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The effect of fertilizers and 2-chloro-6-(Trichloromethyl) pyridine on growth and nodulation of soybeans and growth, nitrate, and potassium accumulation by radish and spinach.

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THE EFFECT OF FERTILIZERS AND 2-CHLORO-6-(TRICHLOROMETHYL) PYRIDINE ON GROWTH AND NODULATION OF SOYBEANS AND GROWTH, NITRATE, AND POTASSIUM ACCUMULATION BY RADISH AND SPINACH

A Dissertation Presented

By

LESLIE JAMES GLOVER

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DEDICATION

To my wife, Mattie Marie (Giles) Glover, through whose love, tolerance, support, and sacrifice this manuscript was made possible. And, for my children (Sandra, Geraldine, Leslie J. II, Leonardo, and Katherine) in the hope that this work will propel their futures into brighter tomorrows.

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ABSTRACT

The Effect of Fertilizers and 2-chloro-6-(trichloromethyl) pyridine on Growth and Nodulation of Soybeans and Growth, Nitrate, and Potassium Accumulation by Radish and Spinach

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The production of protein is of prime importance to all life on earth. Plants are able to synthesize their own protein from the basic building blocks (amino acids). But, animals are dependent on plants for their protein.

Soybeans are a good source of protein for dietary intake. Ordinarily, they are able to grow without N fertilizer since they are legumes and as such are able to fix atmospheric N_2 as a result of the symbiotic relationship between *Rhizobium japonicum* and the host plant in a process called nodulation. However, nodulated soybeans do not always produce an optimum yield. Indeed, it has been shown in some cases that NH_4 and urea applications can increase productivity while having no affect on nodulation or N fixation. But, NO_3 applications have usually retarded nodulation, and hence reduced N fixation. Likewise, nitrates are readily removed from the rooting zone via leaching whereas NH_4 and urea are held by soil colloids.

The objectives of part I and II of this study were to (1) increase soybean yield by N fertilization, (2) see which source of N was best for soybean production, (3) optimize N efficiency by using a nitrification inhibitor (nitrapyrin), and (4) observe the affects of N applications, nitrapyrin (Ns) and inoculum on soybean growth and nodulation. The objectives of part III were to: (1) grow radish and spinach plants that are low in nitrate, (2) see the affects of N, K, and Ns additions on radish and spinach growth, and (3) see the affect of Ns on NO_3 and K accumulation.

Neither Ns nor increasing applications of NO_3, NH_4, or urea fertilizer (25 to 400 ppm) had any affect on pod weight, N content, or nodulation of soil grown 'Amsoy' soybeans. However, NH_4 did decrease soil pH. Likewise, high rates of N (above 400 ppm) decreased the % N in pods (possibly as a result of reduced translocation) and vegetation (due to the dilution effect).

Neither Ns nor increasing rates of $(NH_4)_2SO_4$ (100 to 800 ppm) effected % N in soil grown 'Horosoy' soybeans. Also, Ns caused 'Horosoy' soybeans to retain their cotyledons, to produce lateral growth at the cotyledonary nodes, to have twisted leaves, and the plants to be short and bushy. But,no symptoms of phytotoxicity were observed on Ns treated 'Amsoy' soybeans.

Inoculum played a role in increasing the plant yield, % N, mg N/pot and nodulation of soil grown 'Amsoy' soybeans. Also, it increased pod yield and nodulation of soil grown 'Horosoy' soybeans, but had no effect on yield, % N, or mg N/pot of vegetative and root plant portions.

In part II (hydroponics), inoculum and N additions increased the dry weight of pods, vegetation, and roots for both cultivars as well as the pod number of 'Horosoy' soybeans. Nitrapyrin decreased the root dry weight and vegetative N content, but caused no observable phytoxicity symptoms. Likewise, Ns had no effect on pod or vegetative dry weight, % N, or pod and nodule number of 'Horosoy' soybeans.

In part III, spinach and radish, two fast growing vegetable species, were grown. These crops are important sources of minerals and roughage in the human diet, but they have been shown to accumulate nitrates, and thus they are potentially carcinogens since nitrates can be reduced to nitrites, and nitrites have been implicated as carcinogenic agents.

The use of K fertilizer has been suggested as a means of controlling NO_3 accumulation and Ns and NH_4 applications have been shown to control the NO_3 concentration of the plant. However, no data could be found which employed the three factors together so three greenhouse experiments were conducted in this study to assess the affects of these factors on vegetable growth.

The optimum level of N for radish growth was 200 ppm, but NH_4 levels greater than 100 ppm caused diminished returns on yield. Likewise, high rates of NH_4 depressed root and leaf weight, % K, and mg K/pot. High levels of NO_3 depressed root and leaf weight and hence K content, but it did not depress the K concentration of any plant part. Potassium applications did not affect the weight of leaves, but it increased root weight, the % K in leaves, and K uptake by roots. Nitrapyrin in the presence of NH_4 increased the dry weight of leaves and roots, but it did not affect the % K or mg K/pot. Likewise, Ns in the presence of NO_3 had no affect on either weight, % K, % NO_3, mg K or mg NO_3 per pot.

Spinach weight increased with N and K applications. Nitrate was the most beneficial source of N for increasing weight. The increased weight caused a dilution of the K, Ca, and P concentration. Nitrapyrin increased P concentration, but it had no influence on K or Ca concentration. Likewise, the chemical had no affect on K, Ca, or P content.

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INTRODUCTION

Practically all animal life depends largely on plants as their source of energy. Plants are able to fulfill this important function for animals because they are able to fix energy from the sun via photosynthesis.

Animals depend totally on their environment to provide them with the necessary food for survival. Their populations fluctuate in proportion to their food supply. Unlike other animals, over the years, man has learned to control his food supply and his numbers have increased tremendously in response to an increase in productivity from cultivated crops.

Indeed, the ever growing human population on earth is taxing our food supplies more than ever. Our population grows in geometric progressions while our food supplies grow in arithmatic progressions. Therefore, we could outgrow, or exhaust our food supply in the future.

Although birth control and other measures are being tried in attempts to alleviate this problem, they may not be enough to rectify the situation. Therefore, in an effort to meet the increased demands on our food stocks, we have to produce more food.

The use of nitrogen fertilizer to increased yield of agricultural crops is a well known practice which is employed by many farmers throughout the world. In recent years, pressure has been posed on farmers to utilize fertilizer more efficiently (especially N) than previously. Farmers must maintain this efficient use of N while increasing the yield of crops that they grow. Towards these goals, research plays an important role and it is an instrument that must be utilized to solve our food problems.

Research has shown us that the largest loss of N from the rooting zone of plants is via leaching. The leached nitrates are moved through the soil profile by the movement of water through this medium. Additional N losses occur as a result of denitrification and volatilization. 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), a chemical which is manufactured by the Dow Chemical Company, Midland, Michigan, has shown some promise of being effective in retarding N loss.

Nitrapyrin slows the oxidation of ammonium fertilizers to nitrite and thence to nitrate. Nitrapyrin accomplishes this feat by controlling the population of Nitrosomonas bacteria in the growth medium (5,39,41). Not only does this cut down on losses of N via leaching, but it also retards losses by denitrification and volatilization, since the N would remain in a form (NH_4) that is held by soil colloids as a cation.

Also, since nitrapyrin retards the conversion of ammonium to nitrate, it should serve as an effective means of controlling nitrate accumulation by plants (63,110). This would be a worthwhile function from a health point of view.

Recently, concern has been voiced over the use of nitrites in food products for human consumption. According to news reports, nitrites have been shown to be carcinogenic agents and as such are dangerous to people. By cutting down on the uptake and accumulation of uncombined N (NO_3), the potential for nitrite toxicity is reduced (63). Likewise, if no nitrate is present in the plant tissue, the chance of it being reduced to nitrite when consumed by animals (10) or when stored in cans (87) is eliminated.

To gain some insight into the improved utilization of N fertilizers while attempting to increase the yield of soybean, radish, or spinach, studies were conducted using nitrapyrin and different rates and sources of N on these cultivars as well as applications of K on radish and spinach cultivars.

Soybeans are an important food source in the world market. Some people use them in their original form or as bean sprouts while others use them as food additives. They are quite popular among vegetarians since they have a high protein content. Also, they have a high content of oil which is often used for cooking. In addition, they are used as a source of high protein feed for livestock.

Although radish and spinach are not good sources of protein, they are good sources of minerals, and they also serve as a source of roughage in the human diet. Radish and spinach are fast growing cool season vegetable species; thus, they are often used as vegetables in the diet of many people. However, they have been shown to accumulate nitrates (10,21,22,23,24,25,63).

REVIEW OF LITERATURE

A review of the affects of a nitrification inhibitor (nitrapyrin) and fertilization on certain plant species follows.

Effectiveness of nitrapyrin

The presence of 2-chloro-6-(trichloro-methyl) pyridine (nitrapyrin) in a plant growth medium prohibits nitrification of ammonium and urea fertilizers by controlling the population of Nitrosomonas bacteria (5,39,41). The finding that nitrapyrin retards nitrification (5,19,20,29,39,40,41,42,48,49,52,54,57,63, 76,83,92) seem to be well accepted as fact.

Although nitrapyrin has been shown to retard nitrification, there are some factors that reduce its effectiveness. Large quantities of organic matter in a soil increase nitrapyrin sorption and thereby decrease nitrapyrin activity (19,20,40,41,49). Increases in soil temperature decreases its effectiveness (20,41,48). Nitrapyrin hydrolizes in soils that have a high pH (20,82). Goring (41) said that nitrapyrin sorption is not consistently affected by the clay content of the soil. But, Bundy and Bremmer (20) reported that nitrapyrin is most effective in light textured soils.

Safety of nitrapyrin

Nitrapyrin like any other chemical that is used to aid in food production has to be tested before it can be used commercially. The principal breakdown product of nitrapyrin is 6-chloropicolinic acid (5,7,67,82).

Mullison and Norris (67) said that no appreciable residues of nitrapyrin can be found in crops after its use at the 0.5 lb/A rate since it is degraded to 6-chloropicolinic acid. The 6-chloropicolinic acid is taken up by the plant in small quantities but does not accumulate in the tissue of farm animals (beef calves, pigs, milk of dairy cows, and chickens) to any extent. They found nitrapyrin to be short lived in water (lost via volatization and dehydration). Its hydrolysis is not affected by pH between 3.1 and 8.4. The chemical has an acute oral LD_{50} of 1070 mg/kg for male rats and 1230 mg/kg for female rats. 6-chloropicolinic acid has an acute oral LD_{50} of 2830 mg/kg for male rats and 2180 mg/kg for female rats.

Nitrapyrin has no effect on the soil bacterial population at 1 and 10 ppm and increased the number of colonies at 400 and 1000 ppm (67). Likewise, Laskowski et al (57) indicated that Rhizobium phaseoli growth was inhibited 50% by 1000 ppm of 6-chloropicolinic acid. However, growth of none of the 17 bacterial species studied was affected by nitrapyrin.

A consideration of nitrapyrin effects on legume growth

There is some controversy concerning whether or not nitrapyrin is toxic to plants when it is applied at recommended rates (39,40, 67). The recommended rates are 0.14 to 2.24 kg/ha (40,52) or 1 to 2% of the applied N (39,42).

Phytoxicity depends to a large extent upon the concentration of nitrapyrin as well as the plant species and soil in question (40). Goring (41) observed no reduction in alfalfa growth when 12½ ppm of nitrapyrin on a soil basis was used. But Geronimo et al (40) and

McKeel and Whalley (66) suggested that there was an effect on alfalfa growth. Likewise, Lynd et al (61) pointed out that soil concentrations of nitrapyrin as low as 1 ppm cause severe leaf curling, internode enlongation and abnormal tendril growth of black locust (Robina pseudoacacia).

Geronomo et al (40) stated that when nitrapyrin is applied at recommended rates, there is no effect on soybeans. However, Riley and Barber (83) reported alterations in soybean morphology with as little as 1 ppm of nitrapyrin in the medium. They found stubby and club-like roots with swellings, particularly just behind the root tips, when compared with normal more fibrous roots. Likewise, Parr et al (76) indicated that soybean roots in pots treated with the chemical were stunted and flacid.

The effect of inoculation on growth and nodulation of soybeans and related legumes

Soybeans can be grown without using any N fertilizers since they are leguminous species and as such are able to fix atmospheric N_2 through association with bacteria (Rhizobium japonicum). However, nodulation with certain Rhizobial strains may not cause soybeans to produce the optimum yield that can be obtained from this species.

Indeed, low yields could be due to the fact that not all nodulation of soybeans or other legumes is effective nodulation. Virtanen (101) suggested that nodulation with an ineffective strain of Rhizobium prevented subsequent nodulation with effective strains and, thus, N fixation. Likewise, Munns (70) noticed that developing nodules on Medicago sativa can suppress further infection by suppressing the emergence of root hairs on newly developing roots.

Wright (108) found two biotypes of bacteria that cause soybean nodulation. He classified them as Type-A (those that primarily invade the main root and withstand a pH as low as 4.5) and Type-B (those that have a tendency to produce scattered nodules on the lateral roots of the plants and can withstand pH as low as 4.1). In another study, Wright (109) showed that plants inoculated with type A strains fix two times more N than plants inoculated with type B strains. Other factors that have been shown to affect legume nodulation include: soil population of Rhizobia, weather, hormones, enzymes, pH and calcium content of the growth medium, and interactions between root carbohydrate content and N fertilization. Nodulation failures often result from low Rhizobial numbers in the growth medium (16, 71). Munns (68) noticed that cloudy weather disrupts nodule efficiency. Also, Fahraeus and Ljunggren (35) indicated that polygalacturonase plays a role in root hair infection.

Low pH has been shown to decrease nodulation of soybeans (70,108). Also, calcium has been shown to be necessary for nodulation (2,60). Munns (70) indicated that decreasing the pH from 5.6 to 4.8 increased the Ca concentration required to nodulate 50% of Medicago sativa plants from 0.1 mM to 6 mM. Lowther and Loweragan (60) suggested that the effect of pH and Ca concentration occurs at the stage of nodule initiation. They also observed that once initiated, nodule development proceeds at concentrations of Ca too low for plant growth. They did not attribute the high Ca requirement for root infection (nodule initiation) to either survival of Rhizobium or the effects of Ca on a number of relevant plant processes (tap root length, root hair development, or lateral root initiation). Similarily, Munns (70) pointed out that root extension and root hair production were insufficiently affected by Ca or pH to explain reductions in nodule number.

Decreased nodulation of soybeans in the presence of nitrogenous salts is due to an inadequate carbohydrate supply in the roots because the carbohydrate synthesized is used for top growth and little is available for growth of nodules (94). Latimore et al (58) found that inorganic N did not influence photosynthetate distribution in soybean plants, except in nodules. Nitrogen decreased the incorporation of ^{14}C in nodules during the pod filling stage of growth. They observed a decrease in N_2 fixation when either nitrate or ammonium was the N source. They attributed the decreased fixation to a decreased carbohydrate supply in the nodules.

Hopkins (50), as early as 1910, noticed that inoculated legume crops will get 1/3 of their N needs from the soil and about 2/3 from fixation of free nitrogen. Also, Weber (106) suggested that the amount of N fixed symbiotically ranged from 1 to 142 pounds per acre for soybeans which accounts for 1 to 74% of the total N taken up. This amount is only about half of the amount reported by Bezdicek et al (16) who reported that soybeans fixed about 263 kg N/ha when inoculated with granular inoculum.

The effect of nitrogen fertilization on growth and nodulation of soybeans and related legumes

Although the values for N fixation are high, they may not be large enough to insure optimum plant yield. Some investigators (3,4,14,28,45,46,58,72,73,75,89,94,100,106), who wish to increase soybean yield, do not believe that nodulated soybeans alone produce the maximum amount of yield that can be attained from that species. Towards the end of attaining maximum yield from soybeans, they propose the use of N fertilizers as an added source of nutrients for plant growth.

Results from using N fertilizers in addition to nodulation for soybean growth have been varied and seem to depend on the form of N used. Many investigators (3,4,14,45,58,68,69,72,73,81,93,102) agree that effective nodulation of legumes is prevented when nitrate fertilizer is the N source. High rates of ammonium fertilizer either alone or in conjunction with nitrate fertilizer has also been shown to inhibit nodulation (28,58). Tanner and Anderson (93) showed that nitrates in the external medium catalizes the destruction of IAA while ammonium decreases the amount of tryptophan converted to IAA. They suggested that the destruction of IAA is the reason nodulation is prevented when nitrate or ammonium is the N source.

Results from an experiment conducted by Vigue et al (100) showed that 18mM urea had no effect of nodulation. In the same study, they noticed that 2 mM nitrate nitrogen inhibited effective nodulation. Likewise, Latimore et al (58) noticed that nitrates had a greater effect of nodulation than did ammonium at the same rates, but differences were not significant at the 5% level.

Munns (68) found that 0.2 mM nitrate delayed the appearance of the first nodules by one day, caused the first crop to appear in two minor stages, and reduced the average number of nodules in the first main crop from 25 to 7 per plant. In another study Munns (69) found that nodule numbers were progressively lowered with increasing rates of nitrates and so was the number of root hairs and the percentage of root hairs curled. But, the number of curled root hairs exceeded the number of nodules by at least tenfold at all N levels. He also said that nitrates inhibit the production and curling of root hairs and the initiation and development of infection threads. However, when an initial supply of nitrate is known to be exhausted, an initial delay or inhibition of nodulation can give way to increased nodulation. Furthermore, continued long term nitrate treatment may cause little proportionate reduction in nodule number.

There are many conflicting reports in the literature with regard to whether nitrogen fertilization increases yield of legumes or not. Some researchers (1,4,72,75,89) have found an increase in soybean yield after N was added to the growth medium. However, other researchers (3,28) did not observe an increase in yield with the addition of N fertilizers. Also, some researchers (3,4,14,16, 26,38,58,73,98) believe that N fertilization serves to replace the N fixing ability and the nodulation response of soybeans.

There are conflicting reports on the amount of applied N needed for soybeans to replace that obtained from fixation. Hanway and Weber (46) grew nodulating and non-nodulating 'Hawkeye' soybeans and observed that they produce the same seed yield at 672 kg N/ha.

However, Parker and Harris (75) only observed an increase in yield when N was applied at the rate of 134 kg/ha or less.

The effect of nitrapyrin on spinach, radish and select other plant species

Nitrapyrin applied with ammonium has improved yield of rice, spinach, and sugar beets (99). Swezey and Turner (92) suggested that growth, N uptake based on leaf and petiole analysis, and beet sugar yield were increased by the addition of the chemical to the fertilizer. Yield was not improved by increased concentration of nitrapyrin. They said that the most consistently effective chemical dosage was 1% of the nitrogen.

Mills et al (63) showed that 50 mg of nitrapyrin per pot essentially eliminated the accumulation of nitrate in both roots and shoots of radish. However, the chemical increased the potential for ammonium toxicity.

Zawistowska et al (110) reported a reduction in nutrient concentration by cucumber seedlings grown in the presence of nitrapyrin. Nitrate, potassium and calcium concentrations were restricted 24, 17, and 25% respectively by nitrapyrin relative to untreated controls.

Nitrapyrin has been shown to produce auxin type growth (61, 110). Likewise, Hendrickson et al (48) indicated that potato growth on nitrapyrin treatments was characterized by stunted, dark green tops with bushy rather than vine-like development. However, symptoms were less evident as N rate increased.

The effect of nitrogen and potassium fertilization on nitrate accumulation

The increased use of N fertilizers by farmers has caused some concern about uncombined forms of N in plant tissue. These uncombined forms of N include ammonium, nitrite, and especially nitrate. An appreciable concentration of either nitrite or ammonium can cause severe problems with plant growth. However, large quantities of nitrate can accumulate in plant tissue without the plant itself being adversely affected (10,21,22,24,25,63). Nitrates in the plant tissue can cause problems when this tissue is consumed by animals and reduced to nitrite or when it is reduced in cans after the plants have been processed (10,87).

Nitrates may accumulate in plants, especially vegetables, as a response to a number of factors. These factors include large applications of nitrate fertilizer (21,25,64,79), length of time the plant is exposed to nitrate (9), and changes in photoperiod (24).

There are conflicting trends of thought in relation to what effect K has on the nitrate concentration of plant tissue. Some investigators (23,24,64) have suggested that K causes nitrate accumulation while others (104,105) say that K does not cause accumulation of nitrates by plant tissue. Still others (62,95,103) indicated that K decreases soluble N compounds by aiding in protein synthesis.

The form of N used has a great influence on K uptake and utilization by plant tissue. Kinetic analysis showed ammonium to be strictly competitive with potassium-rubidium (88). Kirkby and

Mengel (55) found a reduction in K uptake when ammonium was in the medium rather than nitrate or urea. Furthermore, Becking (15)found that in older plants ammonium ions in the growth medium exchange for K ions of the root. He indicated that this exchange can be considerable.

When ammonium is absorbed as the principal form of N, a high concentration of K is required for optimum plant growth (13,31,43). Krogmann et al (56) showed ammonium ions to be very effective uncouplers of photosynthetic phosphorylation. They reported 95% inhibition of ATP formation by NH_3 without any inhibition of ferricyanide reduction in the phosphorylating reaction.

The reduction in the amount of ATP formed as a result of ammonium nutrition may be the reason that K uptake is suppressed when ammonium is the N source. Since ATP is required for K uptake, the plants should take up less K when ammonium is in the medium.

Potassium absorption plays a role in the photosynthetic process. Puritch and Barker (80) noticed that ammonium accumulation was accompanied by a loss of chlorophyll and a decrease in photosynthetic activity of tomato leaf chloroplasts. However, chloroplasts may not be the only organelles that accumulate large quantities of K.

Stomates have been shown to open when large quantities of K is in the guard cells of plants (53,85). The closing of the stomates should cause the photosynthetic rate to be decreased since it would lessen the amount of CO_2 taken up from the atmosphere. Terry and

Ulrich (97) presented evidence that showed a decreased in CO_2 uptake per unit area of sugar beet leaf when the plants were grown in K deficient nutrient solution.

PART I

NODULATION AND GROWTH RESPONSES OF SOYBEANS GROWN WITH NITRAPYRIN, INOCULUM, AND DIFFERENT SOURCES AND RATES OF NITROGEN

Abstract: Soybeans (*Glycine max* (L) Merrill) were grown to physiological maturity in pots containing 1 kg of silt loam. In Experiment 1, 'Amsoy' soybeans were grown in four replicates of a factorial design consisting of 5 rates of $Ca(NO_3)_2$, $(NH_4)_2SO_4$, or urea fertilizers and 3 regimes of nitrapyrin (Ns) and inoculum (I) "Ns+I, Ns, and I". In Experiment 2, 'Horosoy' soybeans were grown in five, replicated, randomized, complete blocks consisting of 4 rates of $(NH_4)_2SO_4$ and 3 regimes of Ns and I. Also, a zero N and a control treatment were used. For each experiment, inoculum was applied as a seed coat. The fertilizer was applied to the medium 2 weeks after seedling emergence. Nodules on the roots were counted. The weight and N content of the pods and vegetative plant portions were determined. Also, the soil pH was determined. There was not a significant difference among the N sources with respect to pod weight, N content or nodulation of 'Amsoy' soybeans. However, ammonium fertilizer did decrease the soil pH. Relative to the no-N treatment, increasing rates of N in the growth medium did not appreciably alter pod weight, pod or vegetative mg N/pot, or nodule number of either cultivar. Vegetative weight was significantly increased while the soil pH was significantly decreased by N fertilization. The % N of 'Amsoy' soybeans was significantly decreased by N fertilization. But, the % N of 'Horosoy' vegetation was not affected by fertilization while that in pods was significantly

increased when N was added to the medium. Nitrapyrin had no effect on pod weight, pod or vegetative % N, pod mg N/pot, or nodule number of 'Amsoy' soybeans. However, it did significantly lower vegetative weight and mg N/pot, while soil pH was not affected. The weight, % N, and mg N/pot of 'Horosoy' soybean plant portions were not affected by nitrapyrin in the growth medium. However, nodule number and soil pH were slightly decreased by nitrapyrin applications. Also, nitrapyrin caused the leaves to become twisted and the plants to be short, and bushy. Furthermore, it caused the cotyledons to remain on the plant and destroyed the apical dominance of the plant. Inoculum played a role in increasing the yield, % N, N content and nodulation of 'Amsoy' soybeans. It also significantly increased pod yield and nodulation of 'Horosoy' soybeans but had no effect on yield, % N, or N content of vegetative and root plant portions.

INTRODUCTION

Nodulated soybeans can be grown without using any N fertilizer since they are legumes and as such are able to fix atmospheric N_2. However, it has been shown that nodulated soybeans without added N do not produce the maximum yield that can be attained from this species (4,72,75,89).

The inability of nodulated soybeans to produce the maximum yields could be due to the fact that not all nodulation is effective nodulation. Indeed, Virtanen and Linkola (101) showed that nodulation with an ineffective strain of Rhizobium prevented subsequent nodulation with effective strains and thus N fixation. Nitrogen fertilization may be another factor to be considered as a means of optimizing soybean yield especially in those soils that are low in organic matter and or available N.

Results from using N fertilizers in addition to nodulation for soybean growth have been varied and seem to depend on the form of N used. Some researchers (4,72,73,75,98) have found an increase in soybean yield after N was added, but others (2,13) did not observe an increase in yield.

Many investigators (3,4,14,38,58,73,75,94,98,102,106) agree that effective nodulation is prevented when either NH_4-N or NO_3-N fertilizers are applied either alone or in conjunction with each other as a N source to soybeans at very high rates. Latimore et al (58) noticed that nitrates had a greater effect on nodulation than did ammonium at the same rates but those differences were not significant at the 5% level.

The presence of 2-chloro-6-(trichloro-methyl) pyridine (nitrapyrin) in a soil prohibits nitrification of urea and or ammonium fertilizers by controlling the population of Nitrosomonas bacteria in that medium (5,41). The findings that nitrapyrin controls nitrification seems to be well accepted as fact (5,41, 76,83). However, there is some controversy over whether or not this chemical is harmful to plants. Goring (41) said that no reduction in rate of growth was observed in alfalfa at the 12½ ppm on a soil basis, but Geronimo et al (40) and McKeel and Whalley (66) said that there was an effect on alfalfa. Likewise, Lynd et al (61) reported that soil concentrations of nitryprin as low as 1 ppm causes severe leaf curling, internode enlongation and abnormal tendril growth of Black locust (Robina pseudoacacia). Geronimo et al (40) also found that when nitrapyrin is applied at the recommended rates there is no effect on soybeans. However, Riley and Barber (83) reported alterations in soybean morphology with as little as 1 ppm of nitrapyrin in the medium.

With the foregoing findings in mind, studies were conducted to look at the effect of nitrapyrin on nodulation in the presence of inoculum and N fertilizers added to the medium as either NO_3, NH_4, or urea.

MATERIALS AND METHODS

Experiment 1: The first experiment was conducted in the greenhouse using 1 kg of silt loam soil. The soil has an initial pH of 6.1, a cation exchange capacity of 71 meq/100g of soil, and an organic matter content of 2.4 percent. 'Amsoy' was the variety of soybean used.

Four replicates of each treatment were used. The treatments consisted of three regimes of nitrapyrin (Ns) and inoculum (I) (1) Ns+I, (2) Ns, and (3) I, and five rates (25,50,100,200,400 ppm N) of calcium nitrate, ammonium sulfate or urea. Henceforth, the nitrogen sources will be referred to as nitrate ammonium or urea. The no-N treatment will be called the control. A factorial design was used for treatment identification.

Each pot of soil that was treated with nitrapyrin received 8 ppm. The chemical was mechanically mixed with the soil prior to planting. Inoculum treated seeds were coated with inoculum and lime while the non-inoculated seeds were coated with lime alone. The lime was added to enhance nodulation since other workers (2,60) have shown that nodulation is benefited by calcium and an increased soil pH. The lime coat should serve to increase the Ca content and the pH in the micro-environment near the root surface and thus cause increased infection of the roots by the bacterium.

The pots of soil were seeded with sufficient seeds to insure that a final population of 3 plants per pot would be attained. Two weeks after planting one half of each fertilizer treatment was added. The remaining half of each fertilizer treatment was applied 3 days later (17 days after seedling emergence). Pots were kept moist by using tap water.

Three weeks after planting, the seedling were thinned to 3 plants per pot and allowed to grow until they reached physiological maturity (91 days) as described by Fehr et al (36) (pods yellowing and 50% of leaves yellow). Although others (27,30,37,44,51,65) have used different criteria for physiological maturity, no uniform visual indicator of maturity has been established. Therefore, the one employed by Fehr et al (36) was used in these studies.

At harvest the plants were separated into pods (seeds + pod coats), vegetation (all aerial vegetative parts except pods), and roots. The pods and vegetation were dried in an air circulating oven at 70 C, ground and analyzed for total N content by a modified Kjeldahl procedure. Soil cores were taken from each pot, and the final pH was determined. The pots were submerged in water for a period of time to allow the soil to soften. The roots were then washed from the soil by hand. The nodules were counted, and the roots were discarded.

<u>Experiment 2</u>: The second experiment was conducted in the greenhouse using 1 kg of the silt loam soil that was used in Experiment 1. 'Horosoy' was the variety of soybeans used.

Five replicates of each treatment were used. The treatments consisted of three regimes of nitrapyrin (Ns) and inoculum (I) (Ns+I, Ns, and I) and four rates (000, 100, 200, 400, and 800 ppm) of ammonium sulfate. Also, a control which consisted of no Ns, no I, and no N was used to assess the affect of the native soil Rhizobial population. The experiment was set up in a randomized complete-block design.

The procedures for nitrapyrin application, seed coating, planting, thinning, fertilizing, and harvesting were the same as those explained in Experiment 1. However, the roots were not discarded.

RESULTS AND DISCUSSION

Experiment 1.

Weight of plant parts. Different N souces did not significantly affect plant weight (Table 1). Likewise, nitrogen rates from 25 to 400 ppm did not significantly alter the weight of pods (Table 2). This was in contrast to what was found by others (4,72,73,75,98) who observed increases in seed yield when N was applied. Plant yield was increased by the addition of N to the growth medium. Also, with increasing applications of N above 50 ppm, the vegetative weight of the plants increased significantly. This indicates that N fertilizers do increase yield of 'Amsoy' soybeans but that most of the increase is vegetative and thus unmarketable. However, on soils that are low in available N, the addition of N to the medium may increase the yield of pods because the added N would aid plant growth prior to nodulation and allow the plants to accumulate N in the vegetative portions thus lengthening the seed development period and increasing yield as suggested by Sinclair and DeWit (86).

Nitrapyrin did not affect the dry matter composition of these plants (as indicated by the "Ns+I" treatment vs the "I" treatment) (Table 3). The ratio of pods to vegetation was greater for the "Ns+I" treatment (1) than either "Ns" (.5) or "I" (.9) treatments. The depression in the vegetative weight may be a reflection of nitrapyrin phytotoxicity on soybeans. However, no phytotoxicity symptoms were observed.

Table 1. Nitrogen sources and their effect on the weight of nodulated 'Amsoy' soybeans

N source	Pods	Vegetation	Plants
	g/pot		
		Fresh Weight	
no N	37a**	31a	68a
NO_3	40a	39a	79a
NH_4	41a	40a	81a
Urea	42a	40a	82a
		Dry Weight	
no N	11.7a	12.2a	23.9a
NO_3	12.4a	14.9a	27.3a
NH_4	12.9a	15.8a	28.7a
Urea	13.1a	15.4a	28.5a

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Table 2. Nitrogen rates and their effect on the weight of nodulated 'Amsoy' soybeans

N rate	Pods	Vegetation	Plants
(ppm)	g/pot		
		Fresh Weight	
000	37a**	31a	68a
025	39a	35a	74a
050	40a	34a	74a
100	42a	39ab	81a
200	42a	44bc	86a
400	42a	49c	91a
		Dry Weight	
000	11.7a	12.2a	23.9a
025	12.2a	13.3a	25.5a
050	12.7a	13.8a	26.5ab
100	13.1a	14.7ab	27.8ab
200	13.0a	16.4b	29.4ab
400	13.0a	18.9c	31.9b

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Table 3. The effect of nitrapyrin (Ns) and inoculum (I) on the weight of nodulated 'Amsoy' soybeans.

Regime	Pods	Vegetation	Plants
	g/pot		
		Fresh Weight	
Ns+I	50b**	40b	90b
Ns	21a	32a	53a
I	51b	48c	99c
		Dry Weight	
Ns+I	15.8b	15.2b	31b
Ns	6.6a	13.4a	20.0a
I	16.0a	17.6c	33.6c

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Inoculation had a tremendous impact on the growth of the plants. Pod dry weight in the "Ns" treatment was less than half of that for the "Ns+I" and "I" treatments, however, the vegetative portions of the "Ns" plants was more than three-fourths of that of inoculated plants. This would tend to suggest that plants must attain a minimum vegetative size before the photosynthate can be shunted to the fruiting parts. Also, these points indicate that the native soil population of Rhizobia in this soil is not great enough to initiate sufficient nodulation for maximum yield.

Percent N in plant parts. Relatively speaking, neither nitrogen source had any effect on the % N in pods or vegetation (Table 4). However, as was found by Hanway and Weber (47) most of the vegetative N was translocated to the fruiting parts. Plants that were grown on ammonium had a lower N concentration in both pods and vegetation than did any other treatment. This can be attributed to dilution since these plants also had a higher weight than plants growing on other treatments.

In contrast to what was found by Hanway and Weber (47) there was not a corresponding decrease in % N with increasing N applications from 25 to 400 ppm (Table 5). This suggests that dilution played an important role in the N concentrated by the plant parts. As the yield of vegetation increased, the % N decreased thus indicating a dilution effect.

Nitrapyrin in the presence of inoculum had no effect on the % N present in the soybeans (Table 6). But, when nitrapyrin was applied without inoculum only about 3/4 as much N as was concentrated

Table 4. Nitrogen sources and their effect on the N content of nodulated 'Amsoy' soybeans.

N source	Pods	Vegetation	Plants
	% N		
no N	4.40a**	2.10a	
NO_3	4.33a	2.00a	
NH_4	4.25a	1.84a	
Urea	4.27a	1.98a	
		mg-N/pot	
no N	548a	270a	818a
NO_3	566a	299a	865a
NH_4	579a	287a	866a
Urea	587	308a	895a

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Table 5. The effect of N rate on the N content of nodulated 'Amsoy' soybeans.

N rate	Pods	Vegetation	Plants
(ppm)	% N		
000	4.40a**	2.10ab	
025	4.49b	2.19b	
050	4.48b	1.88ab	
100	4.33a	1.98ab	
200	4.18a	1.92a	
400	3.94a	1.74a	
		mg-N/pot	
000	548a	270a	818a
025	565a	294a	859a
050	593a	265a	858a
100	590a	299a	889a
200	582a	313a	895a
400	555a	319a	874a

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Table 6. Nitrapyrin (Ns) and inoculum (I) regimes and their effect on the N content of nodulated 'Amsoy' soybeans.

Regime	Pods	Vegetation	Plants
	% N		
Ns+I	4.77b**	2.14b	
Ns	3.43a	1.55a	
I	4.65b	2.13b	
		mg-N/pot	
Ns+I	754b	324b	1078b
Ns	231a	193a	424a
I	747b	376c	1123b

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

by pods or vegetation. This suggests that the inoculum stimulated effective nodulation.

Total mg N/pot. The different N sources did not significantly increase the N content of the soybeans above that of the control. But, soybeans seem to take up more urea-N than nitrate or ammonium N.

Application of N did not significantly increase the N content of the soybean plants (Table 5). Nitrogen rates above 50 ppm caused the ratio of pods to vegetative mg N/pot to decrease. This is due to the fact that more vegetative matter is produced as N is added to the growth medium. Likewise, as N was added to the growth medium apparently less N was translocated to the fruiting part. This can be shown by examining the ratios "vegetative mg N/plant mg N" and "pod mg N/plant mgN" which increased and decreased respectively with increasing applications of N above 50 ppm, thus indicating some interference with N translocation.

pH and nodulation. Ammonium fertilizer significantly lowered the soil pH but did not significantly affect the nodulation of the soybeans (Table 7). Blair et al (17) also observed a decrease in the pH of nutrient solution when NH_4 was the principal form of N in the medium. Nitrogen rates above 200 ppm lowered the pH of the soil significantly but not to the point that it would have affected nodulation, i.e., according to Wright (108) Rhizobia can infect soybeans at pH values as low as 4.1. No noticeable change in pH was observed when urea or nitrate was the N source (Table 7).

The presence of nitrapyrin in the medium had no effect on soil pH or nodulation response by the soybeans. However, the pH

Table 7. The effect of N, nitrapyrin (Ns), and inoculum (I) applications on the pH and nodule number of nodulated 'Amsoy' soybeans.

N source	pH	Nod Num
no N	6.09b**	101a
NO_3	6.10b	116a
NH_4	5.84a	101a
Urea	6.02b	115a
N rate (ppm)		
000	6.09b	101a
025	6.04b	115a
050	6.07b	119a
100	6.05b	112a
200	5.90a	111a
400	5.86a	99a
Regime		
Ns+I	5.91a	149b
Ns	6.08b	47a
I	691b	136b

** Means in the same column and followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

was significantly lowered and nodulation was significantly raised by adding inoculum to the growth medium. The lowering of the pH was possibly due to the increased yield obtained with inoculated plants and the resulting exudation of H^+ ions from the plant roots to the soil solution in an attempt to maintain the ionic balance within the cells after absorption of other cations.

Interactions. Although the addition of N fertilizer to the growth medium had no effect on the weight of pods, vegetation, or the entire plant, significant interactions were observed between the amount of N applied and the N-source used (Table 8). There was a significant interaction between these two factors for the fresh and dry weight of pods and vegetation as well as the dry weight of the entire plant. The significant interaction between N rate and source for pods is of particular interest because it indicates that one of the N-sources applied may aid in increasing seed yield. This study indicates that urea may produce a higher yield than the other N sources.

The significant interactions between N rate and source with regard to pH and nodulation can be explained in terms of a decreased pH and nodule number when NH_4 is the principal N source for nutrition of the plants. While the depression in pH by NH_4 is of no consequence in this study since it does not represent the accumulation of many H^+ ions in the rooting medium, it may have an adverse affect in a soil that is buffered to a lesser extent than this one. Too, if nodulation is decreased the amount of N fixed by the plants would probably be reduced.

Table 8. The interactions of nitrapyrin and/or inoculum regimes (NsI), N rate (A), and N source (N) on the weight and N content of nodulated 'Amsoy' soybeans and on soil pH

Pods	Vegetation	Plant	NsI x A	NsI x N	A x N	NsI x A x N
Fr. Wt.	-	-	ns	ns	*	ns
-	Fr. Wt.	-	**	*	*	ns
-	-	Fr. Wt.	**	ns	ns	ns
Dry Wt.	-	-	ns	ns	*	ns
-	Dry Wt.	-	**	ns	**	ns
-	-	Dry Wt.	**	ns	*	ns
% N	-	-	**	ns	ns	ns
-	% N	-	**	ns	ns	*
-	mg N	-	*	*	ns	ns
Nodule number			*	*	*	**
Soil pH			**	**	**	*

The 5 and 1% level of significance is represented by * and ** respectively and ns means nonsignificant.

There were significant interactions between regimes (nitrapyrin and/or inoculum) and N-rates for both vegetation and entire plant growth. This trend can be explained in terms of the significant increase in vegetation when either inoculum or increasing amount of N fertilizer was present in the growth medium. The N concentration and the mg N/pot also showed significant interactions between regimes and amount of N applied. This can be explained in terms of the decreased N concentration in the vegetative plant portions when the rate of N applied was increased and in terms of increased % N when inoculum was applied to the medium. Likewise, interactions were observed for the effect of regimes and N-rate on the soil pH and nodulation of the soybeans. This can be explained in terms of the increased nodulation and decreased pH when inoculum was put into the growth medium as well as the decreased pH and nodulation when the N-rate was increased.

There were significant interactions between regimes and N sources. These two factors showed significant interactions for vegetative weight, mg N/pot, soil pH, and nodulation of soybeans. The significant interaction was probably the result of increased N concentration when inoculum was put into the medium while those of pH and nodulation are manifestations both NH_4 application and inoculum additions.

The three factor interactions for regimes, N rates, and N sources were observed only in the % N of vegetation, the soil pH, and the nodulation of the soybeans. One might say that with regard to the % N in vegetation both regime and N rate played a major role, since either the absence of inoculum in the medium or the presence of large quantities of fertilizer reduces the N concentration of the vegetative plant portions. The presence of NH_4 fertilizer and the addition of inoculum in the growth medium appear to have the largest effect on the values obtained for pH and nodulation. With ammonium fertilization and inoculation, the pH was decreased significantly. However, nodulation was not affected by N rate or source, and inoculum increased the nodule number.

Experiment 2.

Weight. Pod weight of 'Horosoy' soybeans was not increased by N fertilization (Table 9). However, vegetative and root weights were significantly increased with increasing rates of N fertilization. These findings further enforce the argument that N fertilization serves only to increase vegetative plant growth while having no affect on seed production.

The significant interaction between regimes and N rate can be attributed largely to N fertilization. This is apparent since neither regime affected yield.

Contrary to what was observed in Experiment 1, nitrapyrin in the presence of inoculum significantly increased the pod fresh weight above that of the other treatments (Table 10). However, the dry weight of pods for the "Ns+I" treatment was not more than that of the other treatments.

Vegetative and root weights of the treated plants were greater than that of the control. Also, the range tests for fresh and dry vegetative weights of treated plants were not different from each other.

Table 9. The effect of N rate on the weight of nodulated 'Horosoy' soybeans.

N rate	Pods	Vegetation	Roots	Plants
(ppm)		g/pot		
		Fresh Weight		
000	48b**	19b	23a	90b
100	50b	22bc	26ab	98bc
200	48b	23cd	29b	100bc
400	48b	26de	30b	104c
800	45ab	39e	36c	120c
Control	35a	13a	19a	67a
@ Interactions		N x A**		
		Dry Weight		
000	17.0b	8.7ab	3.9a	29.6b
100	18.0b	9.6bc	4.3ab	31.9bc
200	18.3b	10.5cd	4.9bc	33.7c
400	17.3b	12.0d	5.4cd	34.7c
800	16.4b	11.6d	5.8d	33.8c
Control	11.9a	6.6a	3.8a	22.3a

** Means followed by the same letter and in the same column are not different at the 1% level of significance by Duncan's Multiple Range Test.

@ The N x A (Regimes x N-rate) interactions which are followed by ** are significant at the 1% level by F ratios.

Table 10. Weight of nodulated 'Horosoy' soybeans as influenced by nitrapyrin (Ns) and inoculum (I).

Regime	Pods	Vegetation	Roots	Plants
	g/pot			
		Fresh Weight		
Ns+I	51c	23b	25ab	99b
Ns	44b	26b	31c	101b
I	48bc	23b	30bc	101b
Control	35a	13a	19a	67a
		Dry Weight		
Ns+I	18.6c	10.1b	4.3a	33.0b
Ns	15.8b	10.9b	5.1b	31.8b
I	18.2c	10.5b	5.2b	33.9b
Control	11.9a	6.6a	3.8a	22.3a

** Means followed by the same letter and in the same column are not different at the 1% level of significance by Duncan's Multiple Range Test.

However, the fresh weight of roots for non-inoculated (Ns) plants was more than that for the roots of the other treatments while the dry weight was about the same as that of inoculated (I) plants. Therefore, again it appears that nitrapyrin is causing the plants to take up excess water.

Unlike the previous study, the presence of nitrapyrin in the growth medium caused the plants to exhibit phytotoxicity symptoms. The leaves were twisted, and the plants were shorter and more bushy, than those not treated with the chemical. Likewise, the plants lost their apical dominance and began to put on lateral growth from top to cotyledonary nodes. Also, the cotyledons remained on the plants rather than abscise as they did in Experiment 1. These findings suggest that auxin is being destroyed or that the hormonal balance between auxin and ethylene was being altered in some way. The findings are the reverse of those observed for black locust by Lynd et al (61).

The findings of this study in conjunction with those of Experiment 1 indicate that variety of soybeans plays a role in whether phytotoxicity symptoms are masked or not. Visual symptoms of phytotoxicity were observed on the vegetation in Experiment 2 while a loss of vegetative weight was observed in Experiment 1. Also, in Experiment 2, nitrapyrin caused a loss of root weight by the plants. These findings are in agreement with those made by others (61,66,83) and add validity to the assumption that nitrapyrin may be phytotoxic to plants.

Percent N. Contrary to the decreasing trend in the % N of 'Amsoy' soybeans with increasing rates of N fertilizer, the addition of N fertilizer in the growth medium caused an increase in the N concentration of 'Horosoy' pods (Table 11). However, N fertilization had no effect on the % N in vegetative or root portions of the plant.

As was observed for 'Amsoy' soybeans, the N concentration in pods was greater than that in vegetation. This indicates that N was being translocated to the fruiting parts. The root N concentration was greater than that of the stems. This was probably due to a concentration effect since the roots contained less than half of the dry weight accumulated by vegetation. Again it appears that variety plays a role in how N will be distributed in the plant parts. Thus genetic manipulation is apparently the key to getting higher protein beans and may well hold the key to increased production of marketable yield.

As was observed in Experiment 1, nitrapyrin in the presence of inoculum had no affect on the N concentration of vegetative or root plant portions (Table 12). But, the addition of inoculum to the medium did increase the % N in the pods.

Unlike 'Amsoy' soybeans, the addition of inoculum to the growth medium did not increase the % N in the vegetative plant portions above that in the Ns treatment. This suggests that the native soil population of Rhizobia is sufficient to produce enough nodules for optimum N production. However, this increased N production did not cause the plants to produce a higher yield.

Table 11. Nitrogen content of nodulated 'Horosoy' soybeans as influenced by N rate.

N rate	Pods	Vegetation	Roots	Plants
(ppm)		% N		
000	3.82a**	1.52a	2.03a	
100	4.28b	1.53a	1.98a	
200	4.19b	1.41a	2.04a	
400	4.08ab	1.63a	1.98a	
800	4.37b	1.68a	2.11a	
Control	3.95ab	1.82a	2.15a	
@ Interactions	N x A*		N x A*	
		mg-N/pot		
000	673b	134a	80a	887b
100	774b	148a	85ab	1007c
200	767b	147a	100b	1014c
400	711b	198a	106bc	1015c
800	715b	196a	122c	1033c
Control	470a	120a	81ab	671a
@ Interactions	N x A*			

** Means followed by the same letter and in the same column are not different at the 1% level of significance by Duncan's Multiple Range Test.

@ The N x A (Regimes x N-rate) interactions which are followed by * are significant at the 5% level by F ratios.

Table 12. Nitrogen content of nodulated 'Horosoy' soybeans as affected by nitrapyrin (Ns) and inoculum (I).

Regime	Pods	Vegetation	Roots	Plants
			% N	
Ns+I	4.23b**	1.60a	2.04a	
Ns	3.92a	1.61a	2.10a	
I	4.3b	1.45a	1.94a	
Control	3.95ab	1.81a	2.15a	
			mg-N/pot	
Ns+I	787c	164a	87a	1038c
Ns	616b	177a	108a	901b
I	780c	153a	100a	1033c
Control	470a	120a	81a	671a

** Means followed by the same letter and in the same column are not different at the 1% level of significance by Duncan's Multiple Range Test.

Also, the information indicates that 'Horosoy' soybeans are more effective translocaters of N than 'Amsoy' soybeans.

mg N/pot. Weight of plant portion seem to be the over-riding factor in relation to the N content of these soybeans. Nitrapyrin in the presence of inoculum had no effect on the N content of pods and vegetation, however, it did significantly decrease the N content of the roots. Inoculum did increase the N content of pods and roots but had no effect on vegetative N content. The lack of a response in vegetative N content and the small increase in N content of pods when inoculum was put into the medium suggest that the native soil population of Rhizobia was almost sufficient to allow maximum production of quality dry matter. This is in agreement with the findings of Nelson et at (71).

Similar to Experiment 1, the N content of 'Horosoy' pods and vegetation was not affected by N fertilization. This is reflective of the weight of these plant portions. However, the root N content was increased by N fertilization. Again this trend can be attributed to the increased weight of the plant.

There were significant interactions between regimes and N-rates for % N in pods and roots. Likewise, there was a significant interaction between regimes and rates for mg N/pot.

pH and nodulation. As was observed for 'Amsoy' soybeans, nodulation of 'Horosoy' soybeans was not affected by increasing the rate of applied N in the medium (Table 13). Also, pH was decreased with increasing rates of ammonium fertilization.

Table 13. Nodulation of 'Horosoy' soybeans as affected by nitrapyrin (Ns), inoculum (I) and N rate.

Regime	Number Nodules	pH
Ns+I	236b	7.00b
Ns	178a	6.94a
I	312c	7.00b
Control	226ab	7.00ab
@ Interactions		N x A**
N rate (ppm	Nodules	pH
000	223a	6.99ab
100	233a	7.03b
200	247a	6.97ab
400	284a	6.95a
800	224a	6.94a
Control	226a	7.00ab

** Means in the same column and followed by the same letter are not significantly different from each other at the 1% level of significance by Duncan's Multiple Range Test.

@ The N x A (Regimes x N-rate) interactions which are followed by ** are significant at the 1% level of F ratios.

Nodulation of 'Horosoy' soybeans was significantly decreased when the beans were grown with nitrapyrin in the presence of inoculum. This suggests that the presence of nitrapyrin in the medium provided an unfavorable atmosphere for nodulation; however, this may not have been the only cause of decreased nodulation. Indeed, nodulation may have been reduced as a result of decreased root growth for this treatment relative to the inoculated treatment (I).

The presence of inoculum significantly increased nodulation of the plants; however, it had no effect on soil pH. The lack of a pH depression in this study can be attributed to the high initial soil pH.

PART II

NODULATION AND GROWTH OF HYDROPONICALLY GROWN SOYBEANS AS INFLUENCED BY NITRAPYRIN, INOCULUM, AND NITROGEN APPLICATIONS

Abstract: Soybeans (Glycine max (L.) Merrill) from maturity group II were grown to physiological maturity in a modified Hoagland's solution. In each experiment, a randomized complete-block design with 4 replications was used. The 12 treatment combinations for each experiment came from a 3x4 factorial design with 3 regimes of nitrapyrin (Ns) and inoculum (I), 3 sources of N ($Ca(NO_3)_2$, $(NH_4)_2SO_4$, or Urea) and a no N series. Solution and inoculum were changed at 7-day intervals. The pH of the solution was controlled with lime. At harvest, plants were separated into pods, vegetation, and roots. Nodules on the roots were counted, and plant portions were dried and analyzed for N by the Kjeldahl procedure. Neither Ns nor I had an effect on weight, N content or nodulation of 'Amsoy' soybeans. The lack of a response was probably due to reduced growth which was caused by fall growth conditions. Apparently, this was the case since 'Horosoy' soybeans showed growth responses to both factors when they were grown during the following spring. Nitrapyrin increased pod fresh weight (possibly in response to salt accumulation), however, the plants did not show any phytotoxicity symptoms. It also decreased root dry weight and vegetative mg N/pot while having no effect on pod or vegetative dry weight, % N, or pod and nodule number of 'Horosoy' soybeans. Inoculum increased pod weight, % N, pod and vegetative mg N/pot, and nodulation of 'Horosoy' soybeans while having no

effect on vegetative or root dry weight, root mg N/pot, or pod number. Nitrogen fertilization increased dry weight of both cultivars for all plant portions as well as pod number of 'Horosoy' soybeans.

INTRODUCTION

Growing plants in a hydroponic system enables the investigator to look at the affect of a particular factor on plant growth. It is a great diagnostic tool because it allows the investigator to look at the entire intact plant whereas in other systems the roots are usually not visible or ready for inspection.

Soybeans were grown hydroponically to look at the affects of nitrapyrin, inoculum, and N fertilization on nodulation and growth. This system was used to see changes in morphology and to detect physiological changes that the plants may be experiencing in response to the experimental factors that were applied.

Although soybeans can be grown without using nitrogen fertilizers since they are legumes and as such can utilize atmospheric N_2, they have been shown to benefit from the addition of N fertilizers especially when they are grown on soils that are low in available N. Results from applying N fertilizer to the growth medium of inoculated soybeans have been varied and seem to depend in large measure on the amount and form of N used.

Many investigators (32,35,68,69,81,93,100) have observed detrimental affects of nitrate fertilizers on nodulation of legumes. Dixon (32) and Raggio et al (81) found that concentrations of externally applied nitrate ranging from 13 mM to 0.44 mM inhibited nodulation while Munns (68) showed that 0.2 mM nitrate delays the appearance of the first nodules by one day. Some researchers (35,68,69,93) believe that nitrates prevent the curling of root hairs and hence the nodulation process.

Wacek and Brill (102) indicated that nitrate and ammonium prevent nodulation and acetylene reduction in the effectiveness assay. Likewise, Latimore et al (58) showed that both nitrates and ammonium decreased N_2 fixation as a result of decreasing carbohydrate supply to the nodules. Tanner and Anderson (94) also observed a decrease in nodulation in the presence of soluble nitrogenous salts. They said that this was due to an inadequate carbohydrate supply to the roots because the carbohydrate synthesized is used for top growth thus leaving little available for growth of nodules.

Vigue et al (100) noticed effective nodulation in the presence of urea in solution cultures even at concentrations of 18 mM while using 2-liter containers. In the same study they found that 2 mM nitrates inhibited effective nodulation.

Although the likelihood of nitrosomonas bacteria being in a nutrient solution is small, this system can be used to assess the affect of nitrapyrin on the morphology or soybean plants. It is known that nitrapyrin controls the nitrosomonas population in the growth medium. However, reports vary regarding whether or not the chemical is detrimental to plants grown in its presence.

Mullison and Norris (67) said that nitrapyrin degrades readily and does not accumulate readily in plants. They also showed that the chemical does not affect other soil bacteria. Likewise, Latkowski, et al (57) observed that nitrapyrin applications of less than 10 ppm had no effect on Rhizobium species.

Nitrapyrin has been shown to be detrimental to plant growth.

Hendrickson et al (48) indicated that it causes potatoes to become stunted, to have dark green tops, and to have bushy rather than vine-like development. Lynd et al (61) observed severe leaf curling, internode enlongation and abnormal tendril-like stem growth of black locust when nitrapyrin was used. McKeel and Whalley (66) noticed tumor-like swelling behind root tips and small white nodules which were distributed on the root system. Parr et al (76) stated that roots of soybeans grown in pots with nitrapyrin were stunted and flacid. Also, Riley and Barber (83) showed that 1 ppm of nitrapyrin caused soybean roots to become stubby and club-like with swelling behind the root tips.

MATERIALS AND METHODS

Experiment 1: Experiment 1 was conducted in the greenhouse during the fall of 1977. The treatments were set up in a randomized complete block design. Four replicates of the 12 treatments were grown. The legend of treatments is detailed in table 1.

Nitrapyrin was applied at the rate of 1 ppm on those plants that were treated with the chemical. A modified Hoagland's solution was used. The P concentration of this solution was 0.1 mM instead of 1 mM. This was done to ensure that the P concentration would not affect plant growth as indicated by Paulsen and Rotimi (77). Also, preliminary studies indicated that decreasing the P level from 1 to 0.1 mM had no deterimental effect on plant growth. Likewise, Leggett and Frere (59) found that low P levels did not affect soybean growth.

'Amsoy' soybeans were planted in sand that had been heat-sterilized at 200 ^{o}C for 1 hour. They were watered with boiled water and allowed to grow for three weeks.

Seedlings were transplanted at the rate of 2 per pot. Solutions were changed at 7-day intervals,and inoculum was added to the medium of treated plants during each changes of solution. This was done to ensure optimum nodulation. Also, the pH of the solutions was controlled by using lime in the solid phase.

Blair et al (17) and Weissman (107) have observed changes in pH when N is added to the growth medium. Thus, the control of pH in the nutrient solution can be beneficial to soybean production.

Table 1. Legend of treatments for 'Amsoy' and 'Horosoy' soybeans.

	N sources			
Regime	$NaNO_3$	$(NH_4)_2SO_4$	Urea	no N
	treatment number			
Ns+I	1	4	7	10
Ns	2	5	8	11
I	3	6	9	12

Where: Ns = nitrapyrin

I = inoculum

Indeed, Barker et al (7,8) suggested that controlling the acidity in a medium depresses the accumulation of free ammonium in plant tissue by enhancing the conversion of entering ammonium to organic N compounds. The addition of lime also provides calcium to the medium. According to Albrecht and Davis (2) this increases the viability of Rhizobia. Also, Lowther and Loneragan (60) and Munns (70) observed the beneficial affects of calcium on nodulation.

Three-week-old seedling were grown through the grain filling stage. Although the plants did not reach this stage at the same time, they were harvested when the majority became physiologically mature (56 days after transplant). The harvested plant portions were separated into pods, vegetation, and roots. The nodules on the roots were counted. The pods, vegetation, and roots were dried, ground, and analyzed for N content by the Kjeldahl procedure.

Experiment 2: This experiment was conducted in the greenhouse during the spring of 1978. 'Horosoy' soybeans were grown by using the same procedure that were employed in the first experiment. However, the N concentration of the medium was 7.5 mM instead of the 15 mM concentration used in the first experiment. Also, 2-week-old seedlings were grown in solution culture for 63 days instead of the 56 of Experiment 1.

RESULTS AND DISCUSSION

Experiment 1.

Weight. Neither nitrapyrin nor inoculum had any effect on the fresh weight of 'Amsoy' soybean plant portions (Table 2). However, the pod, vegetative, and root plant portions were significantly influenced by nitrogen source.

As expected, the no-N treatment produced the least amount of fresh weight. Urea was slightly less efficient than ammonium in producing fresh weight, and nitrates produced a larger amount of fresh weight for all plant portions than any other N source. This suggests that nitrate is the preferred N source for soybean growth. But, nitrates have been shown to be detrimental to nodulation (32,35,68,69,81,93,100). The detrimental affects of nodulation may be due to physiological changes in the plant as a result of the adequate nutrition that it is receiving from the nitrate fertilizer. Indeed, it has been suggested by some researchers (35,68,69,93) that nitrates prevent root curling.

Roots made the largest contribution to the weight of the plants while vegetation contributed slightly more than pods. The fact that roots contained more fresh weight than vegetation or pods can be attributed to the harvest procedure, i.e., remove roots from solution and blot them dry.

Dry weight followed the same patterns of accumulation as did the fresh weight of 'Amsoy' soybeans with respect to nitrapyrin, inoculum, and N source (Table 3). But, unlike the trend for fresh weight, the dry weight of roots made the smallest contribution

Table 2. The effect of N source, nitrapyrin (Ns), and inoculum (I) on the fresh weight of hydroponically grown 'Amsoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	N source				
	g/pot				
			Pod		
Ns+I	19c**	12bc	2a	10ab	11A
Ns	16c	17bc	14bc	11b	14A
I	17c	10ab	20c	9ab	14A
mean	17B	13AB	12AB	10A	
@ Interaction: N source x Regime **					
			Vegetation		
Ns+I	17cde	14abc	11ab	13abc	13A
Ns	18de	17b-e	14abc	9a	14A
I	22e	18de	17cde	9a	17A
mean	19C	16BC	14AB	10A	
@ Interaction: N source x Regime **					
			Root		
Ns+I	29c	20b	12ab	11a	18A
Ns	29c	21b	17ab	12ab	20A
I	32c	20b	21b	16ab	22A
mean	30C	20B	17AB	13A	

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions with ** are significant at the 1% level by F ratios.

Table 5. The effect of N source, nitrapyrin (Ns) and inoculum (I) on the dry weight of hydroponically grown 'Amsoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	N source				
	g/pot				
			Pod		
Ns+I	3.3c**	1.6abc	0.2a	0.8ab	1.5A
Ns	2.4bc	2.6bc	2.0abc	1.1ab	2.0A
I	2.4bc	0.7ab	2.5c	0.8ab	1.9A
mean	2.7B	1.6AB	1.9B	0.9A	
@ Interaction: N source x Regime **					
			Vegetation		
Ns+I	2.6cd	2.1bc	1.3ab	1.1ab	1.8A
Ns	3.1cd	2.7cd	1.9abc	0.8a	2.1A
I	3.7d	3.0cd	2.8cd	0.7a	2.6A
mean	3.1C	2.6BC	2.0B	0.9A	
			Root		
Ns+I	1.7e	1.1abc	0.7a	0.7a	1.1A
Ns	1.6cde	1.4b-e	1.1abc	0.7a	1.2A
I	1.7e	1.1abc	1.3a-e	1.0ab	1.3A
mean	1.7C	1.2B	1.0AB	0.8A	

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions with ** are significant at the 1% level by the F test.

to plant weight thus indicating that they contained more water than did the other parts.

Percent N. Nitrapyrin and inoculum regimes had no effect on the % N in the tissue (Table 4), but, N source did have a significant affect on the % N present in the plant tissue.

Plants fertilized with ammonium had a significantly higher N concentration in all plant parts than did those grown with any other N source. This can be attributed to the smaller weight of the plants receiving ammonium which would give a smaller dilution effect. The high % N in pods indicates that plants grown on ammonium have a higher edible protein content than plants growing on the other N source. Contrary to expectations, urea fertilized plants had a considerably lower N concentration in their pods than did ammonium treated plants. This can be attributed to the decreased vigor of the "Ns+I" treatment on the urea N source. The poor growth caused less N to be translocated from vegetation and roots than under normal circumstances. Nitrate treated plants had about the same concentration of N in their pods and vegetation as the no-N plants. This was due to a dilution effect since significantly more dry weight was in the nitrate treated plants than any other N source.

Proportionately speaking, more of the N on the no-N treatment went to the production of pods than did that of any other treatment. This suggests that pods are a strong sink for N accumulation. Likewise, the nitrate treatment adds credence to the assumption that pods are a strong sink for N. On the other hand, the ammonium and

Table 4. The effect of N source, nitrapyrin (Ns) and inoculum (I) on the % N in hydroponically grown 'Amsoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	% N				
			Pod		
Ns+I	4.64bc**	5.58bc	1.56a	5.06bc	4.21A
Ns	4.59bc	6.50c	5.75c	5.14bc	5.49A
I	4.54bc	6.62c	5.15bc	3.41ab	4.93A
mean	4.59A	6.23B	4.15A	4.53A	
@ Interaction: N source x Regime **					
			Vegetation		
Ns+I	4.02b	6.11c	7.27c	3.63b	5.25A
Ns	4.11b	6.67c	6.45c	3.26b	5.12A
I	3.65b	6.84c	4.18b	1.54a	4.05A
mean	3.92A	6.54B	5.96B	2.81A	
@ Interaction: N source x Regime*					
			Root		
Ns+I	3.48cd	6.21def	5.05de	2.44abc	4.29A
Ns	4.24cde	6.99ef	4.65de	2.33ab	4.52A
I	3.57bcd	7.49f	4.73de	1.52a	4.32A
mean	3.76B	6.90D	4.81C	2.06A	

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions followed by * and ** represents significance at the 5 and 1% level respectively by the F test.

Urea treated plants do not show such an effect, possibly due to the N concentration in the pods already being very high.

The N concentration in vegetation and roots increase significantly with different N sources. The order of increasing N concentration was no-N $< NO_3 <$ Urea $< NH_4$. Except for the no-N treatment, the increasing N concentration was due to a dilution effect.

Total mg-N/pot. Nitrapyrin and inoculum regimes had no effect on the N content of any plant portion (Table 5). However, different N sources did significantly alter the N content of the tissue. Nitrate N did increase the pod N content above that of ammonium which was about equal to that of urea while the no-N treatment had the lowest N content. Likewise, ammonium was taken up by vegetation and roots to a greater extent than NO_3 which was equal to urea while the no-N treatment again accumulated the least amount of N.

The total mg N/pot for the entire plant shows that the no-N treatment had the lowest N content while those plants growing on nitrate, ammonium and urea took up the same amount of N from the culture.

Nodulation. Nodulation of soybeans was not significantly affected by nitrapyrin and inoculum regimes (Table 6). However, adding N to the medium regardless of source caused a reduction in nodule number. This trend was in direct opposition to what was observed in soil and indicates that some modifying conditions existed in the soil medium that were not in the solution culture.

Table 5. The effect of N source nitrapyrin (Ns) and inoculum (I) on the N content of hydroponically grown 'Amsoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	N source				
	mg/pot				
			Pod		
Ns+I	152bc**	90abc	15a	43ab	75A
Ns	109abc	169c	122abc	54ab	114A
I	109abc	47ab	180c	27a	91A
mean	123B	102AB	105B	41A	
@ Interaction: N source x Regime **					
			Vegetation		
Ns+I	103b-e	135def	95bcd	29abc	93A
Ns	125de	178f	117cde	27ab	112A
I	135def	208f	116cde	11a	118A
mean	121B	174C	110B	25B	
			Root		
Ns+I	57b-e	70cde	35abc	18ab	45A
Ns	69cde	95e	52a-d	15a	57A
I	61cde	89de	60cde	15a	56A
mean	62B	85C	49B	16A	

** Mean followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions followed by ** are significant at the 1% level by the F test.

Table 6. The effect of N source, nitrapyrin (Ns) and inoculum (I) on nodulation of hydroponically grown 'Amsoy' soybeans.

	N-source				
Regime	NO_3	NH_4	Urea	no N	mean
	Number Nodules				
Ns+I	1.3a**	22.3ab	0a	119d	36A
Ns	0a	6.0a	0.3a	40b	12A
I	0.3a	9.5a	68.5bc	92cd	43A
mean	0.5A	12.6A	22.9A	84B	

A review of literature indicated that N nutrition did affect nodulation. All N sources hindered nodulation. Nitrate nutrition was the most detrimental to the nodulation process. This was possibly due to interference with the plants physiological characteristics as mentioned earlier. Another factor that may have contributed to the lack of a delineation between nitrogen sources with respect to nodulation may be the fact that the plants were grown during the fall. This fall growth would cut down on light intensity and duration, and hence, the production and translocation of phytosynthate to roots for Rhizobial nutrition (94).

Experiment 2.

Weight. The presence of nitrapyrin in the medium, the "Ns+I" treatment, increased pod fresh weight significantly over either "Ns" or "I" treatments but had no affect on vegetative or root fresh weight (Table 7). Likewise, the presence of inoculum, the "I" treatment, increased the fresh weight of pods over that of the "Ns" treatment while having no effect on vegetative or root fresh weight (72). These findings are in opposition to what was observed in the first study and in Experiment 1 of this study.

The presence of N increased the fresh weight of pods, vegetation, and roots to a large extent. Urea produced substantially more pod fresh weight than did ammonium which produced the same amount as nitrate. However, the vegetative and root fresh weights were the same among the N sources.

Again as in Experiment 1, the largest portion of the fresh weight was in the roots. The reason being the harvesting proce-

Table 7. The effect of N source, nitrapyrin (Ns), and inoculum (I) on the fresh weight of hydroponically grown 'Horosoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	N-source				
	g/pot				
			Pod		
Ns+I	25bcd**	25bcd	30cd	21cd	25B
Ns	15b	23bcd	19bcd	3a	15A
I	17bc	20bcd	27cd	23bcd	22A
mean	19AB	23AB	25B	16A	
@ Interaction: N source x Regime*					
			Vegetative		
Ns+I	32cd	35cd	37d	14ab	30A
Ns	31cd	42d	34cd	4a	28A
I	40d	36cd	43d	20bc	34A
mean	34B	38B	38B	13A	
			Root		
Ns+I	49de	31bc	35bc	18ab	33A
Ns	64e	66e	62de	4a	49A
I	58de	40bcd	49cde	19ab	42A
mean	57B	46B	48B	14A	

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions followed by * are significant at the 5% level by the F test.

dure i.e., remove roots from the solution and blot dry.

The pattern of dry weight accumulation was the same as that for fresh weight accumulation (Table 8). However, the roots contained less dry matter than the stems and about the same amount as the pods. Too, the "Ns+I" treatment did not contain significantly more dry weight than did the "I" treatment for any plant portion. This indicates that nitrapyrin causes the pods to contain more water than when it is not present. A fact which may be due to physiological changes within the cells of the fruiting bodies (pods) when nitrapyrin is present in the growth medium, i.e., possibly salt accumulation. Again as in Part I, nitrapyrin lowered the vegetative weight of the plant; however, no visual symptoms of phytotoxicity were observed and the differences were not significant at the 1% level.

Nitrogen fertilization increased yield of all plant portions including pods. This is in contrast to what was observed in Part I when soil was the growth medium. The increased yield when N was added in this study and the lack of a response in Part I add validity to the assumption that N fertilization would be beneficial in soils that are low in available N. Also, this study indicates that urea would be the best source of N to apply for optimum seed yield while nitrate would be the worst. These findings are dissimilar to those of Experiment 1 and adds credence to the supposition that adverse weather conditions shaded responses to N fertilizer.

<u>Percent N</u>. The presence of nitrapyrin in the growth medium did not significantly alter the % N in pods, vegetation, or roots,

Table 8. The effect of N source, nitrapyrin (Ns), and inoculum (I) on the dry weight of hydroponically grown 'Horosoy' soybeans.

	N-source				
Regime	NO_3	NH_4	Urea	no N	mean
	g/pot				
			Pod		
Ns+I	6.0cd**	5.7bcd	7.3d	5.0bcd	6.0B
Ns	3.6b	5.3bcd	4.2bc	1.0a	3.5A
I	4.1bc	4.9bcd	6.9cd	5.7bcd	5.4B
mean	4.5AB	5.3AB	6.1B	3.9A	
@ Interaction: N source x Regime*					
			Vegetative		
Ns+I	7.2cde	7.8de	8.1de	4.0b	6.8A
Ns	6.5bcd	9.6e	7.7de	1.3a	6.3A
I	9.4e	8.8de	9.1e	5.0bc	8.0A
mean	7.7B	8.7B	8.3B	3.4A	
@ Interaction: N source x Regime**					
			Root		
Ns+I	4.9cde	5.1c-f	4.3bcd	2.6ab	4.2A
Ns	7.5g	7.0fg	6.5efg	1.9a	5.7A
I	5.9d-g	4.6b-e	5.8d-g	3.4abc	4.9A
@ Interaction: N source x Regime **					

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions followed by * and ** are significant at the 5 and 1% level by the F test.

but inoculum did (Table 9). This indicates that nodulation was effective, because, when no inoculum was present (Ns) the % N of the plant portions was significantly reduced.

As was observed in Part I, the addition of N to the medium had no effect on the % N present in pods, vegetation, or roots. These findings indicate that all three N sources are capable of providing adequate nutrition for the plant. But, urea is apparently the preferred source for increasing seed yield since plants growing on this source had substantially more dry weight than that of other sources thus indicating that it is utilized more efficiently than the other N sources.

Total mg N/pot. Nitrapyrin in the presence of inoculum significantly lowered the uptake of N in the vegetative part of the plant (Table 10). This can be attributed to a combination of reduced yield and reduced N concentration of the vegetative tissue. Pod and root N content were not affected by the presence of nitrapyrin in the medium, but the presence of inoculum in the medium enhanced the uptake of N, as evidenced by the no-N non-inoculated treatments (Ns) vs the inoculated treatments (I) for all plant parts except roots.

The addition of N to the medium did not significantly increase the N content of the pods. However, the N content of vegetation and roots was significantly increased by the addition of N to the growth medium.

Urea and ammonium fertilization increased the uptake of N from the medium to the largest extent while nitrates increased the N

Table 9. The effect of N source, nitrapyrin (Ns), and inoculum (I) on the % N of hydroponically grown 'Horosoy' soybeans.

Regime	N source: NO_3	NH_4	Urea	no N	mean
	% N				
			Pod		
Ns+I	3.26bcd**	3.53cd	3.79cd	4.17d	3.69B
Ns	2.32ab	2.73abc	2.69abc	1.91a	2.41A
I	3.53cd	3.82cd	3.59cd	4.05d	3.74B
mean	3.03A	3.36A	3.36A	3.38A	
			Vegetative		
Ns+I	2.32c	2.72cd	2.24bc	3.27d	2.64B
Ns	1.33a	1.49ab	1.36a	1.27a	1.36A
I	2.41cd	2.82cd	3.02cd	3.02cd	2.82B
mean	2.02A	2.35A	2.21A	2.52A	
			Root		
Ns+I	2.63d	2.79d	2.81e	2.86e	2.77B
Ns	1.57a	1.94ab	2.02abc	1.48	1.75A
I	2.38bcd	2.90e	2.53cd	2.58de	2.60B
mean	2.19A	2.54A	2.45A	2.31A	

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

Table 10. The effect of N source,nitrapyrin (Ns) and inoculum (I) on the N content of hydroponically grown 'Horosoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	N source				
	mg/pot				
			Pod		
Ns+I	189cde**	200cde	280e	206cde	219B
Ns	82ab	141bcd	115abc	19a	91A
I	140bcd	183cde	246e	233de	201B
mean	137A	174A	214A	153A	
			Vegetative		
Ns+I	167b-f	208d-g	179c-f	130bcd	171B
Ns	85ab	142b-e	104abc	24a	89A
I	225efg	247fg	276g	152b-e	225C
mean	159AB	199B	186B	102A	
			Root		
Ns+I	129cd	138d	119cd	75b	115A
Ns	116bcd	134d	130cd	27a	102A
I	140d	132cd	145d	88bc	126A
mean	128B	135B	131B	63A	

@ Interaction: N source x Regime **

** Means followed by the same letter are not different at the 1% level of significance by Duncan's Multiple Range Test.

Capital letters are used to indicate main effects.

@ Interactions followed by ** are significant at the 1% level by the F test.

content of vegetation and roots only slightly more than when no N was present. Also, the N content of pods for those plants growing on nitrates was less than that of the no-N plants.

It appears that the excess N taken up by the no-N plants was accumulated after the plants had stopped growing. If this is true, then it is essential that young plants have an adequate supply of N for growth. Indeed, these results indicate that inoculated soybeans with no added N in the medium produce plants that have low yields but a high protein content due to a lack of plant growth and hence a dilution effect.

Nodulation. Nitrapyrin did not alter the nodule number or the pod number of 'Horosoy' soybeans (Table 11). However, the addition of inoculum did increase the nodulation response but had no affect on pod formation.

The addition of N to the solution did not increase the nodule number; however, pod number was significantly increased by urea and ammonium while nitrates increased the pod number by only a small amount. The increasing pod number corresponds to weight accumulation in the pods (74). However, unlike pod number, the pod weight of soybeans growing on NH_4 was less than that of plants growing on urea. This indicates that the pods of plants growing on NH_4 (.18g/pod) were smaller (less mature) than those growing on urea (.21g/pod). Likewise, the weight of the no-N treated plants (.21g/pod) was greater than the NO_3 treated plants (.19g/pod) again indicating that the NO_3 treated plants were less mature. Therefore,

Table 11. The effect of N source, nitrapyrin (Ns) and inoculum (I) on nodulation and pod formation of hydroponically grown 'Horosoy' soybeans.

Regime	NO_3	NH_4	Urea	no N	mean
	N source				
	Number				
			Pod		
Ns+I	28b-e**	32cde	34de	27b-e	30A
Ns	22bc	36e	27b-e	5a	22A
I	21bc	20b	26b-e	23bcd	22A
mean	23AB	29B	29B	18A	
@ Interaction: N source x Regime **					
			Nodule		
Ns+I	398d	325cd	327cd	385cd	359B
Ns	0.3a	58bc	16a	8a	21A
I	335cd	199bc	300c	434d	317B
mean	244A	194A	241A	275A	

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Capital letters are used to indicate main effects.

@ Interactions with ** are significant at the 1% level by the F test.

the addition of NO_3 or NH_4 fertilizers to the medium slowed the maturation process while urea had no effect on maturation as compared to the no-N treatment.

PART III

THE INFLUENCE OF NITROGEN SOURCE, POTASSIUM FERTILIZATION, AND 2-CHLORO-6-(TRICHLOROMETHYL) PYRIDINE (NITRAPYRIN)ON THE WEIGHT, POTASSIUM AND NUTRATE ACCUMULATION OF RADISH AND SPINACH

Abstract: The optimum use of N fertilizer is of prime importance from an economic, environmental, and health point of view. Vegetables represent the major source of dietary intake of nitrates which can be reduced to nitrites (potentially carcinogenic agents).

The use of K fertilizer has been suggested as a means of controlling NO_3 accumulation in plants. Likewise, nitrapyrin (Ns) and NH_4 applications have been shown to control the NO_3 concentration of plants. However,no data could be found which employed the use of the three parameters together. Therefore, three greenhouse experiments were conducted in this study to assess the affects of these factors on vegetable growth.

During Experiment 1, 'Cherry Belle' radish (Raphanus sativa L.) was grown to market size in pots of synthetic potting mix by using a 5 x 5 x 5 x 2 factorial design that consisted of 5 replications, 5 levels of $(NH_4)_2SO_4$ (0,100,200,400, and 800 ppm), 5 rates of K_2SO_4 (0,140,280,560,1120 ppm), and 2 regimes of Ns (plus and minus). In Experiment 2, 'Cherry Belle' radish was grown in randomized complete-blocks with five replicates of plus or minus Ns treated plants being grown on each regime of N and K (000 N + 000 K, 150 N + 210 K,300 N + 420 K, 600 N + 840 K, and 1200 + 1680 ppm K). The nutrients were applied as half KNO_3 and half $Ca(NO_3)_2$. Two crops of radishes were grown in each experiment and

the plants were separated into leaves and roots. Spinach was grown in Experiment 3 by using a 5 x 4 x 2 x 2 factorial design consisting of 5 replications, 4N regimes (00 N, 200 $Ca(NO_3)_2$, 200 $(NH_4)_2SO_4$ and 200 ppm urea), 2 K rates (000 N and 200 K_2SO_4), and 2 Ns regimes (plus and minus).

The optimum level of N for radish was 200 ppm, but, NH_4 levels greater than 100 ppm caused diminished growth. Likewise, high rates of NH_4 depressed root and leaf weight, % K, and total K content. High levels of NO_3 depressed leaf and root weight and hence K content. But, it did not depress the K concentration of any plant part. Potassium applications did not affect the weight of leaves, but it increased root weight, the % K in leaves, and root, and K uptake. Nitrapyrin in the presence of NH_4 increased dry weight of leaves and roots, but it did not affect % K or K content. Likewise, Ns in the presence of NO_3 had no effect on either weight, % K, % NO_3, K content or NO_3 content.

Spinach weight increased with N and K applications. Nitrate was the most beneficial source of N for increasing weight. The increased weight caused a dilution of K, Ca, and P concentration. Nitrapyrin increased P concentration, but it had no influence on K or Ca concentration. Likewise, the chemical had no affect on K, Ca, or P content.

INTRODUCTION

Spinach (Spinacia oleracea L.) and radish (Raphanus sativus L.) are two fast growing vegetable crops. Nitrogen fertilizer is often applied to these crops to ensure rapid, luxuriant, and succulent plant growth. Although the application of N to the growth medium aids growth of these cultivars, inorganic N, especially NO_3, has been shown to accumulate in the tissue of these crops. This occurs primarily in response to the excessive N applications which are sometimes used on these cultivars.

Uncombined nitrites and ammonium in plant tissue can cause severe problems with plant growth. But, plants can tolerate large concentrations of uncombined nitrate in their tissue without the plant itself being adversely affected (10,21,22,24,63).

Nitrate ions may not harm the plant by being in their tissue. But, they could reek havoc if they are reduced to nitrites by animal consumption or storage in cans for human consumption (10,87).

According to news reports, nitrites have been shown to be carcinogenic agents and as such are dangerous to people. Therefore, in order to avoid the dangerous affects of nitrites, it is imperative that we make optimum use of N fertilizer applications. This would keep the uncombined N level of the plant to a minimum.

One way of achieving optimum efficiency from N applications on these cultivars would be to apply ammonium or urea fertilizers in conjunction with nitrapyrin, a nitrification inhibitor, (29,39,42,52,63,79,92,99,110). Another way is K fertilization since it has frequently been shown to suppress the accumulation of nitrates (92,95,102,104,105).

Although hope exists for controlling the NO_3 concentration of these cultivars by using applications of K and nitrapyrin, some problems have emerged. Some investigators (23,24,64) say that K causes nitrate accumulation while others (104,105) said that it does not. Furthermore, K has been shown to decrease soluble N compounds by aiding in protein synthesis (62,95,103).

Ammonium fertilization has been shown to interfere with K uptake (55,88). Likewise, when ammonium is absorbed as the principal form of N, a high concentration of K is required for optimum plant growth (13,31,43). Also, Becking (15)found that in older plants NH_4^+ ions exchange for K ions of the root.

Nitrapyrin has been shown to affect nutrient accumulation by plants. Mills et al (63) reported an increase in the potential for ammonium toxicity when the chemical was applied. Zawistowska et al (110) indicated that relative to untreated controls nitrapyrin reduced NO_3, K, and Ca uptake by cucumber seedlings 24, 17 and 25%, respectively.

Nitrapyrin has been shown to cause adverse effects on plant growth. Hendrickson et al (48) said that potato growth on nitrapyrin was characterized by stunted, dark green tops with bushy rather than vine-like growth. However, the symptoms became less evident as N rate increased. Lynd et al (61) showed the chemical to produce auxin type growth. Too, in Experiment 2 or Part I, short bushy soybean plants with curled and twisted leaves were observed. The plants also lost their apical dominance and the cotyledons failed to abscise.

The foregoing findings indicate that ammonium and nitrapyrin applications may be harmful to plant growth. However, no information could be found which shows the relationship between N source, K fertilization, and nitrapyrin applications on spinach and radish. Therefore, experiments were conducted to look at the effect of these factors on the growth of the indicated cultivars.

MATERIALS AND METHODS

The experiments were conducted in the greenhouse. The plants were grown in a synthetic potting medium which consists of 7 parts loam, 3 parts peat, and 2 parts sand (v/v/v).

Experiment 1: The first radish experiment was set up in a randomized complete block design with five replicates of each treatment. Nitrapyrin at the rate of 8 mg/100g of soil was used on 25 treatments and no nitrapyrin was applied to the remaining 25 treatments. The legend of $(NH_4)_2SO_4$ and K_2SO_4 combinations for each regime of nitrapyrin (plus and minus) was as follows:

K rate (ppm)	N rate (ppm)				
	treatment number				
000	1	2	3	4	5
140	6	7	8	9	10
280	11	12	13	14	15
560	16	17	18	19	20
1120	21	22	23	24	25

The pots were seeded and allowed to grow for two weeks. They were then thinned to 5 plants per pot. The plants were kept moist by using tap water. They were allowed to grow until they reached market size (52 days). At harvest, the plants were separated into leaves and expanded hypocotyl (root). The plant sections were dried in an air-circulating oven at 70^{o}C, ground, and analyzed for K content.

Two crops of radish were grown on each treatment without any additional nutrients being added during the second growing period (42 days). Crop growth at the second harvest was substantially reduced on the 2 lowest levels of N (100 and 200 ppm). This indicated that the residual level of N was low.

Experiment 2: The second radish experiment was conducted in a manner similar to that of Experment 1. However, the N was supplied as half KNO_3 and half $Ca(NO_3)_2$. The legend of N and K combinations for each nitrapyrin regime were as follows:

ppm N	ppm K	treatment number
000	000	1
150	210	2
300	420	3
600	840	4
1200	1680	5

Due to an experimental error, the rates of N and K were altered from those in Experiment 1. However, this experiment was conducted to assess the affects of nitrapyrin and nitrate applications on the growth, K and NO_3 content of 'Cherry Belle' radish. Furthermore, these data were compared with the data from Experiment 1.

Experiment 3: The spinach experiment was set up in the greenhouse. The procedures were similar to those of the radish experiments. However, three N sources were applied at the rate of 200 ppm N each, and a no-N series was used. The legend of treatments was as follows:

K_2SO_4 (ppm)	N Source: no N	$NaNO_3$	$(NH_4)SO_4$	Urea
	treatment number			
000 K	1	3	5	7
200 K	2	4	6	8

This experiment was done to look at the affect of nitrapyrin and the three N source on the growth of spinach plants. Each N source was applied at the rate of 200 ppm because this appeared to be the optimum level of N for radish growth. Also, the spinach was grown for 56 days, because, they were planted during a time (February 25, 1977) when the light intensity and duration were low. Therefore, growth was slow.

RESULTS AND DISCUSSION

Experiment 1.

Fresh weight. Fresh weight of 'Cherry Belle' radish was affected by N fertilization in both the 1st and the 2nd harvests (Table 1). The fresh weight increased significantly with each increment of N fertilizer until 400 ppm was put in the medium. However, the root fresh weight in the first harvest decreased at high rate of N. This indicates that high rates of N stimulate vegetative yield but NH_4-N suppressed root yield possibly by becoming toxic to the plant. The fresh weight of the entire plant again showed that high rates of N suppressed plant growth. The 800 ppm of N produced less fresh weight than any other N rate while 200 ppm of N produced the most fresh material indicating that it was the optimum level for growing the radish under the experimental conditions used.

As expected the fresh weights of the 2nd harvest (except 800 ppm) were substantially lower than those of the first harvest. Those pots that received 400 and 800 ppm of N produced significantly more leaf and root fresh weight at the second harvest than did any other treatment. This indicates that the N fertilizer from the other treatments had been depleted or lost. Also, the 800 ppm treatment produced substantially more fresh weight during the second harvest than it did during the first. This further enforces the idea that high rates of N depressed plant growth.

Potassium applications had no effect on the fresh weight of leaf

Table 1. Fresh weight of 'Cherry Belle' radish as affected by nitrogen rate.

	Harvest 1:			Harvest 2:		
N rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	g/pot					
000	25a*	54b	80b	1.2	2.0	3.2
100	30b	66c	96d	1.2	3.0	4.2
200	36bc	68c	104e	2.0	6.0	8.0
400	38c	51b	89c	7.0a	33a	40a
800	33b	27a	60a	21b	56b	77b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

tissue during either the first or second harvest (Table 2). The fresh weight of the roots did increase significantly with each increment of K added to the medium. Unlike N, high rates of K do not have a toxic effect on plant growth as indicated by the absence of growth suppression at high K rates.

The fresh weight of the second harvest was substantially lower than that of the first harvest regardless of the K rate. This condition was probably the result of decreased plant vigor due to the depletion of N on those pots that received less than 400 ppm of N. In all likelihood, this reduced vigor caused low yields since the values shown represent an average over all N rates for each level of K applied. Therefore, no valid range test could be performed on these data.

The total leaf fresh weight from both harvests increased with each increment of N fertilizer added to the medium (Table 3). Likewise, an increase in fresh weight per pot was observed with increasing rates of N fertilization. But, the root fresh weight did not increase significantly with each increment of N added. Therefore, the growth of the plant portion usually eaten can be maximized by using far less N than was used in this study.

Increasing the rate of K did not significantly alter the fresh weight of radish leaves. However, the root growth was significantly increased by adding large amounts of K. This indicates that K aids in root development rather than the production of shoot vegetation.

Table 2. Fresh weight of 'Cherry Belle' radish as affected by K rate.

	Harvest 1:			Harvest 2:		
K rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	g/pot					
000	32a*	49ab	81a	6.2	12.7	18.9
140	32a	47a	79a	7.4	22.1	29.5
280	33a	54abc	87ab	7.0	22.0	29.0
560	32a	56bc	88ab	5.2	19.5	24.7
1120	32a	59c	91b	6.7	24.6	31.3

* Means in the same column and followed by the same 1-tter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 3. Total fresh weight of 'Cherry Belle' radish from two harvests as affected by N and K applications.

N rate	Leaf	Root	Pot	K rate	Leaf	Root	Pot
(ppm)		g/pot		(ppm)		g/pot	
000	27a*	56a	83a	000	39a	61a	100a
100	31b	69b	100b	140	39a	69ab	108ab
200	38c	74b	112c	280	40a	76bc	116bc
400	45d	84c	129d	560	37a	76bc	113bc
800	54e	83c	137e	1120	39a	84c	123c

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Dry weight. Dry weight of 'Cherry Belle' radish was significantly influenced by different rates of N during both the 1st and 2nd harvests (Table 4). In the first harvest, leaf weight increased with each N application below 400 ppm while root dry weight accumulation was suppressed at high N rates. A fact which was explained with respect to fresh weight data. Harvest 2 produced less dry weight than harvest 1 except at the high N rates in which case the production of root dry matter for the 800 ppm treatment was more than 2 times greater than that in harvest 1.

Potassium applications did not affect the production of leaf dry matter in the radish cultivar during either harvest 1 or 2 (Table 5). However, with each increment of K applied a corresponding increase in root weight was observed in both harvest 1 and 2. The first harvest produced more dry weight than did the second as a result of decreased plant vigor as mentioned earlier.

The accumulation of dry weight followed the same pattern as was observed for fresh weight accumulation (Table 6). However, the ratio of leaf to root weight was larger for dry weight than fresh weight. Therefore, the radish roots contained substantially more water than did the leaves thus making their yield seem artificially high when compared to leaves.

The addition of N to the medium increased the dry weight of both leaves and roots. With each increment of N, there was a corresponding increase in leaf as well as whole pot dry matter content. However, roots did not show the same trend. Their dry weight increased with the first increment of N (100 ppm) but did

Table 4. Dry weight of 'Cherry Belle' radish as affected by N applications.

	Harvest 1:			Harvest 2:		
N rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	g/pot					
000	1.9a*	3.2c	5.1b	0.4	0.2	0.6
100	2.3b	3.9d	6.2cd	0.1	0.3	0.4
200	2.8c	3.7d	6.5d	0.3	0.6	0.9
400	3.0c	2.8b	5.8c	0.7a	2.5a	3.2a
800	2.9c	1.7a	4.6a	2.3b	3.8b	6.1b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 5. Dry weight of 'Cherry Belle' radish as affected by potassium applications.

	Harvest 1:			Harvest 2:		
K rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	g/pot					
000	2.7a*	2.8ab	5.5ab	0.7	0.9	1.6
140	2.5a	2.6a	5.1a	0.9	1.6	2.5
280	2.6a	3.0abc	5.6ab	0.8	1.7	2.5
560	2.6a	3.4c	6.0b	0.8	1.4	2.2
1120	2.7a	3.3bc	6.0b	0.6	1.8	2.4

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 6. Total dry weight for two harvests of 'Cherry Belle' radish as affected by N and K applications.

N rate	Leaf	Root	Pot	K rate	Leaf	Root	Pot
(ppm)	g/pot			(ppm)	g/pot		
000	2.2a*	3.4a	5.6a	000	3.3a	3.7a	7.0a
100	2.5b	4.1b	6.6b	140	3.3a	4.2ab	7.5ab
200	3.1c	4.2b	7.3c	280	3.4a	4.7bc	8.1ab
400	3.7d	5.2c	8.9d	560	3.2a	4.8bc	8.0ab
800	5.2e	5.5c	10.7e	1120	3.3a	5.1	8.4b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

not increase again until 400 ppm of N was added to the medium. These findings indicate that increasing the N rate for radish above 100 ppm serves to increase the vegetative yield of the plants while causing a minimal increase in root dry weight. Therefore, from an economic standpoint, it would be unprofitable to add N to the medium in excess of 100 ppm due to the diminished returns with increasing N rates. Too, this quantity of N could be used more efficiently than higher rates. This efficient use would cut down on the amount of N left to contaminate water via leaching and surface runoff.

Increasing the rates of K in the medium had no effect on leaf dry weight. However, it did significantly increase the dry weight of roots and the entire pot. This indicates that K enhances the rooting process while N enhances the vegetative process.

Although increasing rates of K caused increased root growth, little benefit was obtained from K applications above 280 ppm. Thus, it appears that this was the optimum level to apply under the test conditions employed in this study.

Percent K. Applications of N up to 400 ppm increased the % K present in both leaves and roots above the level in the zero N treatment (Table 7). Similar to the findings of Halvorson et al (43), the application of N to the growth medium caused an increase in the % K concentrated by the leaves. However, the 100 ppm N treatment contained as much K as any other treatment, and high (800 ppm) rates of N depressed K concentration. But, the depressed K concentration was not accompanied by an increase in weight,

Table 7. The K concentration of 'Cherry Belle' radish as affected by N applications.

	Harvest 1:		Harvest 2:	
N rate	Leaf	Root	Leaf	Root
(ppm)	% K		% K	
000	4.70ab*	4.45a	2.00	3.95
100	5.09b	4.78ab	1.75	3.70
200	5.11b	4.86b	1.80	3.85
400	4.94b	4.79ab	2.29a	3.60a
800	4.39a	4.75ab	2.77b	3.65a

* Means followed by the same letter and in the same column are not different at the 5% level of significance by Duncan's Multiple Range Test.

therefore, it was not a dilution effect.

The % K in the roots increased with N application. However, rates of N above 200 ppm seemed to decrease the % K in the tissue without an increase in tissue weight. The depression in root % K may have been due to the exchange of NH_4 ions of the medium for K ions of the root as suggested by Becking (15) who observed this phenomenon on old plants. Alternatively, NH_4 could have caused a reduction in the energy supply of the plant by uncoupling photosynthetic phosphorylation as suggested by Krogmann et al (56) who showed that ammonium ions inhibited ATP formation by 95% as a result of the uncoupling process. This reduction in energy supply would cause less K to be taken up by the plant since K transport is an active process. Another possibility is that ammonium was competing with K for exchange sites as reported by other workers (55,88). Further evidence that suggests competition between the two ions was reported by Barker et al (12) who found that lesion development in tomatoes is a manifestation of both NH_4 toxicity and K deficiency. Also, it has been shown that when NH_4 is the principal N source a high rate of K is necessary for plant growth (13,31).

Although the % K concentrated by the tissue in this study was reduced by $(NH_4)_2SO_4$ applications, no visual symptoms of NH_4 toxicity or K deficiency were observed. Therefore, it seemed unlikely that the K concentration of the plant was being suppressed to a critically low level. Thus, the uncoupling theory was discareded in this instance because the effects were not severe.

However, no distinction could be made between the competition and exchange hypotheses since the NH_4 concentration was not determined, and the plants were not harvested before they reached market size.

During the second growing period N rate had no apparent affect on the K concentration of the roots. But, high rates of N did increase the % K in the leaves while also increasing the dry weight of the plant tissue. The increase in % K present along with the increased yield indicates that more K was being taken up from the medium by these plant than other plants. This also suggests that the K content of the medium had not been completely exhausted. However, the level had been depleted considerably as evidenced by a comparison between the % K concentrated during the first and second growing periods.

The addition of K fertilizer to the medium significantly increased the % K present in the leaves and roots (Table 8). But, the increase in % K of leaves did not promote an increased yield of this plant portion. Thus, it appears that the plants were luxuriously consuming K as a result of an abundant supply.

The K concentration of roots increased with each increment of K added. Also, the weight of roots increased with K fertilization. These findings add validity to the assumption that K aids in the fruiting process of radish rather than the production of vegetation.

The % K concentrated by each plant portion in harvest 1 was greater than the amount accumulated in harvest 2. This trend can be attributed to the depletion of K from the growth medium during

Table 8. Potassium concentration of 'Cherry Belle' radish as affected by K-rate.

	Harvest 1:		Harvest 2:	
K-rate	Leaf	Root	Leaf	Root
(ppm)	% K		% K	
000	3.43a*	3.70a	1.00	2.98
140	4.29b	4.51b	1.68	3.53
280	4.50b	4.60b	1.71	3.69
560	5.62c	5.09c	2.39	4.03
1120	6.39d	5.74d	3.91	4.47

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

the first growing season.

Although the % K concentrated at harvest 2 was depressed below that of harvest 1, the depression was not sufficient to cause the decreased yield that was observed at each level of K applied. This fact becomes abundantly clear when one looks at the yield obtained at the high rates of N fertilization. Also, these findings further emphasize the supposition that yield retardation in this study was due to N depletion from the root zone.

Total mg K. Nitrogen fertilization increased the K content of the plants during both the first and second harvests (Table 9). During the first growing period, the leaf, root and entire plant K content increased with the first 3 rates of N fertilizer. However, at the 800 ppm N level, the K content was reduced. The reduction was due to reduced growth probably as result of NH_4 toxicity and or competition of NH_4 ions with K ions for exchange sites of the carrier as suggested by others (12,55,88).

During the second growing period, N was noticeably depleted from the growth medium as evidenced by the low yield obtained from the 100, 200, and 400 ppm treatments. This caused less K to be taken up from the medium. However, the 800 ppm N treatment took up approximately the same amount of K that it did during the first growing period, a fact that can be attributed to the amount of residual N and K present and the yield obtained.

With increased K fertilization, there was an increase in the K content of leaves,roots and the entire plant (Table 10). The K

Table 9. Potassium content of 'Cherry Belle' radish as affected by N applications.

	Harvest 1:			Harvest 2:		
N rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)			g/pot			
000	91a*	148a	239a	9	8	17
100	120b	185c	305b	3	10	13
200	145c	174c	319b	5	23	28
400	151c	135b	286b	14a	88a	102a
800	126b	80a	206a	64b	138b	202b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 10. Potassium content of 'Cherry Belle' radish as affected by K applications.

	Harvest 1:			Harvest 2:		
K rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	g/pot					
000	91a*	104a	195a	7	26	33
140	105ab	117ab	222ab	14	57	71
280	116b	136b	252b	14	62	76
560	150c	172c	322c	20	57	77
1120	170d	192c	362d	25	78	103

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

content of the leaves for harvest 1 was almost equal to that of the roots, a fact which can be attributed to the fact that K is a very mobile element. The increased K accumulation in the leaves also suggests that leaves are the sink when K is taken up in excess of plant needs. Indeed, others (53,85,90) have shown that K accumulates in the chloroplasts of leaves. Also, in this study the K content of the leaves was affected more by the % K in the tissue than by the weight of the tissue. Again, this suggested that K was taken up luxuriously.

The accumulation of K by radish roots was more of a reflection of the K concentration than a manifestation of root weight. At the first harvest, K content increased with each increment of K applied in much the same fashion as the % K of roots.

Increasing the N rate significantly increased the K content of the leaves (Table 11). However, in the root, the first increment of N (100 ppm) accounted for about the same amount of K uptake as the highest rate of N (800 ppm). This trend was observed even though the weight of the 800 ppm treatment was substantially greater than that of the 100 ppm treatment. Thus, the % K of the tissue appears to be the overbearing factor influencing the K content of roots.

Applications of K to the medium increased the K content of both leaves and roots. Each increment of applied K caused an increase in K content of the roots. The K content of the leaves increased with the first increment of K, but remained the same for the second while increasing for each of the highest K rates.

Table 11. Potassium content of 'Cherry Belle' radish as affected by N and K rates.

N rate	Leaf	Root	Pot	K rate	Leaf	Root	Pot
(ppm)	g/pot			(ppm)	g/pot		
000	94a*	156a	250a	000	97a	125a	222a
100	122b	195b	317b	140	121b	171b	292b
200	150c	197b	347bc	280	132b	196c	328c
400	169d	223b	392bc	560	166c	229d	395d
800	184d	222b	406c	1120	201d	272e	473e

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

These findings further enforce the idea that N fertilization enhances vegetative yield while K fertilization increases the rooting response.

Nitrapyrin. The presence of nitrapyrin (Ns) in the medium did not affect fresh weight (Table 12). However, when Ns was in the medium, dry weights of leaves, roots, and the entire plant were significantly increased during the first harvest. Likewise, the root and entire plant dry weight per pot (harvest 1 + 2) was significantly increased by having Ns in the medium.

The dry weight of leaves and roots for harvest 2 as well as the leaf dry weight per pot were not altered by having Ns in the medium. The lack of a response at harvest 2 was probably due to the low fertility level of the medium (i.e., most of the nutrients were taken up during the first growing period).

The presence of Ns in the medium did not affect the % K or the mg K/pot of 'Cherry Belle' radish (Table 13). This result was obtained in spite of Ns being implicated as a catylast which increases the potential for ammonium toxicity in plants (31,63). The increased uptake of ammonium would affect the uptake of K by the plants as suggested by others (13,15,31,43,55,88). Therefore, those plants that were treated with the chemical should have a lower K content than the untreated plants. However, this was not the case. Thus, it appears that Ns did not promote the uptake of ammonium ions by the plant to a level above that of the untreated plants. This lack of a delineation was probably due to a small population of Nitrosomonas bacteria in the synthetic potting mixture.

Table 12. The effect of nitrapyrin (Ns) on the weight of 'Cherry Belle' radish.

Plant Portion	Harvest 1: +Ns	Harvest 1: -Ns	Harvest 2: +Ns	Harvest 2: -Ns	Harvest 1+2 +Ns	Harvest 1+2 -Ns
	g/pot					
			Fresh Weight			
Leaf	32a*	32a	6.2a	6.8a	38.2a	38.8a
Root	55a	51a	21.8a	18.6a	76.8a	69.6a
Plant	87a	83a	28.0a	25.4a	115a	108a
			Dry Weight			
Leaf	2.7a	2.5a	0.8a	0.7a	3.5a	3.2a
Root	3.2b	2.8a	1.6a	1.4a	4.8b	4.2a
Plant	5.9b	5.2a	2.4a	2.1a	8.3b	7.4a

* Means in the same row and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 13. The effect of nitrapyrin (Ns) on the K content of 'Cherry Belle' radish.

Plant Portion	Harvest 1: +Ns	Harvest 1: -Ns	Harvest 2: +Ns	Harvest 2: -Ns	Harvest 1+2 +Ns	Harvest 1+2 -Ns
			% K			
Leaf	4.9a*	4.8a	1.2a	1.3a		
Root	4.7a	4.9a	1.8a	2.0a		
			mg K/pot			
Leaf	131a	122a	16a	18a	147a	140a
Root	152a	137a	55a	54a	207a	191a
Plant	283a	259a	71a	72a	354a	331a

* Means in the same row and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Another possibility is that Ns was being absorbed by organic matter in the growth medium. This would allow the ammonium to be nitrified, and hence reduce the potential for ammonium toxicity. However, this was apparently not the case since the nitrate content of these plants was about the same as that of the zero N treatment (0.16%).

Interactions. When either K or nitrapyrin (Ns) was applied alone, there was not a significant affect on the fresh weight of radish leaves (Table 14). However, a meaningful relationship was observed when these two factors were used in conjunction with each other. This relationship was probably fostered as a result of weight depression when Ns was applied without any K fertilizer or with an excessively high K rate (1120 ppm). Likewise, the significant interaction between N and Ns can be attributed to weight reduction when Ns was applied without N fertilizer. These relationships suggest that Ns was toxic to the plants. Therefore, the potential for toxicity does exist. Furthermore, the three factor interaction (K x N x Ns) adds validity to the assumption that Ns is toxic to plants. Both N and K fertilization increased the fresh and dry weight of roots. Likewise, a meaningful relationship was shown to exist between these two factors. This relationship was most apparent for the 400 ppm N and 280 ppm K treatment. Thus, it appears that N and K applications were necessary for optimum plant growth.

Potassium fertilization did not increase the leaf dry weight of radish. However, Ns did increase the dry weight of radish leaves.

Table 14. Interactions for the weight and potassium content of 'Cherry Belle' radish as affected by potassium (K), nitrogen (N), and nitrapyrin (Ns) applications.

Leaf	Root	Plant	K x N	K x Ns	N x Ns	N x K x Ns
Fr. Wt.	-	-	ns	**	*	**
-	Fr. Wt.	-	*	ns	ns	ns
-	-	Fr. Wt.	ns	ns	ns	ns
Dry Wt.	-	-	ns	*	**	**
-	Dry Wt.	-	*	ns	ns	ns
-	-	Dry Wt.	ns	*	ns	ns
% K	-	-	**	ns	ns	ns
-	% K	-	**	ns	**	ns
mg K	-	-	**	*	*	*
-	mg K	-	ns	ns	ns	ns
-	-	mg K	ns	ns	ns	ns

The 5 and 1 percent level of significance are represented by * and ** respectively while ns means nonsignificant.

Likewise, a significant interaction was shown to occur between these two factors.

Nitrapyrin significantly increased the dry weight of leaves on the zero K and all treatments which contained less than 560 ppm K. But, weight was reduced at high levels of K. This occurred even though high rates of K did not depress leaf dry weight. Therefore, it appears that Ns can be detrimental to radish growth when high rates of K are applied to the growth medium.

The significant interaction between N and Ns again reflected the reduced weight of the minus N treatment. But, when N was added to the medium, the dry weight of the Ns treatments was considerably greater than the untreated plants. These findings are consistent with those of Hendrickson et al (48). Also, they suggest that if the chemical is to be applied to this medium for radish growth; it should definitely be applied in conjunction with N fertilizer. The three factor interaction (N x K x Ns) further reflects the idea that fertilization is necessary when Ns is applied to medium for plant growth.

Nitrapyrin applications increased the whole plant weight above the weight of N on treated plants at all levels of K less than 1120 ppm. This trend is probably responsible for the significant interaction of Ns and K on plant weight.

A significant interaction was shown to exist between N and K application for the % K concentrated by leaves and roots. The highest concentration for leaves was 400 ppm N and 1120 ppm K. Also, a similar trend was observed for roots. These results were expected since K was applied to the medium of these plants. Therefore, the potential for increasing K concentration did exist.

A meaningful relationship was observed between N and Ns for the % K in radish roots. The % K concentrated by the roots of Ns treated plants was less than that of non treated plants when the N rate was less than 800 ppm. This trend was attributed to the weight of the plants.

Nitrogen rates below 800 ppm and small K applications significantly increased the K content of the plants. But, the application of Ns had no affect on K content. These factors also exhibited meaningful relationships with each other when the K content of the leaves was examined.

The interaction between N and K applications probably occurred in response to both leaf weight and leaf K concentration. Apparently, this was the case since both of these parameters increased the N content with N applications up to 400 ppm and K rates up to 1120 ppm.

At first glance, it appears that the K and Ns interaction was caused by high rates of K in conjunction with Ns since this regime caused a reduction in K content while the other Ns and K regimes increased the K content above the level found in minus Ns treatment. Furthermore, the differences in K content between the Ns and non Ns treated plants for the 3 lowest K rates did not differ greatly from each other. Upon close examination, it appears that the direct cause and effect relationship can not be attributed to high rates of K since it was shown that radish leaves can concentrate excessive amounts of K. Therefore, it appears that Ns was the causative factor. Indeed, this could have been the case since Ns has been shown to be phytotoxic to plants. Also, the chemical has been known to increase the potential for NH_4 toxicity in radish. The increased NH_4 supply could interfere with K uptake by being antagonistic to K (55,88) or it could exchange for K of the root (15).

The significant interaction between N and Ns regimes seem to reflect the lack of N in the zero N treatment. Apparently, this was the case since Ns without N causes a reduction in K content while the differences between Ns treatments were not great when N was applied. Therefore, N seem to lessen the effects of Ns on K uptake. Also, the 3 factor interaction (N x K x Ns) exemplify the foregone findings.

Experiment 2.

Weight. High rates of nitrates and K fertilizers decreased the fresh weight of 'Cherry Belle' radish (Table 15). The leaf, root, and entire plant fresh weights at the first harvest, were substantially decreased when 600 ppm or more of N and 840 ppm or more K were added to the growth medium. These findings are similar to those of Experiment 1 in which 400 ppm of NH_4 caused a reduction in weight. Therefore, the reduction in weight is probably due to the high rate of N in the medium since increasing the K rate as high as 1120 ppm in the first experiment did not cause a reduction in yield. Thus, it appears that high rates, of either NH_4 or NO_3 fertilizers are toxic to plants.

During the second growing period the yield from replicates of

Table 15. The effect of N and K applications on the fresh weight of 'Cherry Belle' radish.

		Harvest 1:			Harvest 2:		
N rate	K rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	(ppm)	g/pot					
000	000	23b*	58c	81c	0.7	2.3	3.0
150	210	28c	62c	90c	1.9	6.0	7.9
300	420	28c	53c	81c	5.8a	34.2a	40.0a
600	840	22b	40b	62b	15.6b	61.3b	76.9b
1200	1680	13a	13a	26a	21.1c	67.3b	88.4b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

treatment 1 (0 ppm N + 0 ppm K) and treatment 2 (150 ppm + 210 ppm K) was too small for each replicate to be harvested separately. Therefore, all of the plants were harvested, separated into leaves and roots, weighed and devided by the number of replicates (5) in each treatment to obtain a weight figure. For that reason, no statistics were performed on these data.

During the second growing period, the leaf fresh weight of plants receiving 300 or more ppm of N and 420 or more ppm of K increased with each increment of fertilizer added. However, the root fresh weight did not increase above the 600 ppm N 840 ppm K treatment. This was further reflected in the fresh weight of the entire plant. The lack of a yield response at the high fertilizer rate is likely due to N toxicity as indicated earlier. Another possibility is that the plants had obtained their maximum yield as a result of genetic limitations.

The dry weight accumulation showed much the same pattern as the fresh weight (Table 16). However, root weight depression in harvest 1 was observed at N and K rates above 150 and 210 ppm respectively instead of the 300 ppm N and 420 ppm K regime for fresh weight. This suggested that the water content of the roots varied from one treatment to another. The total fresh and dry weight of the leaves increased with each increment of N and K added to the medium (Table 17). The root fresh weight increased at the first three N and K rates. However, at the highest N and K rate, root weight was significantly reduced again indicating that N was at a toxic level.

Table 16. The effect of N and K applications on the dry weight of 'Cherry Belle' radish.

		Harvest 1:			Harvest 2:		
N rate	K rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	(ppm)	g/pot					
000	000	1.7b*	3.7c	5.4c	0.1	0.2	0.3
150	210	2.2c	3.4c	5.6c	0.3	0.6	0.9
300	420	2.2c	2.9b	5.1c	0.7a	2.4a	3.1a
600	840	1.8b	2.4b	4.2b	1.6b	4.7b	6.3b
1200	1680	1.3a	1.0a	2.3a	2.5c	4.5b	7.0c

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 17. The effect of N and K applications on the wright of 'Cherry Belle' radish from both harvests.

		Fresh weight			Dry weight		
N rate	K rate	Leaf	Root	Pot	Leaf	Root	Pot
(ppm)	(ppm)	g/pot					
000	000	24a*	60a	84a	1.8a	3.9a	5.7a
150	210	30ab	68ab	98ab	2.5b	4.0a	6.5ab
300	420	34bc	87bc	121bc	3.0bc	5.2a	8.2bc
600	840	37c	102c	139c	3.3cd	7.1b	10.4c
1200	1680	39c	81abc	120bc	3.8d	5.4a	9.2c

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Total leaf dry weight of radish increased with each increment of nutrients added. However, no meaningful increase in root weight was observed until 600 ppm of NO_3 and 840 ppm K were added to the medium. Also, the highest rate of nutrients caused a reduction in root dry weight. This indicated that the level of N was still too high to allow optimum plant growth.

Percent K. At harvest 1, increasing applications of N and K caused the concentration of K to increase in the plant tissue (Table 18). The % K in the leaves increased when N and K were added to the medium but to a lesser extent than root K concentration at the second and third level of fertilizer application. At the highest level of fertilizer application the % K in leaves is about equal to that in roots.

In contrast to the findings in Experiment 1 when NH_4 was the N source, high NO_3 levels do not inhibit the accumulation of K in the plants. Indeed, the % K in the tissue of NO_3 treated plants is about equal to that of high K fertilization when no N is present. These findings enforce the idea that NH_4 interferes with K uptake.

At the second harvest date, the % K in leaves and roots increased at about the same rates for the three highest regimes of N and K. However, the % K in the roots was larger than that in the leaves. This trend was also observed at the first harvest, and is probably due to a translocation effect (i.e. the translocation of K from vegetative to fruiting parts) since the roots also have a higher dry weight than the leaves. These findings in conjunction with those of Experiment 1, where the leaves contained the highest level of K,

Table 18. The effect of N and K applications on the percent K in 'Cherry Belle' radish.

		Harvest 1:		Harvest 2:	
N rate	K rate	Leaf	Root	Leaf	Root
(ppm)	(ppm)	% K		% K	
000	000	3.65a*	3.66a	2.00	3.68
150	210	4.20b	5.44b	2.93	3.57
300	420	4.46b	6.04c	2.25a	3.69a
600	840	4.46b	6.01c	3.98b	4.40a
1200	1680	6.67c	6.99c	4.85b	5.54b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

suggest that N source influences the distribution of K in plant tissue.

K content. Unlike K concentration, high rates of N and K fertilizer caused a reduction in K content at the first harvest (Table 19). The K content of leaves was increased somewhat with the addition of N and K. However, at high rates of fertilizer, K content of leaves was diminished. Likewise, the highest rate of fertilization decreased the K content of the roots below that of the unfertilized treatment. This trend can be attributed to the weight of the plants growing on these treatments.

At the second harvest, the K content of leaves, roots, and the entire plant were substantially increased with increasing N and K rates. This trend occurred as a result of increased yield and a high % K. The mg K/pot in leaves, roots, and the entire pot were significantly increased by the addition of N and K fertilizers (Table 20). Each regime of N and K increased the K content of the leaves. However, the K content of the roots was increased by each of three lowest regimes of N and K while the highest regime caused a reduction in K content. Likewise, the K uptake per pot was increased with each of the first three regimes of N and K. However, at the highest level of nutrients, the uptake of K was somewhat suppressed. Thus, the propensity for high nutrient levels to suppress K uptake can be attributed to a reduction in plant yield at high rates of N fertilization.

Percent NO_3. The water extractable NO_3 concentration in the plant increased for each increment of N and K applied (Table 21). The increase in NO_3 concentration can be attributed largely to high rates

Table 19. The effect of N and K applications on the K content of 'Cherry Belle' radish.

		Harvest 1:			Harvest 2:		
N rate	K rate	Leaf	Root	Plant	Leaf	Root	Plant
(ppm)	(ppm)	mg/pot					
000	000	62a*	136b	198b	0.2	1.5	1.7
150	210	95b	184c	279c	1.7	4.3	6.0
300	420	101b	172c	273c	16a	89a	105a
600	840	80ab	142b	222b	64b	207b	271b
1200	1680	84ab	66a	150a	122b	249b	371b

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 20. The effect of N and K applications on the combined K content from both harvests of 'Cherry Belle' radish.

N rate	K rate	Leaf	Root	Pot
(ppm)	(ppm)		g/pot	
000	000	62a*	138a	200a
100	140	97b	188ab	285b
200	280	117bc	261bc	378bc
400	560	144c	349d	493c
800	1120	206d	315cd	521c

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Table 21. The effect of N and K applications on the percent nitrate in 'Cherry Belle' radish.

		Harvest 1:		Harvest 2:	
N rate	K rate	Leaf	Root	Leaf	Root
(ppm)	(ppm)	% NO_3		% NO_3	
000	000	0.16a*	0.10a	-	-
150	210	0.74b	0.74b	-	-
300	420	1.49c	1.14b	-	-
600	840	1.68c	1.58c	0.13	0.19
1200	1680	2.50c	2.66d	0.47	0.85

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test. (-) = too little tissue to analyze.

of nitrate fertilizer. This was apparent since those plants that were not fertilized with this N source did not accumulate nitrates to a level above that which was found in the unfertilized treatments.

At the first harvest, the NO_3 concentration was substantially greater than at the second harvest. This trend can be attributed to uptake and subsequent depletion of nitrates from the rooting zone of the plants. This appears to be the major source of loss since plant growth was poor at the second harvest for those treatments that did not receive excessive NO_3 fertilizer. Although depletion appears to be the major source of loss, other factors (leaching and denitrification) may have contributed to the decline of nitrate in the growth medium.

These findings (Table 21) in conjunction with those of Experiment 1 indicate that the level of NO_3 in tissue can be drastically reduced by using applications of NH_4 fertilizer and nitrapyrin. They also showed that when high rates of nitrates are applied little benefit is obtained from applying excessive K. Therefore, it appears that optimum use of N can be made when it is applied at moderate rates and at peak growing periods.

NO_3 content. The NO_3 content in plant tissue followed much the same trend as that of NO_3 concentration (Table 22). However, at rates of NO_3 applications above 300 ppm there was no discernible difference between the mg NO_3/pot for any treatment. This trend can be attributed largely to the depressed growth with high rates of NO_3. Apparently, the high rate of fertilization was toxic to plant growth.

The nitrate content of the second harvest was less than that at

Table 22. The effect of N and K rates on the mg of nitrate in 'Cherry Belle' radish.

N rate	K rate	Harvest 1: Leaf	Harvest 1: Root	Harvest 1: Plant	Harvest 2: Leaf	Harvest 2: Root	Harvest 2: Plant
(ppm)	(ppm)	mg/pot					
000	000	3a*	4a	7a	-	-	-
150	210	17b	25b	42b	-	-	-
300	420	33c	32bc	65c	-	-	-
600	840	30c	36c	66c	4	8	12
1200	1680	31c	24b	55bc	13	24	37

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test. (-) = too little tissue to analyze.

the first harvest. In fact, the nitrate content of these plants was less than that of the lowest N and K regime. These findings indicate that the nitrate level in the medium had been depleted to the point that it was not consumed luxuriously.

Nitrapyrin. Nitrapyrin (Ns) had no effect on the fresh or dry weight of 'Cherry Belle' radish (Table 23). Likewise, the chemical had no effect on the % K or K content of the plant parts or the entire plant (Table 24). Furthermore, the % NO_3 and the NO_3 content was not affected by applications of Ns (Table 25).

These findings were expected since nitrates were added to the medium. Therefore, there was no need to inhibit nitrification. Also, N should be removed from the Ns treated and the untreated pots at the same rate since the form of N would be identical for each of the two regimes.

Experiment 3.

Weight. Potassium fertilization did not alter the fresh weight of spinach (Table 26). However, it did significantly increase the dry weight of spinach.

The different sources had a dramatic effect on the amount of weight obtained from this cultivar. When no N was put into the medium, spinach weight was severely reduced indicating that the N level of the potting mix was not sufficient to produce optimum plant growth.

The plant used both ammonium and urea with equal effectiveness while nitrates produced the most yield that was obtained from this cultivar. These findings add validity to the assumption that nitrates are used most efficiently for the production of vegetation. Also, they show that nitrate is the preferred source of N for crops that are grown

Table 23. The effect of nitrapyrin (Ns) on the weight of nitrate grown 'Cherry Belle' radish.

Plant Portion	Harvest 1: +Ns	Harvest 1: -Ns	Harvest 2: +Ns	Harvest 2: -Ns	Harvest 1+2 +Ns	Harvest 1+2 -Ns
	g/pot					
			Fresh Weight			
Leaf	21	24	8.6	11.4	30	35
Root	51	40	37	32	88	72
Plant	72	64	45	43	118	109
			Dry Weight			
Leaf	2.0	1.7	0.8	1.3	2.8	3.0
Root	2.5	2.4	2.5	2.4	5.0	4.8
Plant	4.5	4.1	3.3	3.7	7.8	7.8

* Means in the same row and at the same harvest did not differ from each other by Duncan's Multiple Range Test at the 5% level.

Table 24. The effect of nitrapyrin (Ns) on the K content of nitrate grown 'Cherry Belle' radish.

Plant Portion	Harvest 1: +Ns	Harvest 1: -Ns	Harvest 2: +Ns	Harvest 2: -Ns	Harvest 1+2: +Ns	Harvest 1+2: -Ns
			% K			
Leaf	4.70	4.60	2.48	2.26		
Root	5.99	5.47	3.02	3.01		
			mg K/pot			
Leaf	91	78	37	49	128	127
Root	150	129	114	109	264	239
Plant	241	207	151	159	392	366

* Means in the same row and at the same harvest did not differ from each other at the 5% level by Duncan's Multiple Range Test.

Table 25. The effect of nitrapyrin (Ns) on the percent nitrate and nitrate content of nitrate grown 'Cherry Belle' radish.

Plant Portion	Harvest 1: +Ns	Harvest 1: -Ns	Harvest 2: +Ns	Harvest 2: -Ns	Harvest 1+2 +Ns	Harvest 1+2 -Ns
			% NO_3			
Leaf	1.19	1.44	0.03	0.21		
Root	1.08	1.40	0.08	0.33		
			mg/pot			
Leaf	23	23	0.7	5.9	23.7	28.9
Root	25	24	3.1	9.8	28.1	33.8
Plant	48	47	3.8	15.7	51.8	62.7

* Means in the same row and at the same harvest did not differ from each other by Duncan's Multiple Range Test at the 5% level.

Table 26. Applications of N and K and their effect on the weight of 'Asgrow's XP 1028' hybrid spinach.

K rate (ppm)	NO_3	NH_4	Urea	no N	mean
	g/pot				
			Fresh Weight		
000	42cd*	30b	30b	8a	27A
200	46d	39c	33b	10a	32A
mean	44C	35B	32B	9A	
			Dry Weight		
000	3.9c	2.8b	2.8b	0.8a	2.6A
200	5.1d	4.4c	3.8c	0.9a	3.6B
mean	4.5C	3.6B	3.3B	0.8A	

* Means followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Capital letters are used for main effects.

principally for vegetative purposes.

Nutrient concentration. Applying K fertilizer to the growth medium increased the % K concentrated by the plant (Table 27). But, K applications lowered Ca concentration while having no effect on NO_3 or P concentration. The lowering of the Ca concentration appeared to be a dilution effect.

The no-N treatment had a larger concentration of K, Ca, and P than any of the N treatments. This probably occurred as a result of low yield on the no-N treatment. Thus, dilution seem to play the largest role in determining the nutrient concentration of the plant.

Nutrient content. The presence of K in the medium significantly increased the K content, but, it had no affect on the Ca or P content of the plant (Table 28). Also, the nutrient content of the plant was clearly a reflection of the percent nutrient concentrated by the tissue since weight was a constant factor in the calculation of each nutrient content.

Plants grown on the no-N treatment accumulated considerably less K, Ca, and P than those growing on any of the N sources. This again reflected the low fertility level of the potting mixture.

Each N source was used with equal effectiveness. Neither N source significantly increased the K content of the plant above the level found in the other sources. However, ammonium and urea caused significantly more Ca to be taken up by the plant than the nitrate N source. Likewise, ammonium caused more P to be taken up than either urea or nitrate.

Table 27. Nutrient content of 'Asgrow's XP 1028' hybrid spinach as affected by N and K applications.

K rate	N sources				
	NO_3	NH_4	Urea	no N	mean
(ppm)	%				
			Potassium		
000	0.71a*	0.84a	0.83a	2.92	1.33A
200	1.90b	2.17b	2.17b	4.34d	2.64B
mean	1.31A	1.50A	1.50A	3.63B	
			Calcium		
000	0.90b	1.34d	1.45d	1.77e	1.37B
200	0.72a	1.10c	1.15c	1.68e	1.16A
mean	0.81A	1.22B	1.30B	1.72C	
			Phosphorus		
000	0.41a	0.72d	0.69cd	0.79d	0.65A
200	0.40a	0.58bc	0.56b	0.76d	0.58A
mean	0.41A	0.63B	0.63B	0.78C	

* Means followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Capital letters are used for main effects.

Table 28. Applications of N and K and their effect on the percent N, Ca, and P in 'Asgrow's XP 1028' hybrid spinach.

K rate (ppm)	N sources				
	No_3	NH_4	Urea	no N	mean
	mg/pot				
			Potassium		
000	28a*	23a	23a	22a	24A
200	95a	93d	78c	39b	76B
mean	61B	58B	50B	30A	
			Calcium		
000	35b	37b	41bc	14a	32A
200	36b	48c	44bc	15a	36A
mean	36B	43C	43C	14A	
			Phosphorus		
000	16b	19c	19c	6a	15A
200	20c	25d	20c	7a	18A
mean	18B	22C	20B	6A	

* Means followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

Capital letters are used for main effects.

Nitrapyrin. The application of nitrapyrin to the medium significantly increased the P concentration of the plant (Table 29). But, the chemical had no effect on the weight, % K or % Ca concentrated by the plant. Likewise, the K, P, and Ca content of the plant was not affected. These findings again indicate that the nutrient content of the plant was a reflection of the nutrient concentrations rather than a manifestation of plant weight.

Table 29. The effect of nitrapyrin on the weight and nutrient content of 'Asgrow's XP 1028' hybrid spinach.

Nitrapyrin	Fresh Wt.	Dry Wt.	K	P	Ca	K	P	Ca
Regime	g/pot		%			mg/pot		
Plus	27a*	2.8a	2.14a	0.68b	1.31a	60a	19a	37a
Minus	33a	3.3a	1.83a	0.55a	1.22a	60a	18a	40a

* Means in the same column and followed by the same letter are not different at the 5% level of significance by Duncan's Multiple Range Test.

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