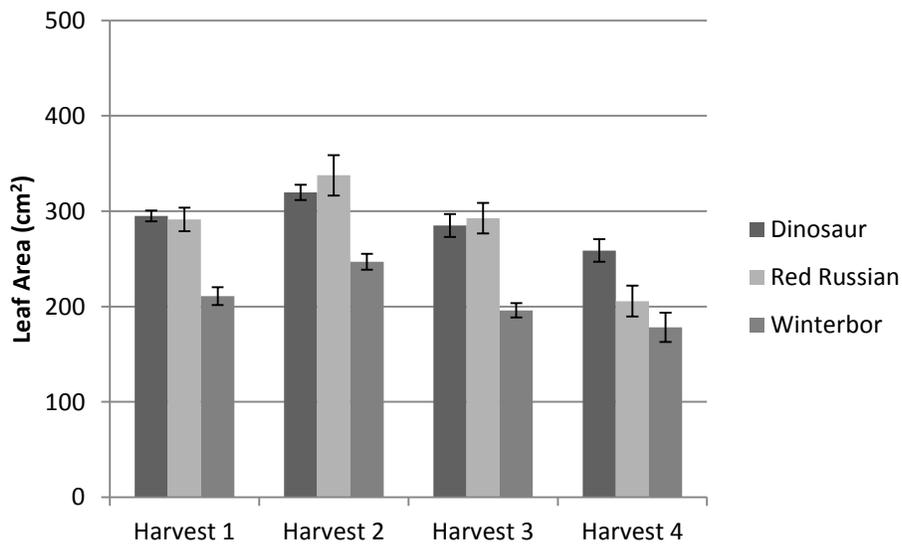


Figure 8. Individual leaf area of three varieties of kale at four harvest times at Spring Kite and Native Hill farms in 2013. Bars represent the standard error of the mean for each sample group.

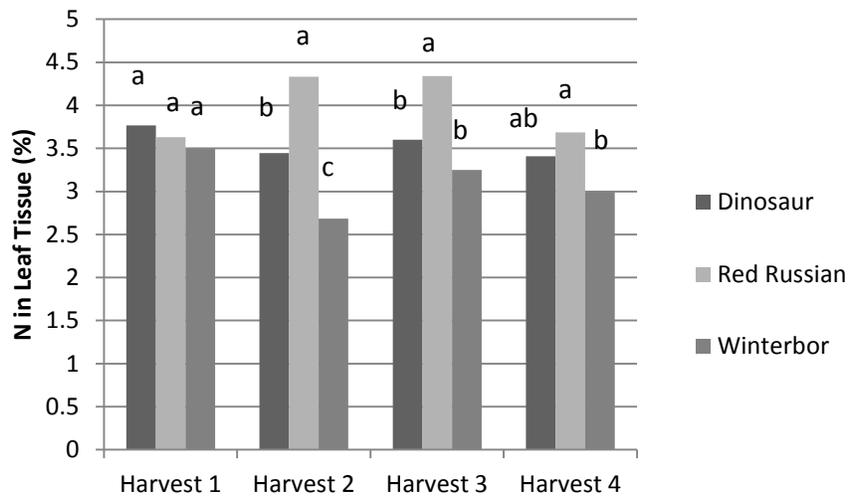


Figure 9. Nitrogen concentration in three kale varieties at four different harvest times at the Horticulture Research Center in 2013. Varieties within harvest with a common letter are not significantly different. Significance in proc mixed ($p < 0.05$).

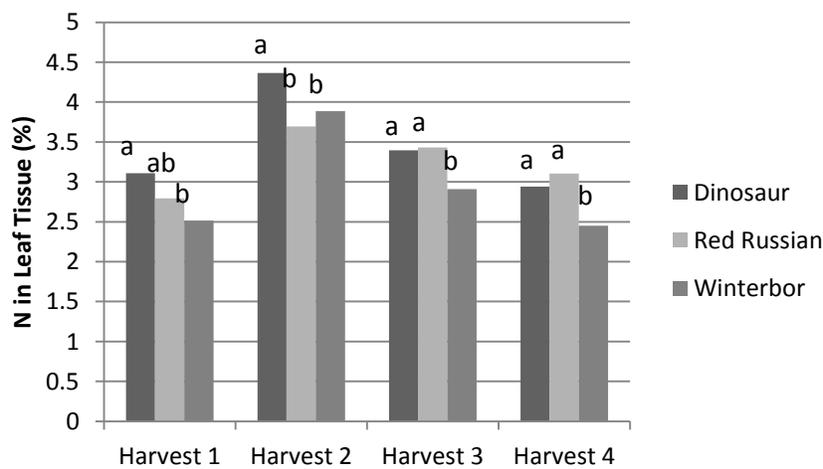


Figure 10. Nitrogen concentration in three kale varieties at four different harvest times at Spring Kite and Native Hill farms in 2013. Varieties within harvest with a common letter are not significantly different. Significance in proc mixed ($p < 0.05$).

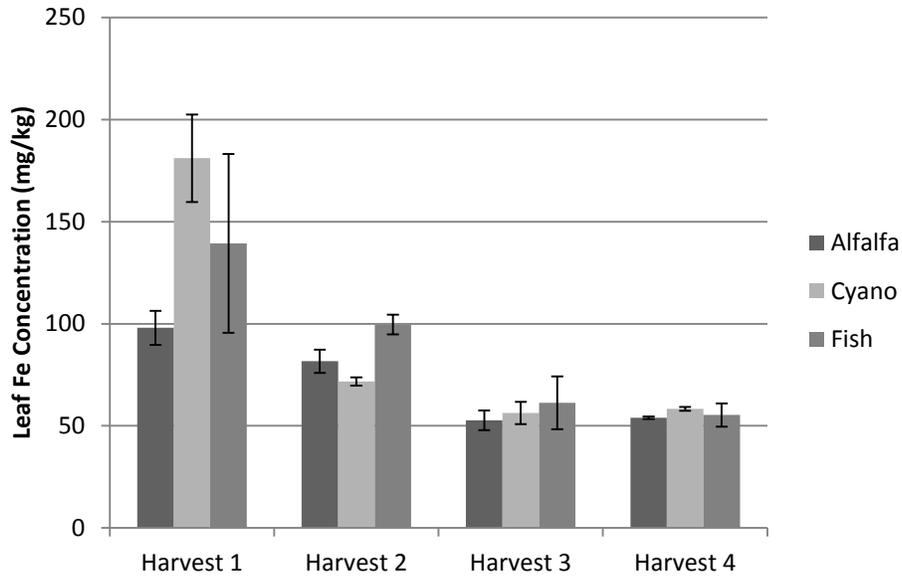


Figure 11. Leaf Fe concentration in Dinosaur kale as influenced by harvest time and fertilizer. Data from the Horticulture Research Center in 2013. Bars represent the standard error of the mean for each sample group. There is a significant difference at Harvest 1 between Cyano and Alfalfa treatment ($p < 0.05$).

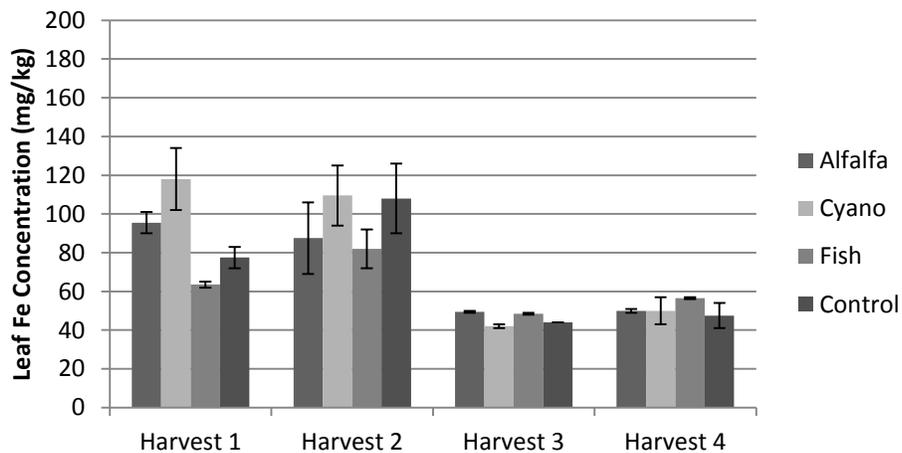


Figure 12. Leaf Fe concentration in Dinosaur kale as influenced by harvest time and fertilizer treatment. Data from Spring Kite and Native Hill Farms in 2013. Control plots only included at SK and NH field sites. Bars represent the standard error of the mean for each sample group.

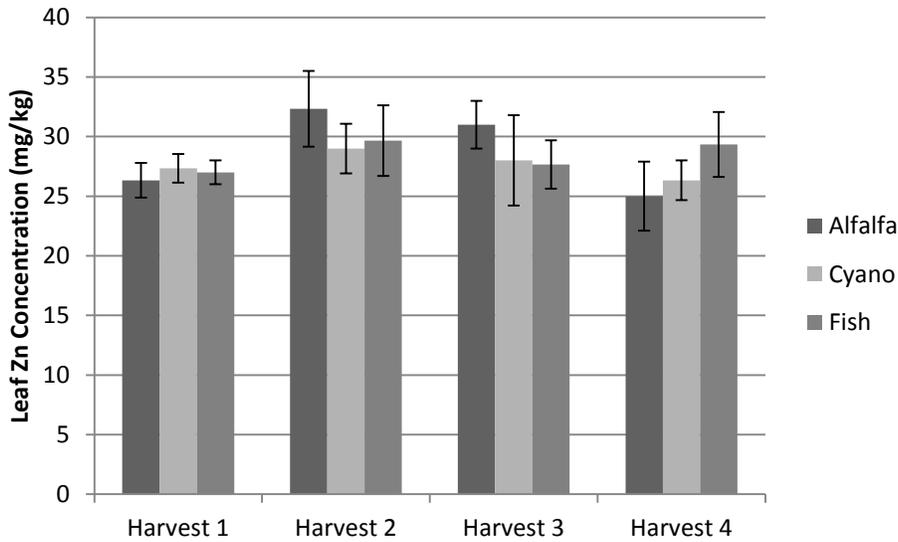


Figure 13. Leaf Zn concentration in Dinosaur kale as influenced by harvest time and fertilizer treatment at the Horticulture Research Center in 2013. Bars represent the standard error of the mean for each sample group.

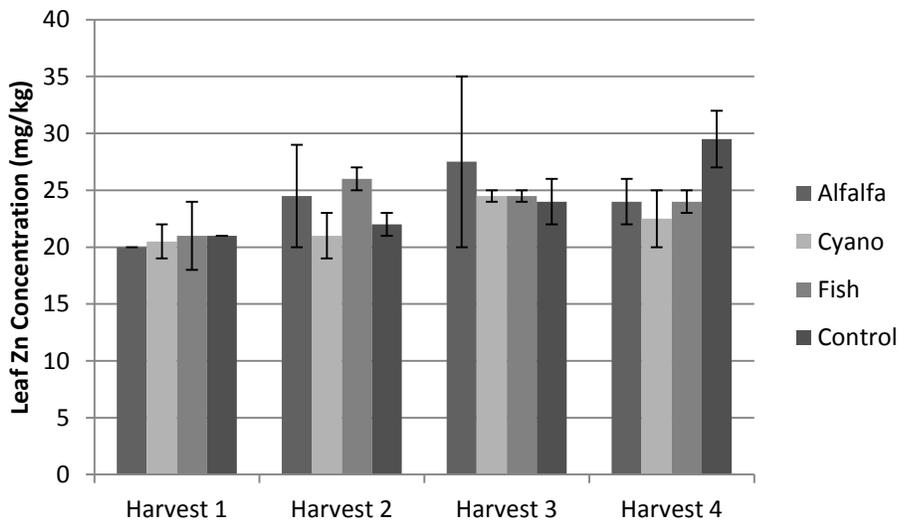


Figure 14. Leaf Zn concentration in Dinosaur kale as influenced by harvest time and fertilizer treatment at Spring Kite (SK) and Native Hill (NH) farms in 2013. Control plots only included at SK and NH field sites. Bars represent the standard error of the mean for each sample group.

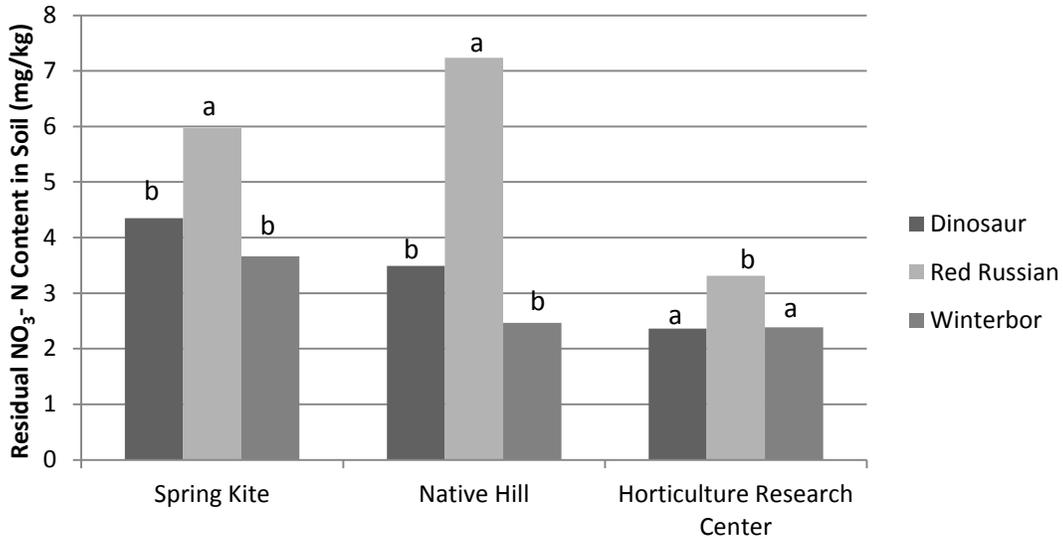


Figure 15. Residual Soil Nitrate by kale variety and growing location in 2013. Varieties within harvest with a common letter are not significantly different. Significance in proc mixed ($p < 0.05$).

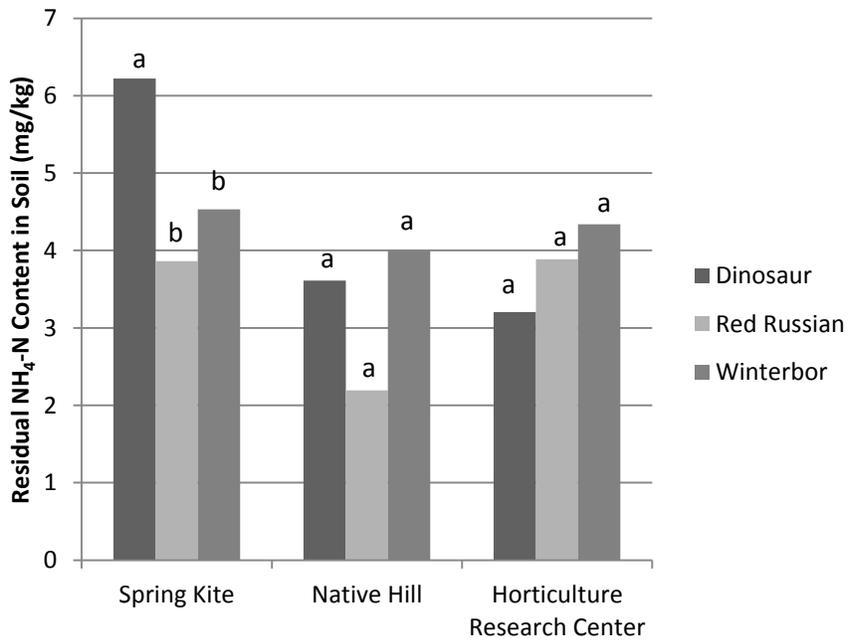


Figure 16. Residual Soil Ammonium by kale variety and growing location in 2013. Varieties within harvest with a common letter are not significantly different. Significance in proc mixed ($p < 0.05$).

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APPENDIX A

FUTURE RESEARCH SUGGESTIONS

While much of this study was new research territory the newest was the use of Lcyb. Research needs to be done on average macro and micro nutrient content in Lcyb in order to understand its effect on plant tissue nutrients and consequently, nutritional value to humans. While Fe and Zn prove important and needed micronutrients in human diets further work could be done on kale's beta carotene and vitamin C content, especially as it relates to fertilizer choices. Lastly, with kales recent boom in popularity there is little known on the basics of kale- most literature cited in this study looked at kales close relatives cabbage and collards. Knott's handbook doesn't even address kale as a separate crop. Finally, future research is needed to develop quick and easy on farm techniques, like the iPhone's GreenIndex, that work well for specialty crops with huge variety morphological differences like kale.