Deficient, Adequate and Excess Nitrogen, Phosphorus, and Potassium Growth Curves Established in Hydroponics for Biotic and Abiotic Stress-Interaction Studies in Lettuce

Douglas Keith Jacobson

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Deficient, Adequate and Excess Nitrogen, Phosphorus, and Potassium Growth
Curves Established in Hydroponics for Biotic and Abiotic
Stress-Interaction Studies in Lettuce

Douglas Keith Jacobson

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

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June 2016

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ABSTRACT

Deficient, Adequate and Excess Nitrogen, Phosphorus, and Potassium Growth Curves Established in Hydroponics for Biotic and Abiotic Stress-Interaction Studies in Lettuce

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Master of Science

Mineral nutrients have marked effects on plant health by providing the building blocks for plant growth, as well as for mitigating abiotic and biotic stress factors, particularly disease development. Even if mineral nutrition field studies are conducted to study pest management, they are at the mercy of complex soil, water, and climatic conditions not amenable to strict experimental control. Therefore, a hydroponic method of growing lettuce was developed and growth curves were established for the macronutrients nitrogen (N), phosphorus (P), and potassium (K). Lettuce plants were grown at varying levels of each nutrient: 2.5, 5, 10, 20, 40, 80, 160, and 320 mg N/L; 0.5, 1, 2, 4, 8, 16, 32 and 64 mg P/L; and 0, 2.5, 5, 10, 20, 40, 80 and 160 mg K/L. Due to inadequate results lettuce was grown again at 0, 10, 20, 40, 80, 160, 320 and 640 mg L K. Optimal levels of N, P, and K were 160 mg/L, 4.0 mg/L, and 80 mg/L respectively. C:N ratios were also looked at for the N experiment. The overall result was consistent with results from similar studies. Unlike similar hydroponic studies done with other plants, micronutrient levels did not become deficient at high phosphorus levels suggesting phosphorus toxicity. These growth curves can be used to test lettuce resilience to various biotic and abiotic stresses.

Keywords: lettuce, hydroponics, growth curves, nitrogen, phosphorus, potassium
ACKNOWLEDGEMENTS

I am thankful for the help and support given to me by my graduate committee as I worked on this project. I would especially like to thank my committee chair, Dr. Brad Geary, for his mentoring and willingness to shepherd me through the research and writing of this thesis. I am grateful to Dr. Hopkins and Dr. Stevens for their expertise, guidance, and support. I am thankful for the many undergraduate assistants who helped with experiment set-up and data collection. I am most thankful for my wife, Geneen, who supported me through long nights, and stressful weeks. I am also thankful to my five children who put up with Dad having to be gone some evenings and working some Saturdays. It’s for them that this was done.
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CHAPTER 1

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important, widely consumed vegetable crop with annual sales near three billion dollars in the United States (US) [(1), (2)]. The US is the second largest producer of lettuce world-wide (3). In 2015 102,587 ha of lettuce were planted of which 101,657 ha were harvested and sold for more than 2.96 billion dollars (2) making lettuce the most valuable vegetable for fresh market in the US (4). Due to the high value of lettuce crops, loss of crop due to biotic and abiotic stresses comes at a high economic cost. Discoloration, abnormal leaf shape, insect damage, disease, nematodes, vertebrate pests, and weeds all cause significant crop reductions (5). Lettuce, along with all plants, is dependent on mineral nutrients for plant growth and overall plant health and quality (1). A number of mineral nutrients are essential to plant growth with nitrogen (N), phosphorus (P), and potassium (K) being considered most important due to the percent found within the plant and the large amount needed for proper plant growth. Although the soil supplies a majority of these nutrients, the reserve is finite and fertilizer additions are essential to maintain adequate nutrition. Fertilizer use in cropping systems is critical for improved production efficiency to sustainably feed an ever-expanding population. Understanding the effects of specific nutrients on plant growth and interaction with various stresses is critical to plant health management.

The relationships and mechanisms by which plant nutrients and plant stressors interact are varied and complex. A particular stressor might inhibit the plant’s ability to absorb an essential nutrient (6) while the absorption of a particular nutrient might allow the plant to escape the effects of a particular stressor [(7), (6)]. Proper plant nutrition is essential to resisting abiotic
or biotic stress factors. If an otherwise healthy plant is deficient in any of the nutrients required for proper growth its susceptibility to stressors is increased [(6), (8)].

Due to their importance within the plant and the quantity needed to maintain plant health, N, P, and K are generally the first to be depleted in the soil (7) and are supplemented by growers. Plants deficient in these three nutrients are less likely to tolerate stress and are more susceptible to disease and other biological threats (5). Likewise, excessive levels of these elements lower plant quality and development (5).

Each of the nutrients—N, P, K—play a significant role within the plant. Nitrogen is essential for the production of amino acids, proteins, enzymes, hormones, phytoalexins and other cellular components, but is often limited in the soil (8). Nitrogen plays an essential role in photosynthesis (9) and promotes growth (8). Of all the mineral nutrients, N is generally found to have the highest concentration within plants, but is relatively transient in the environment and is easily lost to the atmosphere and groundwater (10). Due to its limited availability in soils, N is applied to crops in higher quantities than any other mineral element (7).

Phosphorus deficiency in soils severely limits plant yield (11). Within a plant, P is primarily used for energy transfer and protein metabolism (11). Pyrophosphate bond formation and degradation (hydrolysis) play a key role in the energy balance underlying major plant metabolic pathways (9). Hydrolysis of these bonds releases the energy required for several plant functions such as enzyme activation, N₂ fixation, and the synthesis of organic compounds (9). Continual potassium uptake in plants is generally greater than any other nutrient; additionally, unlike N and P, K does not become part of any plant constituent but rather, remains unattached as a regulator of plant growth (12). Potassium activates at least 60 different enzymes in meristematic tissues (13).
In addition to directly benefitting plants, manipulation of mineral nutrients has considerable effect on biotic stresses. Mineral nutrients influence plant disease development, competition with weeds, and insect and nematode infestations directly and indirectly through growth characteristics, plant metabolites, root exudates and induced biological controls (14). Conventional thought has been that increases in N tend to increase disease while increases in K decrease disease and increases in P can produce either effect. However, such generalizations fail to account for the form of nitrogen, rate or time of application, or soil conditions (15). Nitrogen influences host plant resistance to disease by reducing successful pathogen penetration or by retarding pathogenesis after penetration (15). Both P and K have demonstrated an increase in disease resistance; P by allowing disease escape through vigorous root growth and K has been observed to reduce disease severity in several crops (15). Several studies examine the effects that N, P, and K have on plant disease for a variety of hosts including: cotton (*Gossypium hirsutum* L.) [(16), (17), (18), (19), (15)], eggplant (*Solanum melongena* L.) [(20), (21), (22), (15)], potato (*Solanum tuberosum* L.) [(23), (24), (25), (15)], cabbage (*Brassica oleracea* L. var. *oleracea*) [(26), (27), (28), (15)], and cauliflower (*Brassica oleracea* L. var. *botrytis*) [(29), (15)] to name a few. However, little research has been done on the effects of N, P, and K in lettuce stress interaction—abiotic or biotic. Despite known interactions to plant stressors for other crops, N, P, and K research in lettuce is typically tied to traditional rate-response field trials [(30), (31), (32), (33)] for agronomic analyses rather than finely tuned nutrient studies for biotic stress suppression or enhancement (10). Even if field studies were done to examine disease resistance the lack of uniform conditions in the soils and spatially through the environment can skew results and makes interpretation difficult. Hydroponic studies do not perfectly mirror field conditions. Crops grown in fields receive more light than those in hydroponic studies. Additionally, there is no way to
mimic the soil buffering capacity and microorganism activity in a hydroponic setup. Plants grown hydroponically benefit from more oxygen than those grown in the field. However, despite these differences hydroponic studies can be useful. Growth in a hydroponic system allows for uniformity of root and shoot environments and allows for detection of subtle differences in disease severity that might be lost in field studies (10). Hydroponic studies are particularly useful for lettuce which is grown commercially in hydroponic systems.

In addition, genetic techniques available for identification and quantification of pathogens or the impacts of biotic and abiotic stresses are readily applied to root and shoot tissue more easily accessible in hydroponic cultivation. A system, in which the influence of N, P, and K on lettuce development while under abiotic or biotic stress can be assessed without the many confounding factors associated with soil interactions, would be highly beneficial [(34); (35)]. Identifying N, P, and K, response curves in hydroponic solutions would enable controlled experimental conditions to further study stress factors and their interaction with these elements. Therefore, the purpose of this research was to identify deficient, optimal and excessive N, P and K levels for growth of lettuce in hydroponic solution to allow refined studies on N, P and K biotic and abiotic interactions.

MATERIALS AND METHODS

Nitrogen and Phosphorus

Lettuce (*Lactuca sativa* L.cv. Salinas) was grown from seed (hometownseeds.com) in a solite porous ceramics growing medium (steveregan.com) in an enclosed hydroponic system within an environmentally controlled growth chamber (Mallory Engineering, Inc. Salt Lake City, UT). Plants were grown with a 14-h light period at a temperature of 23±1°C, and a 10-h dark period at 17±1°C. Plants were grown in a 4x4 Latin Square design. The Latin square design was
chosen to minimize potential nuisance variables (36). 14 L buckets containing nutrient solutions were placed in a wooden box and completely covered with an opaque polyethylene lid to prevent light contamination to the roots. Air was supplied at a constant flow (10 psi) to each nutrient solution. Five to six seeds were planted in the solite and allowed to germinate in modified pretreatment Hoagland solutions (37). The pretreatment N experiment nutrient concentrations were (mg/L): 10.94 N; 1.14 P; 27.7 K; 6.2 S; 5.5 Ca; 3.01 Mg; 0.024 Zn; 0.49 Fe; 0.077 Mn; 0.006 Cu; 0.043 B; 4.12 Cl; 0.006 Mo; 0.003 Na. The P experiment concentrations were (mg/L): 42.79 N; 0.45 P; 11.7 K; 4.9 S; 49.6 Ca; 6.29 Mg; 0.024 Zn; 0.49 Fe; 0.077 Mn; 0.006 Cu; 0.043 B; 1.03 Cl; 0.006 Mo; 0.003 Na. The pH for both experiments was maintained between 6 and 7 with 390 mg/L 2-Morpholinoethanesulfonic acid (MES).

Seedlings were allowed to grow for two to three weeks in modified Hoagland pretreatment solution before being transferred to treatment solutions. The N treatment solution consisted of: 2.50 N; 45.12 P; 182.6 K; 127.0 S; 10.0 Ca; 56.94 Mg; 0.506 Zn; 2.50 Fe; 0.876 Mn; 0.114 Cu; 1.749 B; 23.62 Cl; 0.073 Mo; 0.073 Mo; 0.035 Na. The P treatment solution was: 85.07 N; 4.00 P; 69.8 K; 2.9 S; 79.5 Ca; 5.06 Mg; 0.114 Zn; 0.46 Fe; 0.371 Mn; 0.029 Cu; 0.204 B; 1.36 Cl; 0.029 Mo; 0.014 Na. Both solutions again had 390 mg/L 2-Morpholinoethanesulfonic acid (MES) to maintain a pH between 6 and 7.

Once established plants in the N experiment were treated for two weeks with NH4NO3 at rates of 2.5, 5, 10, 20, 40, 80, 160, and 320 mg/L. Plants in the P experiment were treated for two weeks with H3PO4 at rates of 0.5, 1, 2, 4, 8, 16, 32, and 64 mg/L.

At the end of the treatment period the lettuce was harvested by clipping all the plants at the shoot/root interface, placing shoots and roots in separate paper bags, drying at 65°C for at least 48-h, weighing, and grinding (1 mm sieve). Ground plant materials for the N experiment
were analyzed with a N carbon LECO Truspec CN Determinator (LECO Instruments, St. Joseph, Mich., USA) using the total N by combustion method (38). Analysis of micronutrients was not completed for the N experiment due to insufficient plant material (in some treatment groups) for both CN Determinator and ICP analyses. Ground plant material for the P experiment was digested using the minerals by nitric – perchloric acid digestion method followed by ICP-OES analysis (Iris Intrepid II XSP, ICP-OES, Thermo Electron Corporation, Franklin, Maryland, USA) (39). Carbon and N analysis was not performed for the P and K experiments as there was not sufficient plant material (in some treatment groups) to run both the CN Determinator and the ICP analyses.

**Potassium**

Lettuce was grown from seed (hometownseeds.com) in a solite porous ceramics growing medium in an enclosed hydroponic system within an environmentally controlled growth chamber (Environmental Growth Chambers Chagrin Falls, OH). Plants were grown with a 14-h light period at a temperature of 23±1°C, and a 10-h dark period at 17±1°C. Plants were grown in a 4x4 Latin Square design. Plants were grown in 16 L black, square buckets to accommodate the smaller growth chamber and completely covered with an opaque polyethylene lid to prevent light contamination to the roots. Air was supplied at a constant flow (10 psi) to each nutrient solution. Five to six seeds were planted in the solite and allowed to germinate in a pretreatment modified Hoagland solution (36). The solution consisted of the following macro and micro nutrients (mg/L): 42.75 N; 4.87 P; 0.6 K; 4.9 S; 51.8 Ca; 6.57 Mg; 0.024 Zn; 0.49 Fe; 0.077 Mn; 0.006 Cu; 0.043 B; 1.03 Cl; 0.006 Mo; 0.003 Na.

After germination, seedlings were allowed to grow for two weeks in the modified Hoagland pretreatment solution before being transferred to the treatment solution. This solution
consisted of the following macro and micro nutrients (mg/L): 80.07 N; 22.12 P; 0.0 K; 65.7 S; 93.8 Ca; 5.06 Mg 0.114 Zn; 0.46 Fe; 0.371 Mn; 0.029 Cu; 0.204 B; 1.36 Cl; 0.029 Mo; 0.014 Na. pH was maintained between 6 and 7 with 390 mg/L 2-Morpholinoethanesulfonic acid (MES).

Once established plants were treated for two weeks with \(K_2\text{SO}_4\) at rates of 0.0, 2.5, 5, 10, 20, 40, 80, and 160 mg/L. At the end of this experiment it was determined that K rates were too low and a second experiment was done which added 2.0 mg K/L to the pretreatment solution and increased the \(K_2\text{SO}_4\) application rates to 0, 10, 20, 40, 80, 160, 320, and 640 mg/L.

At the end of the treatment period the lettuce was harvested by clipping all the plants at the shoot/root interface, placing shoots and roots in separate paper bags, drying at 65°C for at least 48-h, weighing, and grinding (1 mm sieve). Ground plant material was digested using the minerals by nitric – hydrogen peroxide microwave digestion method (EPA method 3052) followed by ICP- OES analysis (iCAP 7400, Thermo Electron, Madison, WI).

The change in growth chamber was due to the opening of a new Life Science building on the BYU campus and the dismantling of the Mallory growth chambers. Likewise, the change in methodology between the P and K analyses was due to the change in lab facilities and equipment associated with the opening of the new building on the BYU campus. Statistical analysis of yield and percent tissue was by analysis of variance (ANOVA) using the aov procedure of Program R (40). Pairwise comparisons were made using the Duncan-Waller test using the agricolae package in Program R (41).
RESULTS AND DISCUSSION

*Nitrogen Experiment*

Lettuce plants grown hydroponically in a growth chamber were started in a disease free environment, and only N levels varied in this experiment. Increasing levels of N produced more tissue growth up to a clear peak at 80 mg N/L (Figure 1). Shoot growth increased significantly as solution N levels increased up to 40 mg N/L, shoot growth peaked at 80 mg N/L, though this was not significantly more growth than 40, 160, and 320 mg/L (Figure 1). Excessive N levels, 160 and 320 mg N/L, had a downward trend in shoot biomass. Unexpectedly, root biomass did not increase with additional nutrient solution N content and there were no significant differences among treatments (Figure 1). This differs from similar study done on potatoes (10). Work done by Neumann et al. showed that root development of lettuce varied greatly by soil type and that greater root development did not correspond with greater yield (42). It is our hypothesis that root development was uniform due to aqueous environment of the hydroponic solution. This also mirrors work done by Maršić and Osvald who found no significant difference in root biomass of lettuce grown hydroponically in some of their experiments (43). The negative impact of too much or too little N was apparent in the shoots but not roots. However, from these results this hydroponic methodology could be employed with lettuce to study the impacts of deficient and optimal levels of N on disease development. Excess levels of N were not significantly different from the optimum level (80 mg N/L) but the downward trend in biomass suggests stress on the plant due to excessive N. These levels could be used to identify how the plant will respond to other biotic or abiotic stresses when under deficient or excessive N stress.

The percent N in shoot and root tissue was strongly reflective of increasing solution N concentrations. Nitrogen percentages in both root and shoot tissue were similar until 20 mg N/L
when N content in shoots rose dramatically from treatments 20 mg N/L to 40 mg N/L, and roots rose sharply from 20 mg N/L to 80 mg N/L (Figure 2). Shoot N content rose gradually until 80 mg N/L, jumped significantly at 160 mg N/L and dropped slightly at 320 mg N/L. Root N content rose gradually from 80 mg N/L to 160 mg N/L and then rose significantly from 160 mg N/L to 320 mg N/L (Figure 2). The difference in biomass peak (Figure 1) and % N peak (Figure 2) is due to the plant continuing to take up N despite it not being necessary to the plant. The decrease in shoot N content at 320 mg/L suggest that 320 mg N/L is an excessive level of N for lettuce plants.

Deficient and excessive N in plants will likely increase pathogen presence and severity as plants will have to take N from their own cells causing the plant to be weak and unable to mount an adequate defense (44). Stalk rot of corn (Gibberella zae [Schweing.] Petch) is an example of a disease whose incidence increases with insufficient N (45). Corn (Zea mays L.) plants cannibalize physiological ribulose bisphosphate carboxylase (rubisco), phosphoenolpyruvic acid carboxylase (PEP), and proteins as sources of N resulting in increased stalk rot due to decreased plant health (46). Excessive N has been shown to increase foliar diseases and could increase disease severity by providing amino acids to support pathogen survival while weakening the plant at excessive levels [(15), (44)]. Presumably lettuce would follow these patterns but this would need to be confirmed experimentally.

Interesting relationships emerged from the interaction between increasing N and the percent C in the lettuce plants (Figure 3). Expectedly, as N levels increased, % C declined as plants became more succulent until treatment 80 mg N/L (Figure 3). This corresponded to the peak biomass recorded at this treatment level (Figure 1). Increased plant succulence makes the plant a more likely target for insect or disease development as it signals to the pathogen that N is
available [(47), (15)]. As N levels begin to become excessive, the percent C in the shoots increases (Figure 3), suggesting a decrease in plant succulence as excessive N levels in the shoot degrade plant tissue. Percent C in root tissue follows a more upward trend suggesting that root tissues do not experience a similar increase in succulence. However, the overall C to N ratio (plants becoming more succulent) for both roots and shoots increased (Figure 4). This can be a concern because as plant concentration of N increases, the susceptibility to disease also increases (48). This increase happens because N is more easily available in these plants than in plants where N is limited (49). However, depriving plants of N as a way to prevent harm is not a good solution. Nitrogen provides the materials that plants use to grow and recover from injury and maintain balanced plant health (49).

**Phosphorus Experiment**

Lettuce plants grown hydroponically in a growth chamber were started in a disease free environment, and only P levels varied in this experiment. Increasing levels of P produced more tissue growth up to a clear peak at 4 mg P/L (Figure 5). Shoot biomass did not vary greatly between 0.5 mg P/L and 64 mg P/L with all shoot biomass weighing between 8 and 13 g. Biomass peaked at 4 mg P/L which was significantly different than deficient and excessive levels of P (Figure 5). Root biomass fluctuated slightly between treatments but was not significantly (p > 0.5) different. Predicting peak P levels from root biomass was not possible. However, from these results this hydroponic methodology could be employed with lettuce to study the impacts of deficient, optimal, and excess levels of P on disease development.

Percent P levels in both shoot and root biomass confirmed the increase in P levels throughout the treatments (Figure 6). Phosphorus content in shoots remained relatively low in treatments 0.5, 1, and 2 mg P/L. At 4 mg P/L, P content in shoots was significantly different
from the lowest two treatment levels and from the highest four treatment levels (Figure 6). Phosphorus content in shoots rose dramatically from 4 mg P/L to 8 mg P/L and again from 8 mg P/L to 16 mg P/L (Figure 6). This sharp increase in P content corresponded with a decrease in shoot biomass (Figure 5). Phosphorus content in root material was higher than P content in shoot material (Figure 6), but followed a similar trend except for a spike in P root content at 1 mg P/L. Phosphorus content above 0.4 % in both shoot and root tissue seems to indicate less shoot biomass (Figures 5 and 6).

Conventional thought is that as phosphorus levels increase within a plant, plant growth decreases because high phosphorus causes micronutrient deficiency. Work done by Barben et al. on hydroponically grown potatoes for zinc [(50), (51), (52) (53)], copper and iron (51). However, micronutrient analysis of lettuce tissue did not show a similar result. Jones (54) has shown that sufficient nutrient rates for head lettuce are as follows: 1.4-2.25 % Ca; 0.5-2.25 % Mg; 0.2-0.4 % S; 50-500 ppm Fe; 25-250 ppm Mn; 23-100; 7-25 ppm Cu; 25-250 ppm Zn (51). These levels were tested against lettuce grown in California by Hartz (55) who found that nutrient concentrations were generally the same as reported by Jones with the exception of Ca which had lower concentrations (0.4-0.7 %). Results from the phosphorus ICP analysis (Table 1) show that micronutrients are generally within sufficiency ranges regardless of P treatment amount. This suggests that perhaps lettuce biomass declined (Figure 5) due to phosphorus deficiency rather than due to an effect on micronutrients. Due to decades of heavy P fertilizer application to vegetables grown in Salinas Valley, California, many fields in the area have increased soil test P (STP) (56). By establishing excess P levels, researches will be better able to test the effect excessive P has on different stressors in a laboratory setting, providing more relevant information to growers and researchers. The greatest effects of P on plant disease and other stressors are
usually observed when there is a balanced fertility with N and K (15). Research done by Hoque et al. (2010) demonstrates that lettuce yield and quality are not significantly changed by P alone (57). By establishing deficient, optimal and excess levels of P, studies can be done examining the effects of P in combination with other elemental nutrients. For example, P could be increased in order to counterbalance the effect of increased N by decreasing the time at which plant tissue matures (15).

Research shows that a P deficiency in several plants; rice (Oryza sativa L.), bean (Phaseolus spp.), corn, soybean (Glycine max L.), and wheat (Triticum spp.) reduces root development, weakening a plant and leaving it vulnerable to biotic and abiotic stressors (11). In this study, roots were not impacted with deficient or excessive levels but the shoot biomass was significantly influenced by deficient and excessive levels. High levels of P in soil have been associated with increases in some foliar diseases. Sugarcane rust (Puccinia melanocephala Syd & P. Syd) was shown to increase at excess P levels (58). This increase was due to a shorter latent period and increased sporulation (11). Excess P can also induce Zn deficiency; however, in this study a change in Zn levels did not occur, most likely due to sufficient levels of zinc available in the hydroponic solution (59). Testing the influence of disease on lettuce in a hydroponic system would be ideal because N and K levels could be optimal and balanced while P varied and diseases were introduced. Thus, determining an optimal level of P for lettuce is an integral part of a stress management strategy.

Potassium Experiment

Lettuce grown at levels of 0, 2.5, 5, 10, 20, 40, 80, and 160 mg K/L solution did not respond as expected with our initial experiment. Levels remained fairly low (average shoot biomass between 8 and 12 g) until 160 mg K/L when average shoot biomass jumped
significantly to 19 g (Figure 7). Root biomass mirrored this trend (Figure 7). Literature on K and lettuce identified that lettuce, depending on the variety, needs a substantial amount of K, anywhere from 6 to 13.7% content (38). This explained why our initial experiment responded poorly to varying K levels, and it was determined that the experiment would be repeated with quadruple the amount of K.

Lettuce grown at 0, 10, 20, 40, 80, 160, 320, and 640 mg K/L had better biomass yields than lettuce grown in the first K experiment. Shoot biomass of K rates (0 through 40 mg K/L) was unexpectedly high (between 14 and 19 g) (Figure 8). This differed significantly from the results of the first experiment. A few plausible explanations are; first, lettuce grown in the first experiment may have been starved of K at a particularly crucial point in seedling development from which it was not able to adequately recover. Second, during the second experiment, initial K given to the seeds in the pretreatment solution was increased, perhaps masking the intended treatment effects of deficient K levels. Finally, there may have been some cross-contamination of the seedlings in the second experiment. However, we believe the seedlings were starved of K at a crucial point because of the increased biomass in the second experiment. Treatments 80 through 640 mg K/L performed consistent with other experiments and were similar to the N and P results. Shoot biomass peaked at 80 mg K/L steadily fell with 640 mg K/L being significantly different 80 mg/L K (Figure 8). Root biomass varied somewhat between treatments but no clear pattern emerged.

Percent K in shoots and roots generally trended upward, except for 10 mg K/L being an anomaly in the shoot and 40 mg/L K in the root; though neither anomaly was significantly different than the subsequent treatment level (Figure 9). Plants continued assimilating K into shoot and root tissue with 640 mg/L K having a significantly higher percentage than any other
treatment (Figure 9). This suggests that lettuce will continue to draw up K as long as it is available even if it is no longer useful to the plant. With low K percentages, it is possible that the high shoot biomass found in treatments 0-40 mg/L K was due to the ample presence of other nutrients such as N. Though this could not be determined experimentally as there was not sufficient dried tissue (for some treatments) to run a C:N analysis.

Macro and micronutrients were also analyzed, and while micronutrients were added in the same amounts to each treatment, they were found in significantly different amounts between treatments in shoot and root tissue analysis (Table 2). No clear pattern in the significant differences could be determined. Some nutrients were significantly higher in lower treatment levels, but others were significantly higher in mid-level to high levels (Table 2).

The relationship between K and plant stressors, particularly plant disease, has long been studied and established for several crops (15). Increased levels of K have been shown to decrease Mildew (*Bremia lactucae*) in lettuce (60). In many crops, disease resistant plant varieties often contain more K in plant tissues than non-resistant varieties (12). Disease control through K fertilization is often accomplished by increasing K levels within the crops; yet, too much K can be detrimental to crop yield and should be balanced with proper rates of N and P fertilization (15).

CONCLUSIONS

Studies done in a hydroponic growth system provide a more uniform environment, devoid of confounding variables such as weather and soil conditions associated with a traditional field study. This allows researchers to better target the relationship of a specific variable (N, P, or K levels) to a particular plant stressor (disease). Before such work can be done, researchers must know at what rates these elements can be applied to assess nutrient-stress relationships. Depending on the specific objectives, an experiment might look at deficient or toxic levels of N,
P, or K to measure plant response when abiotic or biotic elements are introduced, or exposed to a specific stressor while under deficient or toxic conditions. All of these conditions can be compared optimum nutrient levels serving as the control. Similar experiments could be designed to evaluate plant response to multiple abiotic or biotic elements with or without N, P, or K stress now that deficient, optimal, and excess levels have been identified. Experiments could be designed that look at a combination of nutrients (N and P, P and K, N and K, or N, P, and K) to see if an interaction might afford lettuce increased protection when exposed to an abiotic or biotic stress. The uniform environmental conditions of a hydroponic system will minimize variance in the results of such experiments. This data will benefit researchers in nutrient-abiotic and biotic nutrient interactions because subtle influences of nutrients on plant stress will be detectable.
LITERATURE CITED


43. Maršić NK, Osvald J. Effects of Different Nitrogen Levels on Lettuce Growth and Nitrate Accumulation in Iceberg Lettuce (*Lactuca sativa* var. capitata L.) Grown


51. Barben SA, Hopkins BG, Jolley VD, Webb BL, Nichols BA, Buxton EA. Zinc, Manganese and Phosphorus Interrelationships and their effects on Iron and Cooper in


**TABLE 1-1.** Shoot and root macro and micro nutrient concentrations (in ppm) grown in 0.5, 1, 2, 4, 8, 16, 32 and 64 mg P/L solution. Concentrations with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>S</th>
<th>Zn</th>
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<tr>
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<td>5.0 a</td>
<td>182 a</td>
<td>2.27 x 10⁴ a</td>
<td>2.19 x 10³ d</td>
<td>172 a</td>
<td>188 a</td>
<td>2.12 x 10⁵ c</td>
<td>39.9 a</td>
</tr>
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<td>1 mg/L</td>
<td>7.65 x 10² a</td>
<td>6.1 a</td>
<td>172 a</td>
<td>2.28 x 10⁴ d</td>
<td>2.14 x 10³ d</td>
<td>163 a</td>
<td>222 a</td>
<td>2.26 x 10⁵ c</td>
<td>34.9 a</td>
</tr>
<tr>
<td>2 mg/L</td>
<td>8.53 x 10² a</td>
<td>5.0 a</td>
<td>182 a</td>
<td>2.30 x 10⁴ a</td>
<td>2.83 x 10⁸ d</td>
<td>188 a</td>
<td>352 a</td>
<td>2.13 x 10⁵ c</td>
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</tr>
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<td>4 mg/L</td>
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<td>2.30 x 10⁴ c</td>
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<td>6.9 a</td>
<td>209 a</td>
<td>2.24 x 10⁸ a b</td>
<td>5.03 x 10² a b</td>
<td>259 b</td>
<td>452 a</td>
<td>2.74 x 10⁵ a b c</td>
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<td>5.10 x 10³ a c</td>
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<td>32 mg/L</td>
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<td>8.2 a</td>
<td>276 a</td>
<td>2.09 x 10⁴ a b</td>
<td>4.44 x 10³ a b c</td>
<td>332 a</td>
<td>750 a</td>
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<td>2.98 x 10⁸ d</td>
<td>158 a</td>
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**Shoots**

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<th>K</th>
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<th>Mn</th>
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<tr>
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<td>12.7 a</td>
<td>507 a</td>
<td>2.23 x 10⁴ a b</td>
<td>3.00 x 10³ d</td>
<td>213 e</td>
<td>989 a</td>
<td>4.16 x 10⁵ a b</td>
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</tr>
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<td>1 mg/L</td>
<td>9.08 x 10² a b</td>
<td>17.1 a</td>
<td>1.04 x 10³ a b</td>
<td>2.33 x 10⁴ a b</td>
<td>4.41 x 10³ a b</td>
<td>559 d</td>
<td>1.30 x 10⁵ a b</td>
<td>52.2 a</td>
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</tr>
<tr>
<td>2 mg/L</td>
<td>5.89 x 10³ b c</td>
<td>14.4 a</td>
<td>708 a</td>
<td>1.64 x 10⁴ a b</td>
<td>2.83 x 10³ d</td>
<td>214 d</td>
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<td></td>
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<tr>
<td>4 mg/L</td>
<td>6.95 x 10³ b c</td>
<td>14.4 a</td>
<td>581 a</td>
<td>1.80 x 10⁴ a b</td>
<td>3.48 x 10³ c d</td>
<td>614 b d</td>
<td>4.39 x 10⁵ b c</td>
<td>56.5 a</td>
<td></td>
</tr>
<tr>
<td>8 mg/L</td>
<td>1.28 x 10⁴ a b</td>
<td>14.7 a</td>
<td>681 a</td>
<td>2.35 x 10⁴ a b</td>
<td>5.03 x 10³ a b</td>
<td>360 c d</td>
<td>2.52 x 10⁵ a b</td>
<td>54.5 a</td>
<td></td>
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<tr>
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<td>7.95 x 10³ b c</td>
<td>21.2 a</td>
<td>1.06 x 10³ a b</td>
<td>2.09 x 10⁴ b c</td>
<td>5.10 x 10³ a c</td>
<td>813 a b</td>
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<td>19.3 a</td>
<td>1.16 x 10³ a b</td>
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<td>4.44 x 10³ a b c</td>
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<td>3.47 x 10⁵ b c</td>
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<td>14.7 a</td>
<td>723 a</td>
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<td>2.98 x 10³ d</td>
<td>296 c d</td>
<td>1.36 x 10⁵ a b</td>
<td>50.7 a</td>
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TABLE 1-2. Shoot and root macro and micro nutrient concentrations (in ppm) grown in 0, 10, 20, 40, 80, 160, 320 and 640 mg K/L solution. Concentrations with the same letter are not significantly different at $P < 0.05$, Duncan Waller K Ratio Test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>B</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
<th>S</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/L</td>
<td>55.1</td>
<td>b,c</td>
<td>1.31 $\times 10^2$</td>
<td>c</td>
<td>7.1</td>
<td>c</td>
<td>84 a,b</td>
<td>d</td>
<td>136 b</td>
<td>1.80 $\times 10^1$</td>
</tr>
<tr>
<td>10 mg/L</td>
<td>59.9</td>
<td>c</td>
<td>1.29 $\times 10^2$</td>
<td>c</td>
<td>4.8</td>
<td>b</td>
<td>77 a</td>
<td>b,c</td>
<td>104 a,b</td>
<td>1.21 $\times 10^1$</td>
</tr>
<tr>
<td>20 mg/L</td>
<td>57.1</td>
<td>b,c</td>
<td>1.18 $\times 10^2$</td>
<td>c</td>
<td>3.9 a,b</td>
<td>107 a,b</td>
<td>2.20 $\times 10^1$</td>
<td>c,d</td>
<td>120 b</td>
<td>9.93 $\times 10^1$</td>
</tr>
<tr>
<td>40 mg/L</td>
<td>34 a</td>
<td>b</td>
<td>7.53 $\times 10^1$</td>
<td>a,b</td>
<td>2.8 a</td>
<td>96 a,b</td>
<td>1.36 $\times 10^1$</td>
<td>a,b</td>
<td>88 a,b</td>
<td>3.30 $\times 10^1$</td>
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<tr>
<td>80 mg/L</td>
<td>32.2 a</td>
<td>b</td>
<td>8.27 $\times 10^1$</td>
<td>a,b</td>
<td>2.6 a</td>
<td>71 a</td>
<td>1.05 $\times 10^1$ a</td>
<td>77 a</td>
<td>785 a</td>
<td>2.95 $\times 10^1$ a</td>
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<tr>
<td>160 mg/L</td>
<td>35.4 a</td>
<td>b</td>
<td>6.23 $\times 10^1$</td>
<td>a,b</td>
<td>4.0 a,b</td>
<td>85 a,b</td>
<td>1.51 $\times 10^1$ a</td>
<td>b</td>
<td>66 a</td>
<td>1.51 $\times 10^1$ a</td>
</tr>
<tr>
<td>320 mg/L</td>
<td>43.1 a,b</td>
<td>b</td>
<td>6.81 $\times 10^1$</td>
<td>a,b</td>
<td>3.2 a,b</td>
<td>136 b</td>
<td>1.27 $\times 10^1$ a</td>
<td>b</td>
<td>90 a,b</td>
<td>1.45 $\times 10^1$ a</td>
</tr>
<tr>
<td>640 mg/L</td>
<td>41.2 a</td>
<td>5.51 $\times 10^1$ a</td>
<td>3.5 a,b</td>
<td>118 a,b</td>
<td>1.08 $\times 10^1$ a</td>
<td>106 a,b</td>
<td>7.70 $\times 10^1$ a</td>
<td>4.47 $\times 10^1$ b</td>
<td>6.78 $\times 10^1$ b</td>
<td>53 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>B</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>Mg</th>
<th>Mn</th>
<th>Na</th>
<th>P</th>
<th>S</th>
<th>Zn</th>
</tr>
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<tbody>
<tr>
<td>0 mg/L</td>
<td>48.8 a,b</td>
<td>9.17 $\times 10^1$ a</td>
<td>24.1 a</td>
<td>456 b</td>
<td>688</td>
<td>d,e</td>
<td>124 b</td>
<td>2.71 $\times 10^1$ a</td>
<td>1.35 $\times 10^1$ a</td>
<td>8.09 $\times 10^1$ a</td>
</tr>
<tr>
<td>10 mg/L</td>
<td>56.8 a</td>
<td>8.71 $\times 10^1$ a</td>
<td>21.9 a</td>
<td>699 a</td>
<td>1.28 $\times 10^1$ a</td>
<td>222 a</td>
<td>2.24 $\times 10^1$ a</td>
<td>1.23 $\times 10^1$ a,b</td>
<td>7.33 $\times 10^1$ a</td>
<td>54 d</td>
</tr>
<tr>
<td>20 mg/L</td>
<td>28.9 b,c</td>
<td>6.37 $\times 10^1$ b</td>
<td>12.5 b,c</td>
<td>310 b,c</td>
<td>785</td>
<td>c,d</td>
<td>68 b,c</td>
<td>1.65 $\times 10^1$ b</td>
<td>1.18 $\times 10^1$ a,b</td>
<td>6.68 $\times 10^1$ a</td>
</tr>
<tr>
<td>40 mg/L</td>
<td>24.4 c</td>
<td>4.48 $\times 10^1$ c</td>
<td>9.3 c</td>
<td>191 c,d</td>
<td>745</td>
<td>c,d</td>
<td>46 c</td>
<td>1.05 $\times 10^1$ c</td>
<td>7.72 $\times 10^1$ c</td>
<td>6.22 $\times 10^1$ a</td>
</tr>
<tr>
<td>80 mg/L</td>
<td>38.7 a,b</td>
<td>5.72 $\times 10^1$ b,c</td>
<td>12.7 c</td>
<td>236 c,d</td>
<td>960 b</td>
<td>d</td>
<td>41 c</td>
<td>6.15 $\times 10^1$ c</td>
<td>6.03 $\times 10^1$ d</td>
<td>6.15 $\times 10^1$ a,b</td>
</tr>
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<td>160 mg/L</td>
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<td>5.09 $\times 10^1$ b,c</td>
<td>11.6 c</td>
<td>320 b,c</td>
<td>613</td>
<td>c</td>
<td>27 c</td>
<td>1.48 $\times 10^1$ d</td>
<td>8.78 $\times 10^1$ c</td>
<td>7.35 $\times 10^1$ a</td>
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<tr>
<td>320 mg/L</td>
<td>41.3 a,b</td>
<td>3.48 $\times 10^1$ d</td>
<td>8.3 c</td>
<td>123 d</td>
<td>616</td>
<td>a</td>
<td>22 c</td>
<td>1.32 $\times 10^1$ d</td>
<td>8.00 $\times 10^1$ c</td>
<td>6.99 $\times 10^1$ a</td>
</tr>
<tr>
<td>640 mg/L</td>
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<td>3.42 $\times 10^1$ d</td>
<td>23.4 a</td>
<td>240 c</td>
<td>892 b,c</td>
<td>288 a</td>
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<td>1.16 $\times 10^2$ b</td>
<td>1.11 $\times 10^2$ b</td>
<td>78 c</td>
</tr>
</tbody>
</table>
FIGURE 1-1. Oven dry weight of root and shoot tissue of lettuce grown in 2.5, 5, 10, 20, 40, 80, 160 and 320 mg N/L solution. For comparing root or shoot among solution N levels, bars with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.

FIGURE 1-2. Percent N in the shoots and roots of lettuce grown in 2.5, 5, 10, 20, 40, 80, 160 and 320 mg N/L solution. For comparing root or shoot among solution N levels, bars with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.
FIGURE 1-3. Percent C in the shoots and roots of lettuce grown in 2.5 (1), 5 (2), 10 (3), 20 (4), 40 (5), 80 (6), 160 (7) and 320 (8) mg N/L solution.
FIGURE 1-4. Carbon to N ratio in the shoots and roots of lettuce grown in 2.5 (1), 5 (2), 10 (3), 20 (4), 40 (5), 80 (6), 160 (7) and 320 (8) mg N/L solution.
FIGURE 1-5. Oven dry weight of root and shoot tissue of lettuce grown in 0.5, 1, 2, 4, 8, 16, 32 and 64 mg P/L solution. For comparing root or shoot among solution P levels, bars with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.

FIGURE 1-6. Percent P in the shoots and roots of lettuce grown in 0.5, 1, 2, 4, 8, 16, 32 and 64 mg P/L solution. For comparing root or shoot among solution P levels, bars with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.
FIGURE 1-7. Oven dry weight of root and shoot tissue of lettuce grown in 0, 2.5, 5, 10, 20, 40, 80 and 160 mg K/L solution. For comparing root or shoot among solution K levels, bars with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.

FIGURE 1-8. Oven dry weight of root and shoot tissue of lettuce grown in 0, 10, 20, 40, 80, 160, 320 and 640 mg K/L solution. For comparing root or shoot among solution K levels, bars with the same letter are not significantly different at P <0.05, Duncan-Waller K Ratio Test.
FIGURE 1-9. Percent K in the shoots and roots of lettuce grown in 0, 10, 20, 40, 80, 160, 320 and 640 mg K/L solution. For comparing root or shoot among solution K levels, bars with the same letter are not significantly different at P <0.05, Duncan Waller K Ratio Test.
CHAPTER 2

LITERATURE REVIEW

Since its domestication, lettuce has become an increasingly important agricultural plant. Evidence of lettuce cultivation reaches back 4,500 years ago in Egyptian tomb paintings which depict what appear to be bundles of stem lettuce, similar to the variety still grown in Egypt today (1). Written evidence of lettuce production dates back to 550 B.C. where it is mentioned by the Greek historian Herodotus (1). Lettuce was brought to the Americas by Christopher Columbus, and was grown mainly in home gardens and market gardens through the early part of the 20th century (1). Commercial growth of lettuce began in earnest in the 1920s and expanded rapidly through the 20th century (1). Currently, the United States ranks second in lettuce production worldwide (2), harvesting roughly 162,200 ha in 2011 (3). California provides 75% of that production, growing nearly 121,500 ha of lettuce in 2011 (3). Head lettuce (iceberg) accounts for about half of the lettuce grown in California with leaf types (butter leaf, romaine, etc.) making up the other half (4). Lettuce production is a billion-dollar industry. In 2011, combined head and leaf lettuce production in Monterey County California resulted in 1.2 billion dollars making it the most valuable agricultural crop in the county by more than 500 million dollars (4). This accounts for nearly half of the 2.4 billion dollars generated by lettuce production in the entire United States (3).

In 2011 nearly 121,500 ha of lettuce were harvested in the state of California in several production areas within the state including the Salinas Valley, the Oxnard Plain, the Santa Maria Valley, the San Joaquin Valley, and the Imperial and Palo Verde valleys (1). Variations in seasonal temperatures allow for a continual supply of lettuce year round (1). In California, lettuce is direct seeded on two-row beds in mineral soils of varying texture (1). Plants are
typically over-seeded with a desired spacing of 25-30 cm within the rows after thinning. Rows are typically spaced at 30 cm on top of raised beds (1). Plants can be irrigated by furrow, sprinkler, or drip methods (5), with the latter typically being used extensively after, or shortly before thinning occurs [(5), (1)]. However, some growers have begun using drip line during germination and throughout the growing season of the crop (5). Drip lines are typically installed between 2 plant rows on 1 m wide beds (5). Drip lines also allow growers to better manage plant nutrition through fertigation (5).

*Verticillium* wilt is a major pathogen afflicting crops around the world. *Verticillium* is generally found in the cool, temperate regions of the world [(6), (7), (8), (9)], but has been found in some tropical regions as well (10). *Verticillium* wilt affects a number of major crops worldwide including: artichoke, cotton, potato, tomato, strawberry, cauliflower, cucurbits, olive, eggplant, spinach, peppers, tobacco, cocoa and many other woody and herbaceous perennials [(11), (12), (13), (14), (9), (15), (16), (17), (18), (19), (20), (21)]. There are seven pathogenic species in the genus *Verticillium*, with the two major species, *Verticillium dahliae* and *Verticillium albo-atrum*, causing most of the wilt in agricultural crops [(7), (6)]. Both *V. dahliae* and *V. albo-atrum* are soil borne fungi which enter the plant through the root either directly or through wounds (7). *Verticillium* wilts colonize and are contained within the plants xylem vessels (6). Symptoms of *Verticillium* wilt differ from plant to plant, but generally include wilting of stems and leaves, death in smaller plants or seedlings, stunting, chlorosis or yellowing of leaves, tissue death, and defoliation [(7), (6)].

*Verticillium* wilt is spread in a number of ways. The pathogen can be spread by root contact between plants, air dispersal, water transmission, seed transmission, vegetative transmission, insect transmission and husbandry practices, and agricultural practices [(6), (22),
Verticillium wilt can also survive in soil for many years without a host plant [(7), (6), (9)]. Soil survival is associated with dark thick-walled mycelium in *V. albo-atrum*, and microsclerotia in *V. dahliae* (6). Viable mycelium has been recorded up to 4 years in the soil. The microsclerotia of *V. dahliae* however is much more durable with viable infection in the fields up to 14 years after cropping (6). The durability and longevity of *Verticillium* wilt contribute to its success as a pathogen, and to its threat to agricultural crops.

Until the mid-1990s lettuce was thought to be resistant to *Verticillium* wilt. However, in 1994 several fields on a farm in southern Santa Cruz County, Pajaro Valley, California reported the loss of an entire lettuce crop to an unknown disease (24). Initially, *Verticillium* was dismissed as the cause, although *V. dahlia* was the only pathogen isolated from the plant samples (25). Because lettuce was not thought to be susceptible to the disease, the loss was blamed on herbicides. However, in 1995, *Verticillium* was shown to be responsible for the loss of the crop [(26), (27), (24), (28), (29)]. Since that discovery in 1995, the incidence of *Verticillium* in lettuce has only gone up (Figure 1).

Since its initial discovery in 1995 in California through 2007 the number of fields infected was 64 (24). This works out to an average of 5 new fields infected per year. However, in 2008, 13 newly infected fields were reported (31). This was followed the next year by an explosion in the number of newly infected fields reported with 43 new fields reported in 2009 (30). Finally, the number of newly infected fields reported in 2010 was 30, bringing the total number of lettuce fields infected in California to be 150, representing some 1100 ha (31). A number of these fields experienced infection so severely that the entire crop was lost [(30), (31)]. In the United States, *Verticillium* wilt in lettuce has only been found in California; however,
while it had been reported on the island of Crete (30), it has since spread to Northern Italy and Germany (24), suggesting world-wide spread of the disease.

Symptoms first appear as early as the rosette stage, when the lower whorl of leaves wilt (28), however the most severe symptoms develop closer to market maturity (26). The most common and telling symptom is the greenish-black discoloration in the crown and taproot [(26), (28), (33)]. In many infected fields infection rates of greater than 80% are present resulting in the loss of the entire crop [(26), (25), (28)]. Because lettuce is so important economically, it is vital that effective controls for *Verticillium* wilt be discovered.

One of the most successful controls for *Verticillium* wilt is breeding resistant varieties [(7), (34), (9), (35), (36)]. However, because it was assumed for so long that lettuce was resistant to *Verticillium* wilt, efforts to breed resistant varieties are significantly behind the work that has been done for other crops. For example, researchers have been developing resistant varieties of cotton since the 1930s (9). Work to develop resistant varieties of lettuce, however have only been going on since the mid-1990s (32). While advances in genetic screening methods have led to successes in this effort [(37), (38), (39)], much more work remains to be done.

While resistant varieties of lettuce are developed, other control methods must be developed and implemented in order to minimize the damage done to lettuce fields, especially as the number of fields affected by the disease continues to grow. Another method of control used in the fight against *Verticillium* wilt is crop rotation. For example, when cauliflower fields are rotated to broccoli (a non-host plant) microsclerotia levels in the soil are actively decreased [(40), (18)]. However, this method is not without its drawbacks. While it is true that rotating in a non-susceptible crop will lower the number of microsclerotia, it has been shown that when susceptible hosts are reintroduced there will still be some infection (23). Due to the wide range
of host species, it has been determined that crop rotation is not a viable control method for lettuce (38).

Currently the most widely used control of *Verticillium* wilt is soil fumigation. Chemical fumigation has been used to control *Verticillium* wilt in a number of crops including potatoes [(41), (42)], olives (43), and strawberries (10). However, this method of control is extremely expensive (44). In California, chemical fumigation costs between $800 and $1200 per ha With more than 1100 ha of lettuce fields infected with *Verticillium* wilt this would be a very expensive operation and in fact, chemical fumigation of lettuce is cost prohibitive (38). Additionally, the most effective chemical fumigants are becoming unavailable (44). For example, Methyl bromide, the most heavily used and effective fumigant for *Verticillium* wilt control (10) is scheduled for worldwide withdrawal by 2015 (43).

With these control methods ineffective or unavailable, additional control methods must be developed, especially for the control of *Verticillium* wilt in lettuce. One promising method of control is mineral nutrition.

A number of mineral nutrients are essential to plant growth. They are generally divided into two categories: macronutrients and micronutrients. The macronutrients are: Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Sulfur (S) [(45), (16), (46), (47), (48), (49)]. The first three macronutrients (N, P, K) are considered most important due to the large amount needed for proper plant growth. These three elements are generally the first to be depleted in the soil (45). The remaining three macronutrients (Ca, Mg, S) are typically available in sufficient quantities within the soil for proper plant growth (45). The micronutrients are: Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Boron (B), Molybdenum (Mo),
Chlorine (Cl) Nickel (Ni), and Cobalt (Co) [(45), (50), (51)]. These nutrients are not needed in large quantities for plant growth, and in the extremes can prove toxic to plants (50).

The relationships and mechanisms by which plant nutrients and plant diseases interact are varied and complex. A particular disease might inhibit the plant’s ability to absorb an essential nutrient (51) while the absorption of a particular nutrient might allow the plant to escape the effects of a particular disease [(45), (51)]. Proper plant nutrition is essential to resisting a disease. If an otherwise health plant is deficient in any of the nutrients required for proper growth its susceptibility to disease is increased [(51), (46)]. Proper plant nutrition can also inhibit the pathogens ability to infect the plant (45). One of the advantages to managing disease with nutrients is that to a certain degree growers can control the nutrients available and the timing of their availability to the plant (51). This is especially true in drip irrigation systems through the use of fertigation.

Generally, mineral nutrition and soil fertility affect *Verticillium* wilt in two major ways: 1) by reducing the inoculum density of microsclerotia in the soil and 2) influencing the host plant’s resistance to the pathogen (34). Because *Verticillium* wilt affects so many important crops, a number of studies on nutrition and its effects on *Verticillium* wilt have been done. These studies have examined both the effects of different nutrients on the pathogen directly and the effects nutrition has on the host plant’s response to the pathogen. A majority of these studies have focused on the effects of N, P, and K due to their importance as essential macronutrients. Within studies looking at nitrogen, two different forms of nitrogen were considered (Table 1). Some studies have also looked at the impact of micronutrients on *Verticillium* wilt (Table 2). Due to the wide variety of host plants for *Verticillium* wilt, it quickly becomes clear that there are
no hard and fast rules concerning mineral nutrition and *Verticillium* wilt management. Results tend to vary based on host plant and other growing conditions such as soil pH (52).

*Nitrogen*

Nitrogen is the fourth most abundant plant nutrient and is essential for the production of amino acids, proteins, enzymes, hormones, phytoalexins and other cellular components (46). Nitrogen promotes growth and delays plant maturity and is often limited in the soil (46). Plants uptake two forms of nitrogen from the soil: NH₄ and NO₃ (46). Of all elements it is applied in the highest quantity on crops due to its rapid loss in soils (45).

A number of studies have been done to find the effect that N has on *Verticillium* wilt (Table 1). Generally, *Verticillium* wilt rates decrease when N is made available in the NH₄ form rather than the NO₃ form which generally increases rates of *Verticillium* wilt. Although the exact mechanisms by which NH₄ decreases *Verticillium* wilt are unknown, it is postulated that the change in rhizosphere pH due to the extrusion of H⁺ ions to balance the charge created by NH₄ has a detrimental effect on the pathogen [(55), (34)]. The study of the effect NH₄ has on *Verticillium* wilt in lettuce (38) hypothesized that because lettuce was already grown under low pH conditions the effect was mitigated.

*Phosphorus*

Phosphorus deficiency in soils severely limits plant yield (47). Within the plants P is primarily used for energy transfer and protein metabolism (47). As reported in Table 2, P does not seem to have as great an influence on *Verticillium* wilt as other nutrients do. In many instances an increase in P fertilization independent of other nutrients results in an increase in *Verticillium* wilt rates.
Potassium

In plants the uptake of K is generally greater than for that of any other nutrient; additionally, unlike N and P, K does not become part of any plant constituent but rather, remains unattached as a regulator of plant growth (48). Generally, as reported in Table 2, increased K fertilization corresponds to a decrease in *Verticillium* wilt rates. In particular, cotton plants heavily infected with *Verticillium* wilt show a deficiency of K (61) suggesting a direct effect between K and *Verticillium* within the plant.

Manganese

Of the micronutrients, Mn stands out as being able to decrease *Verticillium* wilt rates in a range of host plants. Within plants, Mn is rather immobile, but plays a key role in important biochemical and physiological processes such as photosynthesis (70). Generally, plant tissues low in Mn are more susceptible to fungal diseases such as *Verticillium* wilt (71). Tissues with higher Mn concentrations resist fungal infections (71). Manganese availability works in tandem with the form of N present and soil pH. Higher Mn uptake is generally found in low pH soils with N present in the ammonia form (NH₄) [(71), (72)].

Sulfur, Copper, Boron, Molybdenum, Zinc

Little research has been done on these elements and their effects on *Verticillium* wilt, except perhaps for Cu (Table 2). Of all the mineral nutrients, only Cu has been shown to kill *Verticillium* wilt directly (50). Sulfur, as a major component in plant defenses (62), has been shown to increase resistance to *Verticillium* wilt, but only in specific plant varieties that are adapted to taking up more sulfur than other varieties (73). Boron caused increased resistance to *Verticillium* in tomato plants but it did not have as drastic an effect on resistance as did Cu and Mn (50). Molybdenum and Zn showed no significant results on *Verticillium* wilt in tomato (50).
Due to the high cost of fertilization, the economic impact of rotating lower value crops, and the ability to target fertilize through drip irrigation systems, managing *Verticillium* wilt through mineral nutrition is a promising avenue to pursue while resistant lettuce varieties are developed. Through fertigation growers could deliver precise amounts of nutrients at optimal stages of development, hopefully mitigating the severity of *Verticillium* wilt. Our study will examine the effects of four nutrients on Verticillium wilt in lettuce: Nitrogen, Phosphorus, Potassium and Manganese. It is our hypothesis that these nutrients, in combination and isolation will mitigate the effects of Verticillium wilt in lettuce crops.
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TABLE 2-1. Effects of Nitrogen (divided by type) on severity of *Verticillium* wilt, arranged by host plant.

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>N (unspecified)</th>
<th>NO$_3$</th>
<th>NH$_4$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton</td>
<td>Increase</td>
<td></td>
<td></td>
<td>(53)</td>
</tr>
<tr>
<td>Eggplant</td>
<td>No effect</td>
<td>Increase</td>
<td>Decrease</td>
<td>(55), (16)</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Increase</td>
<td></td>
<td></td>
<td>(38)</td>
</tr>
<tr>
<td>Olive</td>
<td>Decrease</td>
<td></td>
<td></td>
<td>(43)</td>
</tr>
<tr>
<td>Potato</td>
<td>Decrease</td>
<td></td>
<td></td>
<td>(56)</td>
</tr>
<tr>
<td></td>
<td>No effect</td>
<td></td>
<td>Decrease</td>
<td>(41), (52)</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>Increase</td>
<td></td>
<td></td>
<td>(59)</td>
</tr>
<tr>
<td>Unknown</td>
<td>Decrease</td>
<td></td>
<td></td>
<td>(60)</td>
</tr>
</tbody>
</table>

TABLE 2-2. Effects of various mineral nutrients on severity of *Verticillium* wilt, arranged by host plant.

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>P</th>
<th>K</th>
<th>S</th>
<th>Cu</th>
<th>Mn</th>
<th>B</th>
<th>Mo</th>
<th>Zn</th>
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<tr>
<td>Cocoa</td>
<td></td>
<td>Increase</td>
<td></td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td>(53)</td>
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<tr>
<td>Cotton</td>
<td></td>
<td>Increase</td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(61), (63), (64), (65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(19)</td>
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<tr>
<td></td>
<td></td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(66)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(67)</td>
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</tr>
<tr>
<td>Pistachio</td>
<td>Decrease</td>
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<td></td>
<td></td>
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<td>(68), (1)</td>
<td></td>
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<tr>
<td>Potato</td>
<td></td>
<td>Increase</td>
<td>No effect</td>
<td>Decrease</td>
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<td></td>
<td></td>
<td>(58)</td>
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<td></td>
<td></td>
<td>No effect</td>
<td>No effect</td>
<td>Decrease</td>
<td></td>
<td></td>
<td></td>
<td>(52)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>No effect</td>
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<td>Decrease</td>
<td></td>
<td></td>
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<td>(56), (57)</td>
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<tr>
<td>Tomato</td>
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<td>Decrease</td>
<td>Decrease</td>
<td>Decrease</td>
<td>No effect</td>
<td>No effect</td>
<td>(50)</td>
<td></td>
<td></td>
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</tbody>
</table>

50
FIGURE 2-1. Shows the spread of Verticillium wilt on California lettuce fields since its discovery in 1995 through the year 2010. [(24), (30), (31), (32)]