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Nitrogen fertility studies on the buffalo gourd (*Cucurbita foetidissima*) grown as an annual root crop

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NITROGEN FERTILITY STUDIES ON THE BUFFALO GOURD (*CUCURBITA FOETIDISSIMA*) GROWN AS AN ANNUAL ROOT CROP

by

Terry Lee McGriff

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Date

DEDICATION

This attempt at science is lovingly dedicated to the memory of my mother, Verena Arlene McGriff, whose love and quiet willingness to sacrifice for her children were boundless and constant--and to my father, Marvin M. McGriff, whose concern and assistance in recent years have been indispensable. Both of them have made possible the completion of this work.

ACKNOWLEDGMENTS

No personal endeavor, whether monumental or minuscule, is ever accomplished without the assistance of others. I am undoubtedly indebted to more people than are enumerated here. If anyone be neglected, I apologize for the oversight.

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Now it is onward to the next milestone, hopefully to help make this rotating, revolving sphere we call "home" a more habitable and hospitable place for "our children's children's children."

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ABSTRACT

A fertility study was initiated at the Maricopa Agricultural Center in 1984 to determine how nitrogen levels influenced growth, development, and root yield of the potential domesticate, buffalo gourd (Cucurbita foetidissima). Nitrogen had little or no effect upon the following parameters: seedling emergence, early growth rates, specific leaf weights, individual root weights, consumptive water use, canopy organic N content, and nitrate and ash content of all organs. Percent root dry matter and total carbohydrate content dropped linearly as N was increased, whereas canopy dry matter production, root organic N content, petiole nitrate concentration, and leaf area indices exhibited a positive linear response. A quadratic response curve best fitted fresh weight root yields, total root dry matter and carbohydrate production, water use efficiencies, and final plant populations. A modest amount of N (84 kg ha^{-1}) provided maximum yield response in this feral species grown at 405,000 plants ha^{-1}.

LITERATURE REVIEW

> I think we must retain the belief that scientific knowledge is one of the glories of man. I will not maintain that knowledge can never do harm. I think such general propositions can almost always be refuted by well-chosen examples. What I will maintain--and maintain vigorously--is that knowledge is very much more often useful than harmful and that fear of knowledge is very much more often harmful than useful.
>
> Bertrand Russell

The Roles of Nitrogenous Compounds Found in Higher Plants

Next to carbon, hydrogen, and oxygen, nitrogen is the most commonly occurring element in biological systems (Lehninger, 1982). Nitrogen normally comprises 2-5% of the dry matter of mature plants, the amount varying with species, cultivars within a species, organ, and developmental stage (Marschner, 1986). To these factors might be added the amount and type of N available to the plant, as well as the environmental conditions under which the plant is grown (Goh and Haynes, 1986).

Before being shunted into an innumerable array of compounds, reduced N is initially incorporated into amino acids (AA´s) and the purine and pyrimidine bases, these being the basic building blocks from which all other N-containing compounds are derived (Lewis, 1986). Many functions can be attributed to these relatively simple compounds, either in their free state or after being modified or attached to another moiety. For instance, nucleotides such as ATP or GTP act as phosphorylating agents or as energy carriers, donating a high-energy phosphate bond wherever it is needed. Of the 260 AA´s isolated from plants, only 20 are constituents of proteins (Rosenthal, 1982). Although the roles of most of the remaining 240 are esoteric, functions can tentatively be attributed to a few (Lewis, 1986). For example, canavanine, an arginine analogue found in the jack bean (Canavalia ensiformis), probably acts as a "protective toxin" against various insect predators (Jeffrey, 1987). Seleno-cysteine and seleno-methionine are contained within the tissues of many members of the genus Astragalus (Fabaceae) found growing on soils characterized by a high selenium content. These analogues of the sulfur containing AA´s cysteine and methionine may be naturally occurring insecticides or perhaps a means of safely sequestering selenium, thus allowing the plant to adapt to seleniferous soils (Jeffrey, 1987). As final examples, glycinebetaine and proline are modified AA´s which act as "osmoregulators" in many of the halophytes, accumulating in the cytoplasm during periods of salt or water stress (Marschner, 1986).

Amines and polypeptides (< 100 A residues) are derived from AA´s and serve several functions. Amines are constituents of the membrane phospholipids, e.g., phosphatidylethanolamine, phosphatidylcholine, while many polypeptides chelate and transport metal ions such as Cu, Mn, and Zn throughout the plant via the xylem. A tripeptide, glutathione, functions as a redox agent in chloroplasts; in addition, it acts as a S-transporting agent in the phloem (Marschner, 1986).

Most of the N in the plant is found in the form of proteins, which are usually 16-18% N by weight (Jeffrey, 1987). These versatile biopolymers serve many purposes and may be classified as "enzymic," "structural," or "reserve" (Beevers, 1976). The structural ones are part of the architecture of membranes and cell walls and provide scaffolding for chromosomal organization (histones) (Lewis, 1986). Approximately 60% of the N found in leaves and stems is tied up in proteins serving as either enzymes or structural components of the cell (Haynes, 1986). Reserve proteins are deposited in the developing seed and subsequently used for early embryo growth during germination.

To Beevers´ classification might also be added the categories "recognition" and "translocation." For instance, a phosphate translocator has been identified in the outer envelope of the chloroplast (Marschner, 1986), whereas others have been hypothesized, especially for the uptake of NH_4^+ and NO_3^- (Haynes, 1986). In regards to recognition phenomena, lectins (glycoproteins) have been implicated in the binding of various strains of Rhizobia to the root hairs of their host legumes and in the acceptance or rejection of pollen grains by the stigma of the female parent (compatibility/incompatibility systems). However, these functions have only been confirmed in relatively few situations; the biological significance of most lectins awaits further elucidation (Callow, 1976).

Many of the so-called "water soluble vitamins" (not as far as the plant is concerned, for it can synthesize them) function as cofactors to enzymes participating in basic metabolic steps involving oxidation-reduction reactions, carboxylations or decarboxylations, transfer of amino groups, or transfer of individual carbon atoms. Among them are thiamine, riboflavin, nicotinic acid (niacin), pantothenic acid, pyridoxine, biotin, and folic acid (Lehninger, 1982). All of these cofactors originate from AA´s, nucleic acids, or a combination of the two (Beevers, 1976).

Nitrogen is present in still other primary metabolites (and substances which elicit primary metabolic reactions; i.e., hormones). Porphyrins (tetrapyrrolic compounds) occur in appreciable quantities in membranes: chlorophyll in the thylakoid network of the chloroplast and cytochromes in both chloroplast and mitochondrial membranes, the latter class of compounds being essential for the transfer of electrons generated either through photosynthesis or respiration. Phytochrome, another porphyrin, occurs in a linear rather than a ring form and is responsible for mediating cellular events which are sensitive to quality/quantity of light received by the plant (Lewis, 1986). Finally, two of the phytohormone classes, auxins and cytokinins, include N as a structural component, while a third, ethylene, although not containing N, is derived from the AA methionine (Lewis, 1986).

In addition to these more frequently occurring compounds, many secondary metabolites also incorporate N into their molecular makeup. The most pervasive group is the alkaloids; over three thousand have been isolated, yet physiological roles have only been assigned to a few (Lewis, 1986). Some appear to confer resistance to insect predation. For example, some wild Solanum species are resistant to the potato leafhopper (Empoasca fabae) because of the presence of solanine, tomatine and leptine, and many Nicotiana species possessing the alkaloids nicotine, anabasine, and nornicotine are resistant to such pests as the green peach aphid (Myzus persicae), pea aphid (Acyrthosiphon pisum), and the tobacco hornworm larva (Manduca sexta) (Pathak and Saxena, 1976). The previously mentioned alkaloids solanine and tomatine are also phytoalexins. They have been observed to deter fungal growth in Solanum acaule, a wild relative of the potato (S. tuberosum) (Kuć, 1982).

Two other groups of arcane N compounds deserving of at least a passing glance are the cyanogenic glycosides, found in many genera of plants, and the glucosinolates, confined mostly to the Brassicaceae (Lewis, 1986). Dhurrin (Sorghum bicolor), linamarin (Manihot esculenta), and amygdalin (Prunus spp.) are representatives of the former. Since HCN is only liberated after the plant´s tissues are damaged, it is hypothesized that they function as feeding deterrents to insects. The glucosinolates (N- and S-containing compounds) have been tentatively assigned the same role (Jeffrey, 1987).

From the above seemingly endless march of examples, one is struck by the overwhelming importance of N as a nutrient in plants. It is hardly surprising that after water, N has been declared the most important factor limiting crop yields (Wiersma and Christie, 1987).

Rationale for Conducting Nitrogen Fertility Studies

> He gave it for his opinion, that whoever could make two ears of corn or two blades of grass to grow upon a spot of ground where only one grew before, would deserve better of mankind and do more essential service to his country than the whole race of politicians put together.
>
> Jonathan Swift,
> from Gulliver´s Travels

Future Demands for Nitrogen Fertilizer

The only way in which appreciable amounts of N can initially enter the biosphere is through the fixing of atmospheric N by a limited group of prototrophic microorganisms (Brill, 1980). Because these annual increments are not exhorbitant, humankind has come to rely upon fertilizers to sustain a high level of crop productivity. Even _if_ a soil is relatively high in organic matter, fertilization is often required for modern cultivars to reach their yield potential, simply because the rate of mineralization is inadequate to meet the N demand of the crop. The maximum estimate for annual liberation of N through mineralization is 120 kg ha^{-1}. Unfortunately, many high-yielding crop species require 200-300 kg ha^{-1} in order to yield maximally (Haynes, 1986). An exception to the necessity of applying N fertilizers might be land which has been allowed to fallow, whereby substantial amounts of NO_3^- can accumulate--provided, of course, precipitation has not been great enough to leach the NO_3^- out of reach of the crop´s root system (Haynes, 1986).

In 1974, 40 million metric tons of N were used to help put bread on the table (Brill, 1980); by 1982 this figure had climbed to 60.4 million metric tons (Haynes, 1986); by the year 2000 the demand is estimated to escalate to 200 million metric tons (Lewis, 1986). Over 80% of this demand is currently met by the expensive, energy intensive

Haber Process (Haynes, 1986). This process unites atmospheric N_2 with H_2 (usually supplied by natural gas, CH_4) with the help of a catalyst at temperatures of 400-600 ^{o}C and pressures up to 1000 atmospheres (Lippincott et al., 1977). Every kilogram of N fixed by the Haber Method consumes 1.9-2.0 kg of oil (Sprent, 1987; Haynes, 1986). Unless other means of supplying N economically are discovered, one can only look to the future (when oil becomes scarce and costly) with some degree of apprehension.

Nitrate as an Environmental Pollutant and Health Hazard

Since N is such an expensive factor of production, it behooves the producer to apply it at optimum rates and to make use of cultural practices which assure its efficient use and conserve the remainder for subsequent crops. Oftentimes, however, the producer does not pursue this policy, and as a result, N is lost through leaching of NO_3^- beyond the root zone or by surface runoff (sediment removal). In 1977 such losses were estimated at 682-6820 million kg yr^{-1} of actual N in the U.S. (Canter, 1986).

Excessive amounts of NO_3^- in the root zone can potentially become a health problem to people and livestock. The plant can accumulate dangerously high levels in its tissues, and if the vegetative parts are consumed by people or animals, can lead to a condition known as "methemoglobinemia."

This "blue baby disease" (infants are especially susceptible) is due to the bacterial conversion of NO_3^- to NO_2^- in the small intestine, followed by the absorption of NO_2^- into the bloodstream, where it combines with the normally oxygen carrying hemoglobin, leading to anoxia and sometimes death (Donahue et al., 1977). Overfertilized forages will often accumulate NO_3^- in excess of 1-2% on a dry weight basis, enough to induce methemoglobinemia in livestock (Marshner, 1986). Another detrimental effect of consuming plant materials containing excess NO_3^- is the formation of nitrosamines in the small intestine from the union of the bacterially produced NO_2^- with amino acids. These compounds are known to be carcinogenic (Sprent, 1987; Marshner, 1986). The same repercussions can result from the leaching of NO_3^- into groundwaters or surface waters. Drinking water contaminated with NO_3^- can potentially lead to the same conditions as ingesting plant materials high in NO_3^-. Because of this, the United States Public Health Service has set an upper limit of 45 mg l^{-1} NO_3^- (10 mg kg^{-1} NO_3^--N) in drinking water (Canter, 1986).

The other problem associated with a high [NO_3^-] in surface waters is an environmental one. When NO_3^- and a companion anion, PO_4^{-3}, are present in substantial quantities in slow-moving waters, a condition known as "eutrophication" often results. Prolific "algal blooms" occur on the surface. Prolific birth eventually means prolific death, and as the algae die, they are consumed by saprophytic microorganisms. This depletes the waters of the oxygen supply to levels incapable of supporting much of the original aquatic life. The end result is an unsightly, foul-smelling body of water, relatively devoid of life (Canter, 1986).

A lack of knowledge of the N requirements of a crop in a particular area can lead to the previously described environmental/health problems if the producer applies excessive amounts of N. Such overfertilization also depresses yield and increases production expenses (Marschner, 1986). Of course, one might just as likely fail to apply enough to assure optimum yields. These possible negative effects argue for the need of N fertility studies, especially when new crop species (or new cultivars of established species) are initially introduced into an area.

A Basic Outline of a Nitrogen Fertility Study

Most fertilizer response studies typically consist of applying the nutrient in question at several rates, monitoring the tissue concentration of the nutrient in a selected organ or organs, and attempting to mathematically relate the subsequent yield and/or quality of the product with the amounts of nutrient applied and the amounts which periodically accrued in the tissues of the crop during the growing season (Haynes, 1986). Tissue analysis becomes especially important when multiple applications of a fertilizer are applied by the producer, for it will reveal when a deficiency exists, allowing the grower to then take corrective steps. By convention, the guidelines developed through fertility studies make use of the "critical nutrient concentration" or that tissue concentration which corresponds to 90% of maximum yield (Walworth and Sumner, 1988; Ulrich and Hills, 1989).

This appears to be straightforward--and a simple enough task to perform, but in truth, many factors exist which can complicate such experiments. For instance, the response of the crop species in any given year is dependent upon environmental conditions such as soil type and weather, the genotype of the cultivar, and interactions of other nutrients with the nutrient under study (Walworth and Sumner, 1988). These factors, plus others such as plant age and type of tissue tested, affect the levels of nutrient found within the plant, so the decision of which plant part to make use of and the chemical form of the nutrient to measure is basically one of trial and error (Walworth and Sumner, 1988).

In the case of N, $NO_3^-$is usually the predominant form of available N existing in most agricultural soils, so it is the logical candidate for analysis. Another point in favor of NO_3^-: it is easier and quicker to determine than total N, so one should attempt to correlate this chemical species with yield whenever possible. The use of conducting tissue (stem or petiole) has been found to yield more reliable correlations than leaf tissue, as the [NO_3^-] is relatively low in leaf tissue due to the action of nitrate reductase (Haynes, 1986).

The mathematical expressions used to relate yield response to amounts of N applied or to tissue concentrations take a variety of forms: linear, quadratic, cubic, square root, exponential, and "split line" (linear) functions (Haynes, 1986). Again, because of the complicating variables previously mentioned, it is possible to obtain a different mathematical expression from the same experiment from one year to the next (Marshner, 1986). Harper (1983) simplifies the mathematical matter by classifying yield responses over a wide range of treatments as either "asymptotic" or "parabolic." The parabolic curve exhibits a definite yield decline as the nutrient becomes excessive, whereas the asymptotic curve reflects a relative lack of response of the crop to excessive applications of the nutrient; i.e., at some point the yield levels off and remains more or less constant with additional inputs of the nutrient. Most crop responses to N are parabolic (quadratic) in form, but oftentimes two linear functions, one for either side of the maximum response, will predict yield as well, or better, than the quadratic (Harper, 1983).

In summary, such complicating factors as those previously enumerated argue for repeated trials before recommendations are put forth. Ideally, such studies need to be undertaken for every cultivar developed and every production area in which it is introduced (Haynes, 1986). Until the day arrives when the ability to fix N can be readily conferred to higher plants or free-living, N-fixing microorganisms can be effectively used as "biological fertilizers," e.g., Azospirillum with corn (*Zea mays*) or sugar cane (*Saccharum officinarum*) (Sprent, 1987), the need for this conventional type of N fertility study will continue.

How Certain Root Crops Respond to Varying Amounts of Nitrogen

Root crops figure prominently as staples in sustaining the human race. Thirty crops contribute overwhelmingly in warding off world hunger, and five of these are root crops: sugarbeet (*Beta vulgaris*), sweet potato (*Ipomoea batatas*), potato (*Solanum tuberosum*), cassava (*Manihot esculenta*), and yam (*Dioscorea* spp.) (Harlan, 1976). These five species represent approximately 25% of the total harvested fresh weight of these thirty most prominent crop species (Harlan, 1976).

Since this particular scientific inquiry was focused upon the buffalo gourd, a potential root crop for semi-arid environments, it seemed appropriate to become familiar with the response of certain cultivated species to N in order to determine whether or not a feral species evolving under edaphic conditions of low N availability behaves in a similar manner to increasing N inputs. Sugarbeet and sweet potato were examined for two reasons: they are more important to the U.S. economy than yams or cassava, and although potato is classified as a root crop, it is botanically a tuber, or enlarged, subterranean stem. Thus, even though it is an underground storage sink for excess carbohydrate, the anatomical difference may make direct comparisons more difficult.

Sweet Potato

The scientific reports on N effects on yield in sweet potato are scanty and sometimes conflicting, suggesting that further studies in this area are in order. Moreover, it appears that no one (at least in America) has conducted a "full-fledged" fertility experiment in which many different levels of treatment are evaluated, so as to be able to characterize the yield response over a wide range of available N. As an example of the discrepancies which abound, one source states that past studies demonstrate yield increases with N applications up to 134 kg ha^{-1}. They also claim that others have found N to have no effect on yield (Hammett et al., 1984). Another authority gives a suggested range of 22-146 kg ha^{-1} N with the total recommended amount being split into two or three applications throughout the season (Hill, 1989), while still another recommends <u>no more than</u> 75 kg ha^{-1} on sandy loam soils (Constantin et al., 1984). Against this background of professional strife and confusion, the following information is presented.

Fresh Weight Yields

Leonard et al. (1949) obtained increases in yield up to 179 kg ha^{-1} N, the increases being rapid at first and then leveling off (asymptotic relationship). Constantin et al. (1984) achieved a relatively modest increase in marketable fresh weights (20.7-22.2 Mg ha^{-1}) as N was increased from 0-67 kg ha^{-1}, whereas another study, involving much higher amounts, noted a decline in yields from 46.5-36 Mg ha^{-1} as the N rate was increased from 101-202 kg ha^{-1} (Hammett et al., 1984). In the most interesting of the studies surveyed, Bourke (1985) found it advantageous to supply N up to 225 kg ha^{-1}, obtaining a yield increase (nonlinear) from 5.2-9.0 Mg ha^{-1}, seemingly contradictory to Hammett's work. However, his work was performed on a site in Papua New Guinea on a relatively porous volcanic soil receiving up to 2800 mm of rain annually (Bourke, 1985). Moreover, the N was applied preseasonally in the form of urea, which decomposes rapidly via the action of urease and subsequently is converted to NO_3^- under warm, moist soil conditions (Haynes, 1986). Therefore, much of the N was probably lost to the crop through leaching, in effect "scaling down" the treatments to much more modest levels of available N.

Dry Matter Production

Applying N at low levels appears to raise the root dry matter content slightly (25.6-26.3%) (Constantin et al., 1984). In New Guinea, Bourke (1985) noticed linear increases in both the canopy dry matter and root dry matter on a per plant basis. Both characteristics increased at the same rate so that no change occurred in the harvest index (HI) (72-76%).

Other Growth Data

One study saw an increase in the leaf area index (LAI) from 1.5-2.75 as the applied N levels were elevated from 27-161 kg ha^{-1}. Concomitantly, the number of storage roots per plant was reduced from 6.5-4.5 (Kim et al., 1985). These scientists hypothesized that a certain critical level of free sugars is necessary to stimulate new storage roots to form. Providing more N caused more of the sugars to be diverted to the formation of AA´s, hence less storage organs were formed. Increasing N did not affect the number of storage roots initiated by the plant in Bourke´s study (mean = 2), but an identical change in the LAI occurred as was found in the work of Kim et al. (1.5-2.75). Bourke (1985) also found an increase in the average tuber dry weight through the highest N treatment of 225 kg ha^{-1}.

Tissue Chemical Composition

Both total N of the leaf blades and the [NO_3^-] of the petiole have been used to assess the N status of a sweet potato crop. Healthy, mature leaves usually contain 3.2-4.2% N. Should they drop to 1.5-2.5% N, deficiency symptoms begin to appear (Hill, 1989). In the study of Leonard et al. (1949), the highest yielding treatment (179 kg ha^{-1} N) was found to contain 4.7-5.0% N early in the season, declining to 3.1-3.8% by harvest time. Similar results were achieved at 101-202 kg ha^{-1} N: 3.9-4.5% N in the leaf blades (Hammett et al., 1984). If using petiole NO_3^- concentrations as a guide, one should try to maintain a level of 3500 ppm in the petiole of the first fully expanded leaf (Hill, 1989). One group found levels as high as 13,600 ppm in his highest yielding treatment approximately six weeks prior to harvest (Hammett et al., 1984), a seemingly very high value for so late in the season.

Delivering larger quantities of N to the plant also elevates the N content of the root. Constantin et al. (1984) and Hammett et al. (1984) saw increases of 0.9-1.2% and 1.1-1.4%., respectively, as N was increased. Others have found similar changes to prevail. In treatments ranging from 27-161 kg ha^{-1} N, root organic N increased in a linear manner

(Kim et al., 1985). This same study also discovered that the sugar content decreased linearly with additions of N. Thus root organic N and sugar were inversely related to one another.

In general, it may be said that as N becomes more available, the following compositional changes occur: (1) in the root the protein content becomes greater as sugars, fiber, carotenoids, and vitamin C (ascorbic acid, a glucose derivative) are decreased; (2) the protein content of the vines is also elevated while carbohydrate and oxalic acid levels are reduced (Hill, 1989).

Sugarbeet

In contrast to sweet potato, the voluminous literature on sugarbeet response to N is much more consistent. Most apparent discrepancies in results can usually be explained by drastically different levels of soil residual N from one site to another. This particular difference oftentimes makes interpretation of the response curve difficult--an argument for making as standard practice the addition of the residual NO_3^- to the treatment values, so that interested readers will be aware of the total N (minus the difficult-to-include contribution from mineralization) available to the plots.

Fresh Weight Yields

After surveying several studies, one reaches the general conclusion that the normal yield response of sugarbeet to N is an asymptotic one--provided the range of treatments is broad enough. When a narrower range of treatments is imposed, the response is essentially linear. In most cases where the asymptotic relationship prevailed, a minimum of at least 300 kg ha^{-1} N was necessary to achieve the "tapering off" effect (Halvorson and Hartman, 1980; Johnson et al., 1978; Giles et al., 1975; Lee et al., 1987), although two studies achieved this effect with maximum N treatments of 240 and 208 kg ha^{-1} (Anderson and Peterson, 1988; Halvorson and Hartman, 1988). Again, higher residual levels of NO_3^- will result in the yield response becoming asymptotic at a lower range of treatments. The most dramatic example of an asymptotic yield curve comes from the work of Giles et al. (1975). In the first year this group saw maximum yields of 50 Mg ha^{-1}, with a level response being maintained from 406-616 kg ha^{-1} N (treatment + residual). In the second season the maximum yield of 52 Mg ha^{-1} was maintained through 812 kg ha^{-1} N (treatment + residual)! Other scientists, using up to 270 kg ha^{-1} as their highest N treatment, were able to best describe their response as linear (Ulrich and Hills, 1989; Johnson et al., 1978). Abbott and Nelson (1983) evaluated yield under low levels of

N the first season and higher levels (up to 300 kg ha^{-1}) during the second season. Their results can also be best described by a linear function ($r = .985$, $df = 2$, $p < .02$; correlation derived from their tabular results).

In all of these studies the range of fresh weight root yields was from 34-87 Mg ha^{-1}, depending upon the amount of N available and the location of the experiment. Sites in Colorado, Nebraska, North Dakota, and Montana generally yielded less than ones in Arizona and California, where the growing season is much longer.

Sugar Production

Sugar production is analogous to fresh weight yields in that if the range of treatments is low enough, one relationship prevails (linear), whereas another exists for studies in which higher amounts of N are used (quadratic). Most studies in which the response was parabolic saw maximum sugar production at 100-150 kg ha^{-1} N (Anderson and Peterson, 1988; Halvorson and Hartman, 1980, 1988; Johnson et al., 1978; Giles et al., 1987; Lee et al., 1987). Ulrich and Hills (1989) obtained a linear relationship, as did Johnson et al. in the first year of a two-year study, when he and his colleagues made use of lower N treatments (0-269 kg ha^{-1}) applied to an area relatively low in residual NO_3^- (10-11 ppm) (Johnson et al., 1978). The only exception to these two mathematical relationships was found in a study by Moraghan

(1987) whereby an asymptotic curve best described the results. However, his highest treatment was only 224 kg ha^{-1}. Had he made use of higher application rates, he may very well have achieved a quadratic response.

Maximum sugar production in the sugarbeet, as found in these studies, spanned a range from 8,300-12,300 kg ha^{-1}.

Dry Matter Production

The total amount of dry matter contributed by the canopy tends to increase in a linear fashion with greater applications of N--at least through intermediate levels of 220-240 kg ha^{-1} (Anderson and Peterson, 1988; Moraghan, 1987). Moraghan (1987) also examined root dry matter production, as influenced by N applications, finding an asymptotic relationship. Different results were obtained by another group using applications of N up to 336 kg ha^{-1}. They described topgrowth as increasing asymptotically, and root dry matter first increased, then declined at the highest levels, the data being best described by a quadratic function (Lee et al., 1987). These same researchers also showed that as N is greatly increased beyond levels optimum for sugar production, a higher percentage of the photosynthate gets shunted into a vigorously growing canopy rather than into the root for storage.

Other Growth Data

Data on various growth parameters are almost non-existent in the recent literature. In one study in which the LAI was monitored, all N treatments peaked in July, ranging in values from 2.0-3.3 (Lee et al., 1987). The N level considered optimum for sugar production (112 kg ha^{-1}) produced as high a LAI as the highest application rate of 336 kg ha^{-1}. However, the latter maintained this LAI of 3.3 for two months, but the optimum treatment started to drop almost immediately after attaining this value. By September both of these treatments had returned to a value of 2. This indicates that higher than optimum N applications do not affect the <u>rapidity</u> of canopy response but will maintain the canopy at a much higher LAI for a considerable period of time (leaf area duration).

Tissue Chemical Composition

One parameter which responds very consistently to applications of N is sucrose concentration. All studies examined reported a linear decrease in percent sucrose as the availability of N became greater (see any reference previously cited). The only exception to this almost universal trend came from Johnson and cohorts (1978). In the second year of their research a typical linear response was observed, with the sucrose concentration dropping from 15.7-13.3%. During the first year such was not the case. Sucrose levels remained constant over the first couple of treatments before slowly declining from 16.7-15.4%. No explanation was offered for this deviation from linearity, but they did report very low residual NO_3^- values of 10-11 ppm. Photosynthesis may have been somewhat compromised at the lowest treatment due to an inadequate amount of N for chloroplast development. Therefore even though the second increment of N would have caused a larger absolute amount of photosynthate to be diverted to the production of nitrogenous compounds, the same proportion may have been available for storage as sugar because of an increase in photosynthesis. This is one possible explanation why the concentration of sucrose remained constant over the first two treatment levels.

The "purity index," or proportion of the sugar which can actually be extracted, is also affected by the amount of N available to the plant and, in a way, is a type of indirect measure of the organic N present in the root. A high content of organic N makes sugar extraction much more difficult and growers may be penalized for this (Archer, 1985). This purity index, like the percent sucrose, tends to decrease in a linear manner (Anderson and Peterson, 1988; Lee et al., 1987; Halvorson and Hartman, 1980, 1988).

As with the growth parameters, almost no information is available in the more recent literature on the effect of N applications upon the amount found in the organs of the plant. Only one study supplied any information on the amount of N taken up by the crop, and even this researcher did not report the percent N in the organs or the relationship which existed; it had to be inferred from the tabular data. Analysis of his work shows that N accumulates in both the root and canopy in a linear manner as the rate of application is increased. Increases of .48-.61% and 1.5-2.0% occurred in the roots and canopy, respectively, as the N application rate was raised from 0-224 kg ha^{-1} (Moraghan, 1987).

Sugarbeets are considered to be N deficient whenever the petiole NO_3^- content drops below 1000 ppm (Ulrich and Hills, 1989) during any period of the plant´s ontogeny. Nevertheless, under certain environments a recommendation of 1000 ppm at the end of the seaon may not apply. An experiment conducted in Nebraska obtained somewhat different results: 2000 ppm NO_3^--N at the season´s end was found to be associated with their highest yielding treatment (Anderson and Peterson, 1988). One study found midseason (February sampling) concentrations of petiole NO_3^- to correlate best with end-of-season yields (Johnson et al., 1978). Their yields were best related to petiole NO_3^- by means of a quadratic function. In conclusion, it might be noted that early season [NO_3^-] in the petiole may be quite high under conditions of heavy fertilization; Moraghan (1987) observed up to 17,000 ppm NO_3^- at the time of "canopy closure."

The Buffalo Gourd as a Possible New Crop for the Arid and Semi-Arid Regions of the Earth

About the Plant

The buffalo gourd (*Cucurbita foetidissima*) is a perennial, prolifically vining member of the squash and gourd family (Cucurbitaceae), indigenous to vast stretches of the arid American west and northwestern Mexico. More specifically, its range extends northward from Guanajuato, Mexico across the Great Plains of North America to South Dakota. It is also distributed westward across New Mexico, Arizona, and into southern California (Vasconcellos et al., 1981).

It may be found in the wild as an individual plant or occur in large, relatively homogeneous colonies. Such colonies can be formed because this species enjoys the capability of rooting adventitiously from nodes along the vine whenever moist conditions prevail (Vasconcellos et al., 1981). Bemis et al. (1978) have speculated that this may be the primary mode of reproduction in the wild.

Buffalo gourd is a sexually dimorphic species--populations contain individuals which are either monoecious, bearing both male and female flowers on the same plant, or gynoecious, bearing only pistillate flowers (Scheerens et al., 1989). All other species within the genus *Cucurbita* are monoecious (Vasconcellos et al., 1981). Therefore, Bemis has tentatively applied the label of "monogynodioecious" to describe this condition (Hogan and Bemis, 1983). Research has shown the gynoecious character to be conditioned by a single gene whose allele is dominant to monoecy (Dossey et al., 1981).

The hard-shelled fruit, or pepos, develop from pistillate flowers which have been pollinated by species of solitary bees belonging to the genera Peponapis or Xenoglossa (De Veaux and Schultz, 1985). The mature fruit are oblong or globose and tend to be 5-7 cm in diameter (Vasconcellos et al., 1981), although the range can actually be extended to 4-9.8 cm (personal observation). Housed within the pepos are 200-300 seeds, weighing approximately 4 g per 100 seeds (Bemis et al., 1978a) and containing 32.9% protein and 33% oil (Scheerens et al., 1978), or viewed more from a botanical perspective, they are one-third seed coat and two-thirds embryo (Hogan and Bemis, 1983).

The perennial nature of the buffalo gourd is due to its large, starch-laden, heavily suberized taproot, which can survive winter temperatures as low as -25°C (Vasconcellos et al., 1981). The root is also the plant's chief means of adapting to xeric environments (Bemis et al., 1978). If the environment becomes too adverse, the plant simply slips into a dormant state. The starch and water reserves contained in the root allow the crown to initiate new growth when conditions again become favorable for growth.

Dittmer and Talley (1964) studied four species of desert gourds, the buffalo gourd among them, noting the massive size of the taproot and the extensive branching of the laterals. They confined their excavations to a depth of 1.2 m, as they were mainly interested in describing the gross morphology of the enlarged portion of the taproot and the more prominent laterals. Since then, excavations of a more ambitious nature have shown the root system to extend to a depth of at least 2.5 m during its first season of growth (Nelson et al., 1989). One can imagine how extensive the root system could become after several seasons of growth, especially if growing in a sandy wash where they are often found.

This rapidly developing taproot contains appreciable quantities of starch (50-56% on a dry weight basis) (Bemis et al., 1978b). Cucurbitacins, tetracyclic triterpenoids which impart an extremely bitter taste, are also present in the root (Hogan and Bemis, 1983), but they can be readily removed from the starch by thorough washing during its isolation (Bemis et al., 1978b).

It is the oil within the seeds and the starch within the roots which have primarily commanded the attention of plant domesticators for over four decades.

Potential as an Oilseed Crop

Curtis (1946) was the first scientist to popularize the attributes which make the buffalo gourd a likely candidate for domestication as an oilseed crop. His survey of plants, encompassing a range from north Texas westward to southern California, revealed a species average of 60 fruit plant^{-1}, 12 grams of seed fruit^{-1}, and oil and protein contents of 34% each. He estimated an annual yield of 1709 kg ha^{-1} from simply harvesting feral plants and suggested the possibility of using this species to alleviate the extreme shortage of vegetable oil which at that time existed in such areas as Mexico, northern Africa, and other countries bordering the Mediterranean Sea.

Shahani et al. (1951) performed the first detailed analysis of the seed oil, finding it to be composed almost entirely of palmitic, stearic, oleic, and linoleic acids. After bleaching, refining, and deodorizing, the oil was found to be of acceptable blandness and very stable, but he suggested that the resulting color was too dark for consumer acceptance.

Subsequent and more detailed studies have demonstrated the suitability of this oil as an edible vegetable oil. It is high in linoleic acid (49.5-68.8%), low in free fatty acids (0.5%) and conjugated trienoic acids (0.5%), low in total carotenoids (3.6 ppm) after refining, and contains about the same amount of phospholipids (2.9%) as cottonseed or soybean oils (Scheerens and Berry, 1986). Moreover, the safety of the unrefined oil has been confirmed in mouse feeding trials. Normal growth occurred through the highest level of incorporation into diets (11%) (Vasconcellos et al., 1981). Chick feeding trials further demonstrated a lack of toxic constituents (Scheerens and Berry, 1986).

Oleic and linoleic acids, the two fatty acids comprising the greatest proportion of the soil, are inversely related to one another (Scheerens et al., 1978). Acting on this information, Gathman and Bemis (1983), through genetic studies, revealed that these two fatty acids are highly heritable. Given this fact, and the fact that tremendous variation was exhibited by the progeny in the crossing program (12.9-61.7% for oleic, 27.4-73.0% for linoleic), along with the already demonstrated strong inverse correlation, it should be possible to develop lines with diverse oil properties (Gathman and Bemis, 1983).

The protein residue remaining after extraction of the oil has been deemed as safe for livestock or incorporation into food products. As with most plant proteins, buffalo gourd seed protein is deficient in certain essential amino acids, but when mice were fed whole seed as their protein source supplemented with the amino acids methionine, threonine, lysine and valine, they performed as well as controls fed whole egg (Scheerens and Berry, 1986). Seed levels of various antinutritional factors such as phytate, lectins, protease inhibitors, and flatulent sugars were negligible (Scheerens and Berry, 1986), and no signs of toxicity have surfaced during the feeding trials (Bemis and Hogan, 1983).

At this stage of the plant's domestication, the yields regularly attained by the currently available synthetic population are not nearly as promising as the products themselves. Nelson et al. (1988) evaluated the yield of this synthetic population at two locations and for 2 years at one of the sites. At the 360 m elevation site, yields were deplorably low the first year (226 kg ha^{-1}) but improved considerably during the second season (748 kg ha^{-1}). This is to be expected, since many first season plants fail to produce pistillate flowers (Nelson et al., 1988). First year yields were more respectable at the 610 m elevation (933 kg ha^{-1}), but the crop also consumed more water (870 mm vs. 645 mm used by first year plants at the 360 m elevation). This increase in yield at the higher elevation could have been anticipated. Buffalo gourd does not normally grow below 670 m in Arizona (Nelson et al., 1989), so it is quite likely that the higher temperatures encountered at the 360 m site were instrumental in suppressing its inherent yield capabilities.

The outlook for its development as an oilseed crop should not be construed as hopelessly dismal! Past evaluations of diverse sources of germplasm have held out the promise of acceptably high seed yields. Small plot yields at the University of Arizona from hybrids of various accessions have climbed as high as 3274 kg ha^{-1} (De Veaux and Schultz, 1985). Prior to this, Shahani (1951) attained accession yields up to 2175 kg ha^{-1}--still adequate for commercial purposes. In addition to these preliminary trials, variation in yield parameters has been quantified by a number of studies involving both annual and perennial stands. Curtis, working in Lebanon on a project sponsored by the Ford Foundation, observed that the best yielding gynoecious plants produced significantly more fruit than the highest yielding monoecious ones (272 as opposed to 209). Also, in monitoring a population of 712 second year plants, he found the fruit number per plant to vary from 0-271 (Bemis and Hogan, 1983). Another study, making use of 85 accessions, discovered high levels of variation (CV = 20-37%) in a number of important yield parameters such as seed size, seed number $fruit^{-1}$, and seed weight $fruit^{-1}$. Also, the number of fruit $plant^{-1}$ ranged from 0-300 (Scheerens et al., 1978). A later study, utilizing plants descended from these original accessions, confirmed Curtis´ observation that gynoecious plants tend to outyield monoecious ones and discovered that four carpellate fruit (four seed "chambers" within the ovary) produce about 23% more seed than three carpellate fruit. This same study uncovered a number of important correlations between yield parameters, invaluable knowledge for breeders interested in developing improved lines. Seed weight plant^{-1} was highly correlated with the fruit number plant^{-1} ($r = .81$), while the seed weight fruit^{-1} appeared to be related to both the fruit diameter and the seed number fruit^{-1} ($r = .61$ and $.85$, respectively) (Scheerens et al., 1989). This information, all taken together, suggests that a serious effort at breeding high-yielding lines would be amply rewarded.

Besides the need to produce higher-yielding lines, another major obstacle to commercialization exists: this feral species is susceptible to a variety of pathogens. At a number of locations, past research plots have fallen prey to Texas root rot (*Phymatotrichum omnivorum* (Alcorn et al., 1986), powdery mildew (*Erysiphae cichoracearum*) (Mulrean and Russell, 1982), and *Erwinia carotovora*-*Fusarium solani* complex and root-knot nematode (*Meloidogyne javanica*) Rosemeyer et al., 1982). As if these were not enough with which to cope, strains of five different viruses have been isolated from various plantings of buffalo gourd: cucumber mosaic, watermelon curly mottle, lettuce infectious yellows, squash mosaic, and watermelon mosaic (Rosemeyer et al., 1986).

Viral symptoms include severe foliar distortion and often a failure of the plants to flower and set fruit.

It is these last five pathogens that make it difficult to maintain perennial plantings and hence, to maximize yield--at least with wild germplasm. Annual plantings yield less because a substantial number of plants fail to initiate any female flowers during their first year of growth (28-41%) (Scheerens et al., 1989). Viral infections do not normally become rampant in an established planting until the second or third year of its existence--just at the time when yields should be rising (personal observation).

All the diseases that beset the buffalo gourd, with the exception of the viruses, also can be viewed as impediments to the use of this species as a root crop--a possibility to which we can now turn.

Potential as a Root Crop

The feasibility of developing the buffalo gourd as a root crop was not explored until after the mid 1970s, when an interdisciplinary plant domestication group at the University of Arizona was formed (Scheerens et al., 1987). Prior to this, its potential as an oilseed was emphasized.

Under widely spaced plantings (2000-3000 plants ha^{-1}) in sandy soils, root growth can be phenomenally rapid. Three-year-old roots have been reported to reach crown diameters of 20-30 cm (Berry et al., 1978) and weigh up to 40 kg (Hogan and Bemis, 1983). Under a more intense cultivation regime of 10,000-14,000 plants ha^{-1}, typically used when evaluating a stand for its seed potential, roots may attain 1 kg during their first season's growth and up to 3 kg during the second year (Scheerens et al., 1983).

In the perennial root, dry matter tends to remain constant throughout the season (26.4%), but the starch component may vary dramatically, depending upon the physiological stage of the plant (Berry et al., 1978). The starch content in this study peaked in August at 52% (dry weight basis). Throughout the season major starch declines were observed at shoot initiation in early spring (down to 35.6%), flower initiation in late spring (down to 18.5%), and senescence in late autumn (down to 38%). The first two drops in starch content were due to competition between sinks, whereas in the case of senescence, it was merely noted that a similar plummeting of starch reserves had been found in other root crops (Berry et al., 1978). My own speculation is that a certain amount of starch is hydrolyzed to sugar, thus decreasing the osmotic potential of the cells, allowing them to better withstand the colder, more dessicating conditions encountered in the upper soil profile during the winter months.

In a study in which compositional changes in annual roots were monitored, dry matter and starch increased up to

2.5 months of age. The values then remained essentially constant for the remainder of the season (26.2% dry matter, 18.5% starch on a wet weight basis), except for a brief decline at 3.5 months, probably due to the onset of flowering (Scheerens et al., 1983). Even though the starch concentration may reach a maximum value by early August, it would not be advantageous to harvest at this time. Nelson et al. (1983) have reported that over 50% of root growth of annual plants will occur after early August.

An intensive evaluation of the starch isolated from the root of buffalo gourd has shown it to be acceptable for incorporation into food products. Proximal analysis reveals the starch to be relatively free of any impurities such as protein, lipids, or phosphorus (Hogan and Bemis, 1983). Also, it forms an attractive, opaque gel when mixed with water; gelatinizes over roughly the same temperature ranges as other commercial starches such as corn, potato, and tapioca; and exhibits a very desirable pasting behavior; i.e., it develops a relatively high viscosity as it is heated, then is able to maintain this viscosity over a wide temperature range as it is cooled (Scheerens and Berry, 1986). The only undesirable characteristic this cucurbit starch manifests is poor stability when subjected to a cycle of freezing/thawing. This problem, however, can be rectified by modifying the starch with hydroxypropylene (Scheerens and

Berry, 1986). In addition, sensory evaluation studies have demonstrated consumer acceptance for this starch when made into puddings (Scheerens and Berry, 1986), and its safety has been ascertained through mouse feeding studies, whereby the starch, after being autoclaved to break down the granules, was found to be more digestible than potato starch and on par with tapioca and corn starch (Dreher et al., 1981). Mice placed on diets containing gourd starch exhibited similar weight gains by consuming approximately the same amount of feed (feed efficiency) as those on the diets utilizing tapioca and corn starches.

Another possible use for the root may be as a bio-fuel--the starch and sugars present could become substrates for fermentation into ethanol. Scheerens and other colleagues (1987) hydrolyzed root slurries with commercially available enzymes and subsequently fermented them with the yeast Saccharomyces cerevisiae. They found no inhibition of enzyme activity or yeast growth, and the ethanol yields obtained were 82-87% of the theoretical maximum. Moreover, this level of efficiency was attained by the use of a yeast strain specifically developed for potato slurry fermentation (potatoes were used as a control). The only drawback noted was that substantial quantities of water had to be added to the gourd root mashes to achieve a desirable consistency for rapid yeast growth, whereas potato mashes were suitable for fermentation without the addition of water (hence, no dilution of the substrate).

Interestingly, the unimproved synthetic population currently available already produces roots and starch in sufficient quantity from annual plantings to make it attractive as a root crop (see Results and Discussion)--quite the opposite situation that exists with seed yields. Ironically, the future of buffalo gourd as a root starch crop may ultimately depend upon the development of high seed yielding lines, for in order to maximize root yield, high-density populations of 317,000-676,000 plants ha^{-1} must be established [550,000 was determined to be the optimum population in a 1981 study by Nelson et al. (1983)]. If one assumes a 75% emergence rate, then one would need to overplant by 33%. If one further assumes an average weight of 4 g per 100 seed and a seed yield of 933 kg ha^{-1} (obtained in a 1981 Marana, Arizona planting), after performing the mathematics, one reaches the rather dismal conclusion that the seed harvested from a 1 hectare planting will only establish roughly 32 hectares of plants for harvest as a root crop.

The root yield potential of this unimproved germplasm appears even more attractive when planted at elevations similar to where the species is found in the wild (> 670 m). The highest root yields realized by Nelson et al. (1983) at Mesa, AZ, a 370 m site, were approximately 32.5 Mg ha^{-1}, attained at a population of 320,000 plants ha^{-1} (data interpolated from Figure 3). When a similar study was performed at Middle Verde, AZ (920 m), this same group was able to achieve almost identical yields (32.7-34.5 Mg ha^{-1}) at much lower populations of 138,000-177,000 plants ha^{-1} (Nelson et al., 1983; Table 2). Such results are encouraging, as they suggest that much smaller quantities of seed could be used to establish acceptably high yields at elevations where buffalo gourd is indigenous.

In summary, it may be said that one can only speculate what the maximum yield capability of this wild cucurbit is. Variation in such yield parameters as root size and weight, percent dry matter, and percent starch has never been adequately quantified under <u>high density plantings</u>. One preliminary study was conducted at three separate locations to determine the strength of the relationship between starch content and total dry matter content of the root. The correlations ranged form 0.7-0.8 and were significant, but the sample size used at each site (n = 10) is hardly large enough to reliably ascertain the variation existing within the species (McGriff, unpublished data). Therefore, no predictions can be currently made about the possibility of improvement through selection and hybridization.

Research Objectives

At the time this study was undertaken, interest in development of the buffalo gourd as a starch crop for arid environments had overshadowed its development as an oilseed, partially due to a greater interest in the starch displayed by industry. Therefore, it was decided to concentrate upon some important aspect of its culture (nitrogen fertilization).

This experiment had several purposes. The primary purpose was to examine the effect of nitrogen upon the yield response of the buffalo gourd grown in an irrigated agricultural setting. Second, would it be possible to relate the N nutritional status of the plant to its yield via tissue analysis? Another practical consideration was consumptive water use. Would the different levels of applied nitrogen encourage this plant to deplete soil moisture at different rates, thus causing water to become a limiting factor during its growth? Another objective of this study was to describe this plant's developmental response to available nitrogen through the use of such measurements as leaf area index, specific leaf weights, height, dry matter partitioning, inorganic/organic nitrogen partitioning, and total mineral content of various organs. Towards these ends, the following activities were undertaken.

MATERIALS AND METHODS

> We must dive into the bottom of things by repeated and different experiments, and, as it were, force nature to yield herself up to our inquiries.
>
> Dictum of the Academy of Surgery of Paris, 1765

Experimental Setting and Design

This fertility study on the buffalo gourd (*Cucurbita foetidissima*) was conducted at the University of Arizona's Maricopa Agricultural Center in 1984, at an altitude of 360 meters, and on a soil type known as Trix clay-clay loam [fine-loamy, mixed (calcareous) hyperthermic Typic Torrifluvent].

The five nitrogen (N) treatments consisted of 0, 84, 168, 252, and 336 kg ha^{-1} of actual N (henceforth identified as N0, N1, N2, N3, N4) applied in the form of granular urea $CO(NH_2)_2$. The Latin Square design was selected because soil heterogeneity or other environmental variables might contribute to much of the experimental variation. If this proved to be the case, the variation could be partitioned in two directions.

The plots consisted of ten beds 30.5 m long and .86 m from center to center, giving a total plot width of 8.6 m. The reason for such large plots was a simple one: the buffalo gourd plant occupies considerable space. Even under the competitive conditions of the dense plantings employed for root production, the vines often grow to a length of 2.5-3.0 m. Such prolific growth would result in plants from adjacent plots encroaching on each others´ territory for a distance of at least two beds. Designing plots ten beds wide would provide six center beds from which to obtain various measurements throughout the season as well as end-of-season yields.

It was felt that the large size of the plots precluded any interference of one plot with another. Therefore, treatments were systematically assigned to each block rather than randomizing them (Figure 1). This facilitated the preplot application of fertilizer and allowed one to immediately orient himself as to treatment and block throughout the course of the season, even after the vines had piled up high enough to obscure plot markers. (Implications of this decision are to be found in Results and Discussion.)

Also included in the experiment were six planted beds on the west side and four on the east (the rows running in a north/south direction), giving a total planted area of about 0.9 ha.

Pre-Plant Preparation

During the summer and fall of 1983, Sudangrass (*Sorghum sudanense*) was grown as a cover crop, then cut and

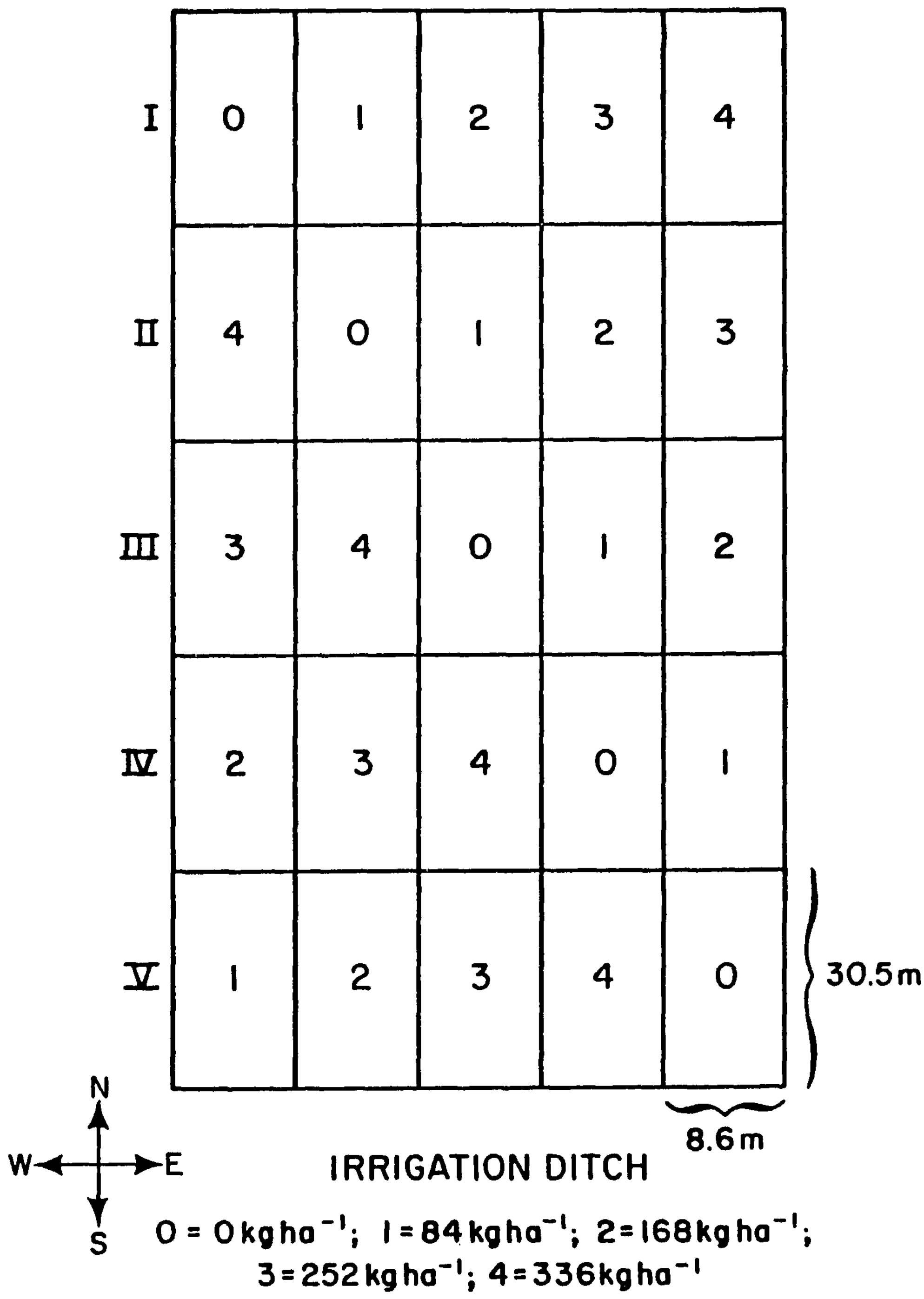

Figure 1. Field plan for 1984 buffalo gourd nitrogen fertility study.

removed from the field with the purpose of depleting the soil N reserves as much as possible. The following spring the field was plowed and bensulide (O,O-bis(1-methylethyl)S-[2-[(phenylsulfonyl)amino]ethyl]phosphorodithioate) applied preplant at the rate of 6.7 kg ha^{-1} a.i. and incorporated by discing. The field was then divided into five sample areas (four quadrants plus a center), and duplicate soil samples were obtained from each area to a depth of 120 cm at 30 cm intervals. These duplicates were composited and transported to the University of Arizona Soil and Water Testing Laboratory for the following analyses: pH, Na^+, K^+, soluble salts through determination of electrical conductivity (EC_e), exchangeable sodium percentage (ESP), P by means of CO_2 extraction and Technicon determination of the orthophosphate, and NO_3^--N through CO_2 extraction and subsequent reduction of NO_3^- by the Technicon Method. Following the soil sampling, plots were marked off, the appropriate amount of urea applied to each plot with a grass seeder, and phosphorus fertilizer broadcast to the entire experiment at the rate of 80 kg ha^{-1} of actual P. Immediately afterwards, the fertilizer was incorporated by discing, the field was furrowed out, and beds shaped without benefit of a pre-plant irrigation.

Establishment and Maintenance of Plots

Planting was accomplished on May 2 by International Harvester 185 planters whose seed boxes were equipped with 40 cell grain plates. These plates, in combination with a wheel-to-box gear ratio of 14:20, delivered approximately 737,000 seed ha^{-1} (three rows per bed) of Arizona Synthetic #1. Two irrigations followed in quick succession to assure adequate stands, each furrow receiving water. Between May 14 and May 25 the plots were observed frequently for seedling emergence.

After May 25, the stands were considered fully established. At this time 5.1 cm diameter access tubes were installed for neutron probe use prior to the next irrigation. These access tubes were installed in holes drilled with a hand-held, engine-driven auger to a depth of 220 cm. Three treatments were selected for the study of consumptive water use (N_0, N_2, N_4), each being replicated three times for a total of nine tubes.

The hydroprobe (Campbell Pacific Nuclear model #503) was calibrated at two separate sites. At each calibration site a "standard count" was obtained prior to lowering the radioactive emitter/sensor down the access tube. Readings were then taken at 20 cm intervals to a depth of 80 cm and divided by the standard count so as to acquire a "probe ratio." At each 20 cm interval, duplicate bulk densities and percent soil moistures (by weight) were also obtained, this information being necessary to compute the amount of H_2O by volume (θV) present. The average θV at each interval was then related to its accompanying probe ratio through a regression equation, thus allowing probe readings to be converted to θV throughout the season.

Another set of soil samples was also taken to determine the amount of water held at field capacity and the permanent wilting point (-.03 MPa and -1.5 MPa, respectively). This information was needed to determine the amount of available water supplied by this soil type. Once the amount of available water on a volumetric basis was known, an arbitrary minimum amount could be chosen as the criterion for requesting an irrigation. In this study it was decided to irrigate when 50% of the available H_2O was depleted from the upper 120 cm of the soil profile. This prevented water stress from becoming a complicating factor in evaluating the potential yield of this species.

Plots were kept relatively weed-free throughout the season, initially, by running sweeps through the furrows and manually removing weeds later on when plant growth made it impossible to move equipment through the field without causing damage to plants. No alleys were put in; instead, adjacent plots were allowed to grow together to mimic a normal field sitation.

Early Season Measurements on Juvenile Plants

Stand Counts

Stand counts were obtained from all plots on May 25. A 1 m^2 area was randomly selected from each of three central beds within each plot and seedling counts made. All three subplots were averaged to obtain the value for each plot.

Plant Heights

On June 7 and June 29, measurements of vine length were made from the ten plots situated in replicates 1 and 3. Two of the center beds were randomly chosen and twenty plants from each bed were randomly selected for measurement. Averages and standard deviations were calculated for each subplot and the subplots averaged to provide one value for each plot.

Dry Matter Partitioning

One other parameter examined early in the life of the plant was dry matter partitioning. On June 7, two 0.5 m^2 samples per plot were obtained from replicates 2 and 4, the taproots being dug to a depth of approximately 23 cm. Plants were then separated into roots and shoots, transported to the laboratory, and oven-dried at 65°C until a constant weight was achieved. The ratio of shoot dry weight to root dry weight and the harvest index (HI) of each subsample were calculated and averages obtained.

Measurements Made Periodically Throughout the Season

Petiole Sampling and Analysis

Sampling dates were June 7, July 28, and August 23. Only two replicates were sampled during the first period. However, every plot was sampled in July. Intuition dictated that this period would be the most crucial, for the plant should be into its log phase of growth by then, having completed over one-third of its development. At this time, detecting and correcting a nitrogen deficiency might allow the plant to recover and realize its maximum yield potential. Thus during this period, the need for reliable data would be the greatest. During the August sampling the number of replicates sampled was reduced to three.

The procedure for collecting and processing petioles consisted of the following: (1) 25-30 plants were selected at random from the six central rows of the plot; (2) petioles from three adjacent nodes were collected, beginning with the first fully "unfurled" leaf blade and proceeding to the next two more mature ones; (3) the petioles then were promptly placed in plastic bags, returned to the lab in a cooler, dried at 65^{o}C overnight, and ground in a Wiley Mill to pass through a #30 mesh sieve. (During the second and third samplings, the second fully unfurled leaf was taken as the youngest sample instead of the first. The first fully unfurled leaf was simply too small to yield a satisfactory amount of material for analysis from a sample size of 25-30, after being oven-dried.) After processing, the samples were subjected to NO_3^- analysis through the use of an Orion Ion-analyzer (Model #407) equipped with a NO_3^- specific electrode. The voltage reading from each sample was converted to a $[NO_3^-]$ value through a regression equation developed by relating the voltage reading of a series of "standard" solutions to their $[NO_3^-]$. The large number of samples (150) mandated that each one be analyzed once rather than in duplicate.

Leaf Area Indices and Specific Leaf Weights

Leaf area indices (total leaf area per given area of soil surface, LAI) and specific leaf weights (weight of leaf dry matter per area of leaf, SLW) were determined on three separate occasions: June 7, June 17, and August 17. Undoubtedly, this number of samplings was inadequate for fully characterizing canopy development, but damage due to a variety of pathogens (identified in later section) in late August made further data collection of this type futile. Two 1 m^2 subsamples were obtained from each plot in replicates 2 and 4 and transported to the lab on ice. Here a ten leaf subsample was temporarily removed from every plot subsample for the determination of SLW. A #5 cork borer (diameter = 1 cm) was used to punch out three disks from each of the ten leaves. The 30 disk groups were then oven-dried overnight at 65^{o}C and weighed. Next, the ten leaf subsamples were returned to the original plot subsample and also dried overnight (or until a constant weight was achieved). SLW was found by dividing the weight of dried disks by the total area of these 30 discs (24.9 cm^2; previously determined by a Licor Leaf Area meter). The LAI for each sample was calculated by dividing the total dried leaf weight by the SLW of that sample, then multiplying by 24.9.

<u>Consumptive Water Use (CWU)</u>

Commencing on June 6, CWU was monitored at intervals of 7-10 days at all nine access tubes. To monitor CWU throughout the entire 220 cm soil profile, measurements or "counts" were taken at 20 cm intervals. The ratio of the count to a "standard" value (taken that day <u>and</u> at each tube) was calculated, then related to θV through the regression equation mentioned previously. The θV value obtained at each interval was considered to be the amount of water in the 20 cm <u>immediately</u> <u>above</u> the point at which the reading was taken, so that the amount found at 20 cm applied to the interval from 0-20 cm, the amount at 40 applied to the interval 20-40 cm, and so on. The amounts present at each of the intervals were then added together to give the total amount of water present in the 220 cm profile at each site. In a similar manner, θV would be measured the following week, the difference between the volumes representing the CWU during that particular time period. Probe readings were always made just prior to and as soon as possible following every irrigation. The amount of water used during the time period surrounding each irrigation was estimated by taking a weighted average of the period immediately before and immediately after the irrigation. Also, seasonal precipitation was considered as part of CWU and was recorded from rain gauges located in an experiment adjacent to this fertility study. Each rainfall event was credited to the CWU for the measurement period spanning the event. All calculations were performed on a Digital Rainbow 100 computer by programs written in BASIC. These programs allowed one to calculate CWU for each site and alternatively, to find the average CWU for each 20 cm interval.

Visual Observations

Throughout the season each plot was scrutinized closely for any overt deficiency symptoms which could conceivably appear at the lower levels of fertilization. A second reason for close inspection of the plots was the desire to know whether or not differences in N availability would result in changes in plant ontogeny such as alteration of internode length, time to flowering or fruit set, and delayed or early senescence.

As fate had it, another reason for closely examining the plants' progress was the unfortunate fact that three different diseases struck this experiment in late August and rendered many of the plots useless for the collection of yield or growth data. It then became necessary to visit the experiment almost daily to follow the progression of the epidemic. This was done so as to be able to quickly commence with an early harvest--should the entire experiment appear to be in danger of succumbing to these pathogens--thus salvaging as much information on yield as possible.

End of Season Field and Laboratory Measurements

During the last week of September, after the disease complex [consisting of powdery midew (*Erysiphae* spp.), an unidentified stem rot, and a root rot--probably an *Erwinia*/*Fusarium* partnership] had caused an alarmingly high incidence of death (> 50% in many plots) it was deemed necessary to begin an early collection of canopy material for dry matter and N analysis and roots for yield and various end-of-season chemical analyses. These data were collected approximately a month before senescence, so one can assume that the various yield parameters reported would have been more impressive if the crop would have had this additional time to develop.

Canopy Nitrogen Partitioning and Mineral Analysis

Twenty healthy plants from each treatment in replicates 4 and 5 were randomly selected and a one meter section of vine of intermediate age cut away. These sections of vine were bulked, bagged, and transported to an uncooled greenhouse, where they were carefully washed with tap water until all soil adhering to the vine pubescence had been removed. Each sample was separated into leaves and stems, placed on clean tables, and allowed to air dry for several days. They were then taken to the lab and ground in a Wiley Mill so as to pass through a #30 mesh sieve. Next, they were oven-dried overnight at 65°C and their dry weights recorded, thus allowing for the computation of percent of total canopy weight contributed by each plant part. Later, these samples were analyzed for their N content in order to quantify how much total soil N was removed by the canopy during the course of a growing season and also to find out how this species partitions N into its organic and inorganic forms once it has been absorbed by the plant. Nitrate analysis was performed with the Orion NO_3^- electrode in the same manner as the petiole samples. Organic N was quantified via the time-honored Kjeldahl Method (micro) employing standard digestion, distillation, and titration apparatus. Finally, the total mineral content was determined gravimetrically after ashing in a muffle furnace for 10-12 hours at 450°C. All analyses (NO_3^- excepted) were done in duplicate and the results expressed on a dry weight basis.

Yield, Dry Matter Partitioning, and Chemical Determinations on Root Material

Yield data were gathered on October 10, approximately ten days after canopy samples for tissue analysis were collected. It was not possible to sample randomly, given the high probability of selecting an area where the root rot pathogen (the primary pathogen) had exacted its toll. To have sampled randomly in this situation would have introduced bias rather than eliminated it, because yield obtained from a severely diseased area would have been abnormally low, thus not representative of the potential of the crop in a normal year. Therefore, a 2 m^2 area (encompassing two adjacent beds) relatively free of disease was selected for harvesting from each plot. The top growth was first cut away, bagged, and taken to drying chambers at the Maricopa Agricultural Center for determination of total dry matter. Roots were manually removed from the soil at a depth of approximately 23 cm and counts made of both the healthy and diseased ones. Those free of root rot were taken to the lab, weighed, and a subsample of 25 randomly chosen for moisture determination and other analyses. After a thorough washing, each sample was diced into small pieces and homogeneously mixed. Moistures were determined in duplicate, the remainder subsequently lyophilized and ground to a fine powder with a Waring blender. Nitrate, organic N,and ash content analyses were performed as previously described. In addition, starch and sugar were quantified by a spectrophotometric procedure using anthrone as the reagent to effect the color change (Clegg, 1956; Yemm and Willis, 1954). Again, all analyses were done in duplicate with the exception of NO_3^-, which was performed only once on each sample. Root yield was expressed as both total weight per unit area and as weight per root, both on a fresh weight and dry weight basis. In addition, "potential yields" were derived by multiplying the average fresh (or dry) weight per root by the total number of roots occupying the 2 m^2 harvest areas (healthy, diseased, and dead). Such an action seemed justified since the purpose of the study was to define the potential yield of the species--and certainly a population reduced to 30-60% of its original stand is not going to yield to its potential! The potential yield data can be considered reliable because the average root weight of the healthy roots was based upon relatively large sample sizes of 30-64. All of the following computations and statistical analyses are based upon the potential yields rather than actual yields.

Data Analysis

Analysis of variance, simple regression, and correlation were applied wherever it was deemed worthwhile. In the case of regression and correlation, the averages of all replicates were used when relating any independent variable to the treatment variable. When relating any other variable to another (such as root sugar content to root starch content), all data pairs from all blocks were used. In such cases one was attempting to uncover a relationship that was not directly related to the treatments imposed. Using more data points increased the likelihood of obtaining a significant association between the two variables. However, in the former situation the variation due to blocks will oftentimes result in a poor correlation between the treatment variable and the other variable of interest, even when the same trend exists in all of the blocks. For such cases it seemed sensible to eliminate this variation by using the average value association with each treatment level.

RESULTS AND DISCUSSION

> The search for truth is in one way hard and in another way easy, for it is evident that no one can master it fully nor miss it wholly, but each adds a little to our knowledge of nature, and from all the facts assembled there arises a certain grandeur.
>
> Aristotle

Comments on the Experimental Design

The failure to randomize treatments in a field setting may have introduced some bias in the form of a "correlated yield response," occurring when the same treatments always appear adjacent to one another (Steel and Torrie, 1960). Since plots next to one another always yield more alike than those further apart (Steel and Torrie, 1960), there is the possibility that significant differences will not be found among those plots close together when actual differences exist (Type II error). Conversely, those plots always kept further apart in a systematic arrangement may appear to be significantly different from one another when,in reality, they are from the same population (Type I error) (my interpretation). Thus this shortcoming in the experimental design may have compromised to some degree many of the following statistical analyses--at least those which only showed significance at the .05 probability level.

Preseason Soil Analysis

Table 1 shows how the residual NO_3^- was distributed throughout the soil profile at the inception of this study. The [NO_3^-] in the upper 30 cm is relatively homogeneous, while the other levels exhibit a wider degree of variability. Despite this greater variability in the lower levels, the average for the entire 120 cm profile is amazingly constant over the entire experiment (CV = 8.6%). Using the grand mean of 6.11 ppm and the average bulk density of 1.61 g cm^{-3}, one can calculate the total residual NO_3^- initially available to the crop, which is found to be the equivalent of 120 kg ha^{-1} of N.

The only other parameter deserving of comment is the ESP. A soil is classified as sodic if the ESP value is greater than 15 (Donahue et al., 1977), and there were several areas throughout the field which exceeded this value. Just focusing on the upper 30 cm, one finds values of 4.5-6.6 prevailing in the southern end of the field, increasing to 11-12 near the center of the field, remaining at that level in the northeastern corner, then dramatically rising to 23 in the northwest corner. More crusting of the soil surface was noticed toward the northern (lower) end of the field during the period of seedling emergence. This was probably caused by both the loss of crumb structure associated with a rise in Na^+ content and the tendency for water to pond over the tops

Table 1. Preseason distribution of NO_3^- throughout the soil profile of the 1984 buffalo gourd N fertility study.

Sample Site	0-30	30-60	60-90	90-120	Mean
	Interval Sampled (cm)				
	ppm NO_3^--N				
N.E. Quadrant	13.2	4.5	2.4	2.0	5.5
N.W. Quadrant	12.2	6.9	3.1	2.0	6.1
Field Center	13.7	6.6	3.7	2.3	6.6
S.W. Quadrant	11.2	5.3	3.1	2.8	5.6
S.E. Quadrant	13.0	6.5	4.3	2.8	6.6
Mean	12.7	6.00	3.4	2.4	6.1
CV (%)	8.0	17.2	20.6	16.0	8.6

of the beds at the lower end in fields which are not completely level. This more extensive crusting in the downfield direction probably was responsible for much of the variability in stands noted in the following section.

Early Season Growth Measurements

Stand Establishment

Data on seedling emergence are presented in Table 2. A gradual decrease in germination is evident as one moves from the head to the tail end of the field (from block 5 to block 1). Also, there is a tendency for the values to drop as one moves from the periphery toward the center. This variation due to location is significant, as evidenced by the F values found in Table 3. On the other hand, treatment effects are nil, the five means being virtually identical. The grand mean divided by the seeding rate (46.2/73.7) gives an overall emergence of 62.6%, a result to be expected given pre-season germination percentages of 70-80%.

Plant Heights

The early June sampling reveals essentially no differences between treatments or blocks, as can be seen from Table 4. The coefficient of variation (CV) for all means is 8.3%, indicating little difference from plot to plot. It is interesting to note that the within-plot variation (data not shown) is more than three times as high as the variation

Table 2. Initial stand establishment in the 1984 buffalo gourd N fertility study.[a]

	Columns					
Blocks	1	2	3	4	5	Mean
	Plants m^{-2}					
1	41	38	37	43	50	41.8
2	42	45	36	40	49	42.4
3	48	40	43	54	51	47.2
4	49	46	45	54	50	48.8
5	55	53	47	48	52	51.0
Mean	47.0	44.4	41.6	47.8	50.4	46.2

	Treatment Means[b]					
	N_0	N_1	N_2	N_3	N_4	Mean
	47.0	46.6	46.0	46.6	45.0	46.2

[a] Spatial arrangement of the plot averages is identical to the plot plan appearing in Figure 1, i.e., block 1 = northernmost block.

[b] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

Table 3. ANOVA of seedling emergence in the 1984 buffalo gourd N fertility study.

Source	df	SS	MS	F	P
Total	24	751.2	31.3	-	-
Treatments	4	10.5	2.6	0.16	NS
Rows	4	326.9	81.7	4.98	< .05
Columns	4	217.2	54.3	3.31	< .05
Error	12	196.6	16.38	-	-

Table 4. Early season heights of juvenile plants in 1984 buffalo gourd N fertility study.

	June 7			June 29		
Treatments[a]	Block 1	Block 3	Mean	Block 1	Block 3	Mean
	cm					
N_0	4.8	5.2	5.0	6.8	8.3	7.6
N_1	4.2	5.6	4.9	7.1	7.2	7.2
N_2	4.7	4.5	4.6	8.4	8.0	8.2
N_3	5.0	4.6	4.8	8.6	13.7	11.2
N_4	4.5	4.7	4.6	6.7	12.6	9.7
Mean	4.6	4.9	4.8	7.5	10.0	8.8
CV (%)			8.3			27.6

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

among plots, with CVs ranging from 16-34%. This higher within-plot variation can probably be attributed to both the high degree of genetic heterogeneity of the synthetic population used in this study, and the large size of the plots.

Greater differences are apparent between both the treatment means and the block means in the later sampling period, but no real treatment trend exists. Although much more variation is apparent between plots, the within-plot variation is still greater, presumably for the same reasons as before. For this sampling, the within-plot CVs ranged from 32-67%, whereas the CV of the plot averages was only 27.6%.

In contrast to the wider spacings employed when planting buffalo gourd as an oilseed (10,000-20,000 plants ha^{-1}), these dense plantings result in very slow initial vine elongation. The leaves, however, expand rapidly, resulting in early ground coverage.

Dry Matter Production in Young Plants

Total dry weights and harvest indices (HI) for young plants collected on June 7 (13-15 days old) appear in Table 5. Treatment values for total dry weight vary considerably (statistical significance undetermined), but no real trend presents itself. Block differences are also readily apparent. The range of HI values exhibited by the samples in this experiment diverge considerably from those obtained in a

Table 5. Early season dry matter production in the buffalo gourd, June 7.

	Total Dry Weight[a]			Harvest Index[b]		
Treatments[c]	REP 2	REP 4	Mean	REP 2	REP 4	Mean
	- - - - gm^{-2} - - - -					
N_0	66	109	87	.17	.24	.20
N_1	49	107	78	.17	.26	.21
N_2	77	68	73	.20	.20	.20
N_3	105	90	97	.19	.20	.20
N_4	74	91	83	.17	.20	.19
Mean	74	93	84	.18	.22	.20
CV (%)			23.7			14.9

[a] Includes both roots and shoots.

[b] HI = wt. roots/wt. roots + wt. shoots

[c] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

later greenhouse study conducted to characterize water use and dry matter partitioning in both cultivated and xerophytic cucurbits (Nelson and Scheerens, 1986). These scientists found an average HI of .60 for young buffalo gourd plants. Several explanations exist for these contradictory results. Among them are the following: their plants were at a different developmental stage when harvested (60 days old); the plants were "pampered" by being in a controlled environment, never having to compete for water or essential nutrients; and the sequestering of plants in individual pots made it possible to collect the <u>entire</u> root system, as opposed to excavating only the upper part of the taproot, as was done in this study.

Measurements Made Periodically Throughout the Growing Season

Leaf Area Index/Specific Leaf Weight

The SLW data are displayed in Table 6. All three sample periods share a common feature: little or no variation exists between replicates. Greater variation is present between treatments, suggesting a possible treatment effect (standard ANOVA not performed). However, the treatment values from the first two sampling periods simply seesaw in an unpredictable manner, giving no indication of a trend. Only in the last set of samples is there a slight tendency for the SLWs to drop as N is increased.

Table 6. Seasonal changes in specific leaf weight in the buffalo gourd.[a]

Treatments[b]	June 7 Rep 2	June 7 Rep 4	June 7 Mean	June 17 Rep 2	June 17 Rep 4	June 17 Mean	August 17 Rep 2	August 17 Rep 4	August 17 Mean
	----------------			mg cm^{-2}			----------------		
N_0	7.5	7.9	7.7	8.8	8.5	8.7	7.0	7.0	7.0
N_1	7.1	7.4	7.3	9.5	9.0	9.3	6.2	7.0	6.6
N_2	7.5	7.5	7.5	9.0	8.3	8.7	6.7	6.1	6.4
N_3	7.9	8.0	8.0	8.9	8.7	8.8	6.3	5.7	6.0
N_4	8.1	7.6	7.9	7.9	7.3	7.6	6.4	6.0	6.2
Mean	7.6	7.7	7.7	8.8	8.4	8.6	6.6	6.4	6.5

[a] Dry weight per unit leaf area.

[b] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

If one examines the grand means of the three sampling periods, one notices a relatively modest 12% increase from the first to the second period, followed by a more substantial decrease of 33% from the second to the third. This pronounced decline in dry matter per unit leaf area later in the season may be the result of the root becoming a more influential sink for the photosynthate being produced. Prior work has shown that as much as 56% of root growth from a May planting occurs after mid-August (Nelson et al., 1983).

On the other hand, leaf areas show an increase throughout the season, first doubling (94% increase) during a ten-day period, then trebling (191% increase) over the course of the next two months (Table 7). Unfortunately, it was not possible to secure samples at later dates to characterize the rate of decline as the plants approached normal senescence, but the August sampling probably approximates the season's maximum values. If this is true, then a LAI of 3.7, representing that attained at the optimum N fertility level (N_1), could probably be viewed as a rough indicator for predicting end-of-season maximum yields.

Focusing on the June 7 data, one notices a precipitous decline in the N_4 treatment, apparent in both replicates. This high of a level of nitrogen (336 kg ha^{-1}), was apparently somewhat toxic during the juvenile stage. At this point the other treatments have exerted no real effect.

Table 7. Seasonal changes in leaf area index in the buffalo gourd.[a]

Treatment[b]	June 7 Rep 2	June 7 Rep 4	June 7 Mean	June 17 Rep 2	June 17 Rep 4	June 17 Mean	August 17 Rep 2	August 17 Rep 4	August 17 Mean
N_0	0.7	0.7	0.7	1.1	1.3	1.2	2.2	3.0	2.6
N_1	0.7	0.8	0.8	1.1	1.2	1.2	3.9	3.4	3.7
N_2	0.7	0.8	0.8	1.1	1.1	1.1	4.0	3.7	3.9
N_3	0.8	0.8	0.8	1.6	1.5	1.6	5.1	5.3	5.2
N_4	0.5	0.6	0.6	1.7	2.1	1.9	4.1	4.9	4.5
Mean	0.7	0.7	0.7	1.3	1.4	1.4	3.9	4.1	4.0
r			--			.804			.873
P			--			< .10			= .05

[a] LAI = ratio of leaf area/given area of ground.

[b] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

Correlation analysis performed on the data from June 17 yielded an r value whose significance level was $.10 < p < .05$. The N_3 and N_4 means have become more distinct from the lower N treatments, which are still essentially identical to one another. The N_4 plants now appear to be stimulated by the additional nitrogen rather than repressed.

The August 17 sampling date displays an interesting feature. Even though all the means have diverged from one another, the N_3 and N_4 treatments have switched order, thus making a linear correlation barely significant. If the N_4 treatment was generally inhibitory to normal growth, then the ranking displayed on June 7 would have prevailed both on June 17 and August 17 as well. Instead, the N_4 treatment in the June 17 sampling had surpassed all the others, leading one to believe that the only way in which the N_4 treatment could lose its highest ranking at an even later date would be through sampling error.

A graphical representation of the seasonal changes in LAI appears in Figure 2.

Factors Affecting Petiole NO_3^- Levels

Nitrogen treatment, time of season, and age of petiole were found to influence the $[NO_3^-]$ within the petiole. Table 8 presents the data from each of the three sampling periods while seasonal changes are summarized in Table 9.

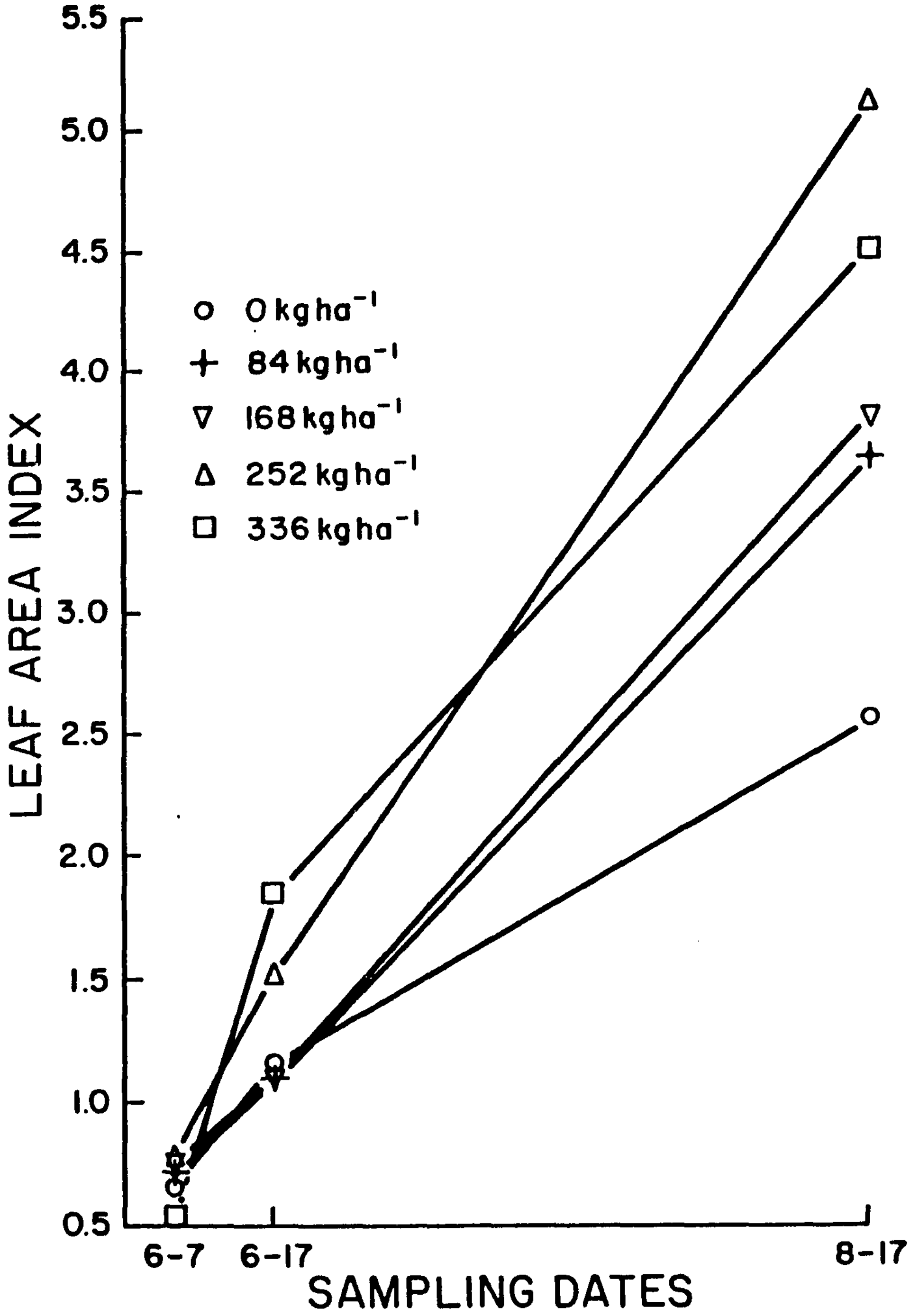

Figure 2. Seasonal changes in leaf area of the buffalo gourd due to varying levels of soil nitrogen.

Table 8. Petiole [NO_3^-] in the buffalo gourd, as influenced by rates of N application and petiole maturity.[a]

	Treatments[b]						
Sample Date	N_0	N_1	N_2	N_3	N_4	r^d	P
	- - - - - - - ppm NO_3^--N - - - - - - -						
June 7[c]							
Petiole 1	3405	11336	13126	19630	20081	.961	< .01
Petiole 2	3352	12745	18904	22689	29690	.989	< .01
Petiole 3	3094	14456	23073	30915	33422	.979	< .01
Mean	3284	12846	18368	24411	27731	.991	< .01
r^e	-.934	.998	.995	.966	.969		
P	NS	< .05	= .05	NS*	NS*		
July 28							
Petiole 1	901	1973	4986	7609	10365	.991	< .01
Petiole 2	918	2267	4892	10189	12208	.979	< .01
Petiole 3	765	2765	5756	12274	16652	.980	< .01
Mean	861	2335	5211	10024	13073	.985	< .01
r^e	-.810	.989	.812	.998	.972		
P	NS	< .10	NS	< .05	NS*		
August 23							
Petiole 1	402	452	1090	2577	4689	.930	< .05
Petiole 2	394	466	1184	2652	5141	.926	< .05
Petiole 3	414	519	1441	3878	7048	.931	< .05
Mean	403	479	1238	3036	5626	.956	< .02
r^e	.596	.947	.969	.890	.942		
P	NS	NS*	NS*	NS*	NS*		

a Values for each petiole represent means of all replicates.
b 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.
c Petiole 1 = youngest, Petiole 2 = intermediate age, Petiole 3 = oldest.
d All correlations in this column are petiole [NO_3^-] vs. level of fertilizer N.
e Petiole [NO_3^-] vs. age of petiole; * signifies that all relationships judged nonsignificant would be significant if subjected to rank correlation (r_s).

Table 9. Seasonal decline in petiole NO_3^- in the buffalo gourd.[a]

Treatment[b]	June 7	July 28	Aug. 23	r[c]	P
	Sampling Dates				
	- - - - -ppm NO_3^--N- - - - -				
N_0	3284	861	403	-.983	$\doteq$ .10
N_1	12846	2335	479	-.981	$\doteq$ .10
N_2	18368	5211	1238	-.994	$\doteq$ .05
N_3	24411	10024	3036	-.999	< .05
N_4	27731	13073	5626	-.999	< .05
Mean	17328	6301	2156	-.998	< .05

[a] Each treatment value for each sample date consists of the average of all nodes over all replications.

[b] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

[c] Petiole $[NO_3^-]$ vs. time of season.

The level of N fertilizer applied had the most profound influence upon petiole [NO_3^-]; there exists a clear-cut distinction between all treatments in every sample period. On all three sample dates the average [NO_3^-] of petioles of all maturities correlated as well with N levels as the values for each petiole considered separately, suggesting that, when collecting petioles for analysis, one could simply gather a population of "young" petioles without regard to their exact stem position. (The possibility of using petiole [NO_3^-] as a predictor of yield will be discussed in the section on yield.)

The relationship between petiole [NO_3^-] and the age of the petiole was a more ambiguous one. Early in the season when soil NO_3^- was readily available, older petioles consistently held a higher [NO_3^-]. The June 7 sampling shows this trend in all but the N_0 treatment. Although the differences between successive petioles within the other four treatments are rather large, they are not described by a simple linear relationship, as indicated by the fact that the r values are nonsignificant as often as they are significant. The July 28 data displays the same trend: a tendency for the [NO_3^-] to increase in the older petioles, but not necessarily in a linear manner. Again, this tendency disappears in the N_0 treatment. Also, the differences between petioles are not as pronounced as found in the June 7 sampling. The tendency of the more mature petioles to act as storage sinks for NO_3^- was reduced as soil NO_3^- became scarce; less uptake by the root system resulted in the NO_3^- being apportioned more evenly among the developing leaves. Finally, the age of the petioles has even less of an influence on petiole [NO_3^-] in the last sample period, the differences all but disappearing until treatments N_3 and N_4 are reached.

Table 9 reveals that all treatments exhibit a marked decline in their petiole [NO_3^-] as the growing season progresses. Treatments N_2-N_4 show a strong negative linear relationship, whereas treatments N_0 and N_1 demonstrate a decline in petiole NO_3^- with time, but not in a linear manner, as evidenced by r values significant at only the .10 level. This decline in petiole [NO_3^-] reflects the reduced availability of soil NO_3^- as the season progresses due to leaching, denitrification, and prior uptake by the crop.

Water Usage

Surprisingly, neither treatments nor location affected water use of the buffalo gourd in this study. Average daily use values of each treatment monitored, accompanied by the total seasonal CWU, are to be found in Table 10 (also see Figure 3). All treatments and all replicates gave similar values for average daily use and total use for the entire season.

Table 10. Average daily and total consumptive water use in the buffalo gourd at three different levels of applied nitrogen.

	Treatments[a]			Replicates			
Period	N_0	N_2	N_4	1	3	5	Mean
	- - - - - - - - - -	- - mm day^{-1}	- - - - - - - -				
May 22-25[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
26-31	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Jun 01-06	4.3	4.3	4.3	4.3	4.3	4.3	4.3
07-12	6.9	5.4	6.9	6.0	6.7	6.5	6.5
13-18	9.6	7.9	9.2	8.9	8.9	8.8	8.9
19-25	12.2	10.4	11.4	11.7	11.2	11.2	11.3
26-02	5.3	3.7	6.0	5.9	4.1	5.0	5.0
Jul 03-07	7.1	6.7	7.4	9.1	5.3	6.9	7.1
08-14	9.0	9.7	8.8	12.4	6.4	8.8	9.2
15-27	9.0	9.3	8.8	10.1	7.4	9.0	9.0
28-02	4.9	5.2	4.1	5.0	4.3	4.9	4.7
Aug 03-07	6.1	6.8	6.8	6.1	6.8	6.8	6.6
08-13	7.7	8.3	9.4	7.5	9.2	8.6	8.4
14-22	5.2	5.1	4.3	4.6	4.4	5.6	4.9
23-27	6.1	7.7	8.7	9.7	5.7	7.2	7.5
28-30	10.7	12.2	9.9	9.9	9.9	12.9	10.9
31-04	5.2	2.9	3.6	3.5	4.5	3.8	3.9
Sep 05-11	4.6	1.8	5.5	4.0	4.3	3.6	4.0
11-29	3.7	2.8	3.7	3.0	2.8	4.5	3.4
30-10	1.6	2.0	2.2	1.8	1.9	2.0	1.9
Mean[c]	5.8	5.6	5.9	6.0	5.3	5.9	5.7
Seasonal							
Total[d]	827.3	798.5	843.2	856.3	753.6	839.8	819.7
% of Mean	100.9	97.4	102.8	104.4	91.9	102.4	100.0

[a] 0, 168, and 336 kg ha^{-1} N.
[b] Values are extrapolated from graphical data for first three samplings.
[c] Averages are weighted to take into account sample periods of varying length.
[d] Includes 119.6 mm of rain (14.6% of total CWU).

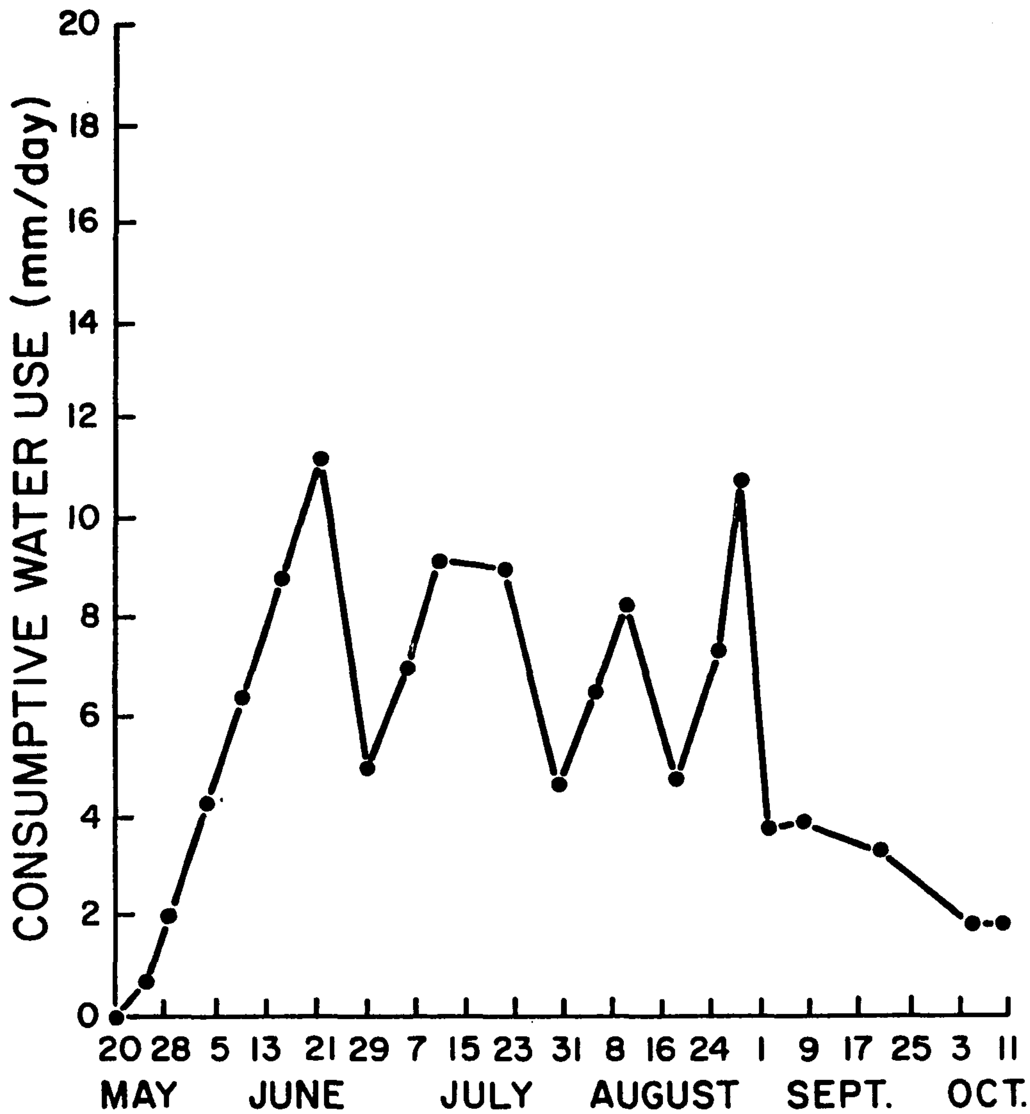

Figure 3. Seasonal changes in average daily water use in the buffalo gourd, Maricopa, Arizona, 1984.

There was considerable fluctuation in usage from one sample period to the next, apparent in all treatments and all replications. This was anticipated, for the amount of available water in the soil at any point in time will influence the rate of uptake by the plants. As the H_2O potential of the soil is lowered, the root system does not extract H_2O as rapidly (Harpstead et al., 1988), so usage will be relatively high immediately following an irrigation, then will drop off rapidly as moisture is depleted from the root zone. The only exception to this general rule occurred during August 28-30. At this time average daily use was highest just before an irrigation because of 26.7 mm of rainfall, which had to be credited to the plants' CWU for this particular period.

The most puzzling results to contemplate are the lack of differences between the various N treatments. Given the fact that the higher applications of N gave much higher leaf areas for transpirable H_2O loss to occur, one would have thought that these more rapidly growing canopies would have utilized more H_2O. Since this was not the case, one can tentatively conclude that the transpiration <u>per unit leaf area</u> is less in the higher N treatments. In fact, it must be reduced by approximately the amount the leaf area is increased by in order for CWU to remain constant from one treatment to the next. This apparent reduction in transpiration rate was not substantiated through measurement, but is certainly conceivable, for the denser canopies of the higher N treatments would have a buffering effect on transpirable loss from leaves at the lower levels of these canopies.

CWU values measured in this experiment were much higher than those determined in a 1985 study in which the same synthetic variety was used (Nelson et al., 1989). Those scientists recorded root yields of 24.1, 25.3, and 27.8 Mg ha^{-1}, using 514, 652, and 726 mm H_2O, respectively.

Table 11 provides a description of how different levels of the soil profile contribute to the overall CWU of the plant. By recording the amount of H_2O depleted from different depths one can get a rough idea of the development of the root system throughout the plant's life. This plant's root system appeared to be well-developed, even in the juvenile stage, for the June 12 sampling shows 7.1% of the CWU coming from a depth of 160-220 cm--an admirable feat for plants approximately 3 weeks old! This proportion was more or less maintained throughout the season. The preponderance of H_2O used came from the upper 120 cm of the profile (almost 82% when discounting precipitation), a result to be expected, since only two irrigations exceeded this level. Thus, in this region of the profile, the higher H_2O potentials would inevitably result in a greater rate of uptake, <u>even if</u> the root system were homogeneously distributed throughout the entire profile. [However, such an extensive root system is

Table 11. Proportion of water depleted from different levels of the soil profile by the buffalo gourd throughout the 1984 growing season.[a]

Profile Depth (cm)	Selected Sampling Dates				
	June 12	July 14	Aug. 13	Oct. 10	Oct. 10[b]
	- - - - - - - - - % Total CWU - - - - - - - - - - -				
0-20[c]	20.8	30.1	35.2	40.8	29.9
20-40	18.2	15.3	12.2	11.4	13.4
40-60	23.3	13.9	11.7	10.1	11.9
60-80	13.4 (75.7)[d]	13.7 (73.0)	11.7 (70.8)	9.8 (72.1)	11.6 (66.8)
80-100	4.6	9.6	8.6	7.5	8.8
100-120	2.3	5.1	5.2	5.2	6.2
120-140	3.0	3.6	4.9	5.1	5.5
140-160	6.7 (16.6)	3.5 (21.8)	4.2 (22.9)	4.7 (20.5)	3.4 (26.5)
160-180	2.2	1.7	2.1	2.8	3.4
180-200	3.4	1.7	2.0	2.3	2.7
200-220	1.5 (7.1)	1.2 (4.6)	1.8 (5.9)	2.0 (7.1)	2.4 (8.5)

[a] CWU = Consumptive water use.

[b] % CWU if precipitation is discounted.

[c] All precipitation added to this interval.

[d] Values in parentheses represent totals for intervals 0-80, 80-160, 160-220.

obviously of benefit to the plant during times of moderate H_2O stress, although under conditions of severe stress the plant drops its leaves and enters into dormancy until the stress is relieved (John Nelson, personal communication).] Similar depletions were noted in a later study (Nelson et al., 1989), whereby the upper 80 cm was responsible for providing 65-70% of seasonal CWU under a similar irrigation regimen.

Chemical Composition of Plant Tissues

The Partitioning of Nitrogen Among Various Parts of the Plant

For the most part, soil N had little effect upon the amount of nitrogenous compounds accumulated by this species. The data in Table 12, in conjunction with the ANOVAs in Table 13, demonstrate that increasing levels of N did not significantly change the amount of organic N in the leaves or stems. On the other hand, the roots responded in a definite linear fashion to increments of N fertilizer. No mean separation procedure was employed for the percent N in the roots because the correlation coefficient was significant. When graded levels of a quantitative variable correlate well with the response variable, then all treatment means are considered significantly different from one another (Chew, 1976).

Table 12 also reveals that each organ is quite distinctive in the amount of organic N compounds which accrue.

Table 12. Effect of soil N availability upon the partitioning of organic N in the buffalo gourd.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Leaves	- - - - - - - -	- - -	% N (dry weight)	- - -	- - - -	- - - -
REP4	4.33	4.30	4.48	4.21	4.51	4.37
REP5	4.29	4.39	4.93	4.68	5.10	4.68
Mean	4.31	4.35	4.71	4.45	4.81	4.53
		Range = 4.21-5.10				
Stems						
REP4	1.88	1.86	1.82	1.97	1.95	1.90
REP5	1.92	1.76	1.68	1.98	2.31	1.93
Mean	1.90	1.81	1.75	1.98	2.13	1.91
		Range = 1.68-2.31				
Roots[b]						
REP4	0.94	1.22	1.13	1.52	1.48	1.26
REP5	0.89	0.89	1.11	1.34	1.41	1.13
Mean	0.92	1.06	1.12	1.43	1.45	1.19
		Range = 0.89-1.52				

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

[b] Linear relationship between N treatment and organic N content highly significant: $r = .964$, $p < .01$.

Table 13. Effect of soil N availability upon the partitioning of organic N in the buffalo gourd/ANOVA.

Source	df	SS	MS	F	P
Leaves					
Total	9	.7830	.0870	-	-
Treatment	4	.3924	.0981	2.64	NS
Block	1	.2421	.2421	6.52	NS
Error	4	.1485	.0371	-	-
Stems					
Total	9	.2570	.0285	-	-
Treatment	4	.1766	.0441	2.26	NS
Block	1	.0029	.0029	0.14	NS
Error	4	.0780	.0195	-	-
Roots					
Total	9	.5173	.0574	-	-
Treatment	4	.4427	.1106	13.71	< .05
Block	1	.0423	.0423	5.24	NS
Error	4	.0323	.0081	-	-

By simply examining the grand means of each organ, one sees that the root contains about 1.2% N, the stem approximately 60% more, while leaf tissue contains almost four times the amount of N found in the root.

Turning now to NO_3^- partitioning (Table 14), one notices a tendency for organ-specific accumulation also, although it is not as pronounced as with the organic N fraction. The stems contain more NO_3^- than either the leaves or roots; not surprising, since nitrate reduction occurs in either the roots or leaves, or both (Haynes, 1986). Considerable overlap exists in the range of values for the roots and leaves, but this is because the roots, in response to the treatments, accrued NO_3^- comparable to leaf tissue at the higher levels. The N treatments did not produce a predictable pattern of response in the leaves; instead, the endogenous NO_3^- levels merely fluctuate.

One other item deserving of mention is the extremely high [NO_3^-] found in all three organs from treatment N_4 of replicate 5. These values are much higher than those of the replicate 4 counterpart, possibly due to sampling error, e.g., sampling in a "hot spot" or area of higher soil [NO_3^-]. However, if sampling from an area of higher soil N is the explanation for the extremely high [NO_3^-] of the organs, then why was the organic N fraction of the roots from this plot not higher in replicate 5 than in replicate 4? (Recall that

Table 14. Partitioning of NO_3^--N in the buffalo gourd as influenced by N availability.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Leaves	- - - - - ppm NO_3^--N (dry weight) - - - - -					
REP4	307	415	311	312	515	372
REP5	520	312	208	520	1642	640
Mean	414	364	260	416	1079	507
	Range = 208-520[b]					
Stems						
REP4	832	1835	616	832	1334	1090
REP5	1140	726	810	1096	3925	1539
Mean	986	1281	713	964	2630	1315
	Range = 616-1835[b]					
Roots						
REP4	68	72	57	389	411	199
REP5	72	62	103	131	906	255
Mean	70	67	80	260	659	227
	Range = 57-411[b]					

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

[b] Unusually high values for N_4 in replicate 5 are omitted from the ranges as they do not reflect "normal" treatment effect; see text for details.

this parameter was affected by varying the amount of N.) If the above explanation is correct, this suggests that the organic N in the root had reached a threshold difficult to surpass, possibly because the root NO_3^- reductase had become saturated with its substrate. It has been amply demonstrated that the conversion of NO_3^- to NO_2^- is usually the first rate limiting step in the overall reduction process (Haynes, 1986).

Total Mineral Content

Once again, varying the soil N produced no discernible trend in the characteristic under consideration, and once again, the different plant parts concentrated different quantities, as indicated by their nonoverlapping ranges (Table 15). These results vary considerably from those previously garnered from an earlier study in which the ash values were leaves, 28.5%; stems, 17.8%; and roots, 3.8% (Berry et al., 1976), perhaps because their work made use of two year old plants under much wider spacings. The values of Berry et al. and those displayed in Table 15 at first seemed exceedingly high, but tables containing ash contents of various foods showed plants to be highly variable, ranging from 2-4% (dry weight basis) for many types of seeds up to 15-20% for the vegetative parts of cole crops (Joslyn, 1970). The 1976 study on buffalo gourd composition listed leaf values much higher than even those found in the published

Table 15. Total mineral content of various plant organs of the buffalo gourd.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Leaves	- - - - - - - -	-	% Dry Weight	- - - - - - - -	-	
REP4	17.2	21.1	16.8	18.8	18.4	18.5
REP5	19.2	16.8	19.7	16.8	17.2	17.9
Mean	18.2	19.0	18.3	17.8	17.8	18.2
			Range = 16.8-21.1			
Stems						
REP4	13.4	15.1	14.2	13.6	13.5	14.0
REP5	14.2	12.0	11.6	13.3	14.1	13.0
Mean	13.8	13.6	12.9	13.5	13.8	13.5
			Range = 11.6-15.1			
Roots						
REP4	5.8	5.5	4.0	6.1	4.9	5.3
REP5	5.0	5.2	6.1	6.1	6.1	5.8
Mean	5.4	5.4	5.1	6.1	5.5	5.6
			Range = 4.0-6.1			

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

tables. They could possibly be explained as an artifact in the preparation of materials for analysis. The leaves are covered by a dense pubescence to which much soil adheres, oftentimes making the complete removal of contaminating minerals an impossible task.

The ranking of these different organs according to their mineral content is probably identical to what would be found in most taprooted plant species. The root would possess smaller concentrations of minerals because the stored carbohydrate would have a diluting effect. The leaves, being the major organs of synthesis of the plant, would require the highest concentration for such reasons as the following: (1) phosphorus for phosphorylation and energy transfer reactions within the chloroplast, (2) magnesium as a structural component of the chlorophyll molecule, (3) potassium as an osmoregulatory agent in the opening and closing of stomata, (4) chlorine for the water splitting reaction in photosystem II and for maintenance of cellular electroneutrality, (5) other minerals such as iron, zinc, copper, and manganese to act as cofactors for proteins involved in either electron transport or as enzymes for the numerous other synthetic reactions taking place (Bidwell, 1974).

Root Dry Matter and Carbohydrate Content

As is apparent from Table 16, the percent dry matter in the root steadily declined as the nitrogen level of the soil was increased. This effect is much more pronounced in replicate 5 than in replicate 4. Total dry matter did not correlate well with either total carbohydrate (CHO) or organic N, or a combination of the two. This is somewhat surprising, since they are two of the major constituents of the root.

No predictable relationship existed between soil nitrogen and root sugar content, but a dramatic block effect was evident with replicate 4 plots averaging only 58% of the amount of replicate 5. The only exception was treatment N_3, which yielded a value 18.1%, comparable to those found in the treatments of replicate 5. This high a level of sugar has never been detected in buffalo gourd roots before, although the values in this study represent the first sugar analyses obtained from such closely spaced populations. Typical values found in the past from <u>both</u> perennial and annual plantings have ranged from 3.2-4.0% on a dry weight basis (Berry et al., 1978; Scheerens et al., 1983, data derived from Table 1). Because of the great discrepancy between this study and previous work, and because of the one unusual value of replicate 4, all sugar analyses were performed again,

Table 16. Effect of soil nitrogen levels upon root dry matter and carbohydrate content of the buffalo gourd.

	Treatments[a]							
	N_0	N_1	N_2	N_3	N_4	Mean	r	P
	%							
Dry Matter								
REP4	24.5	24.4	24.0	22.8	22.3	23.6		
REP5	24.0	23.2	20.4	20.6	16.2	20.9		
Mean	24.3	23.8	22.2	21.7	19.3	22.3	-.968[c]	<.01[c]
Sugars[b]								
REP4	9.0	9.7	10.0	18.1	9.1	11.2		
REP5	22.2	17.9	18.1	16.5	21.2	19.2		
Mean	15.6	13.8	14.1	17.3	15.2	15.2	-.831[d]	< .01[d]
Starch[b]								
REP4	48.2	45.3	48.5	38.6	45.5	45.2		
REP5	39.5	44.9	41.6	40.3	33.0	39.9		
Mean	43.9	45.1	45.1	39.5	39.3	42.6	-.831[d]	< .01[d]
Total CHO[b]								
REP4	57.2	55.0	58.5	56.7	54.6	56.4		
REP5	61.7	62.8	59.7	56.8	54.2	59.0		
Mean	59.5	58.9	59.1	56.8	54.4	57.7	-.908[e]	< .05[e]

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.
[b] Values for sugar, starch, and total carbohydrate (CHO) are expressed on a dry weight basis.
[c] N treatment vs. dry matter content.
[d] % sugar vs. % starch.
[e] N treatment vs. CHO.

yielding identical results to those obtained in the first run.

These extremely high concentrations of sugar could have been a manifestation of the root rot disease. The root sugar content may influence the susceptibility to pathogens in some root crops. For example, a high sucrose concentration is associated with increased resistance in sugar beets to *Phoma betae*, the causative agent of storage rot (Vidhyasekaran, 1988). The abnormally elevated sugar levels existing in both replicates might be a plant response to the root rot pathogen, but this does not explain the major discrepancy between the replicates. Both replicates display similar attrition rates (expressed as percent survivors from the initial populations; Table 19 of the following section), meaning that the severity of disease was the same in both blocks.

Whatever the reason for the above block differences, it must have exerted its effect through the hydrolysis of starch to sugar (or the inhibition of the conversion of sugar into starch), for a strong inverse relationship was found between these two classes of carbohydrates ($r = -.831$, $p < .01$). The higher sugar levels of replicate 5, associated with lower amounts of starch than in replicate 4, resulted in similar total carbohydrate levels for both replicates.

Looking at the values for starch, one notices a quadratic relationship existing for both replicate 5 and the averages of the treatments. However, replicate 4 gives the now familiar "seesaw function," making it impossible to make a definite statement about a treatment trend. Nelson et al. (1983) asserted that spacing and planting dates did not modify the root starch content. It appears as if N fertilization is another cultural practice which does not modify in a predictable manner the starch content of this feral species.

Total CHO behaves in much the same manner as starch in that both replicates do not present the same treatment trends. There is some fluctuation among the lower N treatments in both replicates, but the overall effect, as displayed by the averages, is one of decreasing CHO with increasing soil N. Even though the average values give a significant correlation, one cannot feel confident about the exactness of this inverse relationship because of the fluctuations found in both replicates.

In examining the total CHO and organic N percentages from all ten plots, one finds a highly significant inverse relationship ($r = -.792$). This appears logical, for as larger quantities of reduced N begin to accumulate in the root tissue, more carbon skeletons must be utilized in the formation of amino acids or other N carrier molecules; hence less sugar/starch is available for storage.

End of Season Yield and Dry Matter Partitioning

Proportion of Canopy Weight Contributed by Leaves and Stems

The data on the proportion of leaves and stems presented in Table 17 were originally collected to ascertain the amount of N taken up by seasonal canopy growth, once the percent N of the leaves and stems was known. However, it also serves to further characterize growth and development in this species.

This plant, during its first season of growth, produced slightly more leaf matter than stem matter, but no treatment trend was evident. Higher N inputs increased the LAI (Table 7), but apparently the stem tissue increased at roughly the same rate as leaf tissue when N became more available. These results vary considerably from those of a prior study that reported 61% leaves and 39% stems (Berry et al., 1976). However, this earlier study used second year plants established at much wider spacings. That plants under less competitive circumstances produce larger leaves is immediately apparent when one has the opportunity to observe them in both high- and low-density populations.

Table 17. Relative contribution of leaves and stems to canopy dry weight in the mature buffalo gourd.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Rep 4	- - - - - - % of Canopy Dry Weight - - - - - -					
Leaves	51	55	52	48	5	52
Stems	49	45	48	52	44	48
Rep 5						
Leaves	54	54	51	56	52	53
Stems	46	46	49	44	48	47

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

Nitrogen Effects on Yield and Yield Components

Table 18 records the fresh weight yields and number of healthy roots taken from each sample site. Although statistical analyses were performed upon potential yields extrapolated from these figures (making use of total populations, including dead or dying roots), a strong belief in adherence to research integrity demanded that the actual yields be reported.

Yield on a per unit area basis, whether considering fresh weight or dry weight, is a function of individual root weight and the number of roots per unit area. Table 19 reports the values of these yield components, while Table 20 gives the results of ANOVA for these components.

Individual root weights, measured on both a wet weight and dry weight basis, appeared to respond in a quadratic manner to the N treatments. Nevertheless, the ANOVA did not find any significant treatment effects. Another salient difference was apparent when inspecting the individual root weights: the mean of block 4 was noticeably less than the mean of block 5 in the fresh weights (not statistically significant), but the block means for the dry weights were almost identical. This can be explained by returning to Table 16 and observing that the mean percent dry matter in block 4 is greater by 2.7% (23.6% vs. 20.9%). So, although block 4 is noticeably lower in fresh weight values,

Table 18. End-of-season plant populations and fresh weight root yields of the buffalo gourd based upon disease-free plants.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Final Populations[b]	- - - - - - - - Plants m^{-2} - - - - - - - -					
REP 4	23 (43)	32 (64)	23 (47)	15 (33)	19 (42)	22.4 (46)
REP 5	32 (62)	31 (56)	24 (45)	19 (40)	15 (31)	24.2 (47)
Mean	27.5 (53)	31.5 (60)	23.5 (46)	17.0 (38)	17.0 (37)	23.3 (46)
Root Yields	- - - - - - - - - Mg ha^{-1} - - - - - - - - -					
REP 4	10.2	19.0	13.1	6.3	8.4	11.4
REP 5	18.8	19.5	14.8	11.6	7.0	14.4
Mean	14.5	19.3	14.0	8.9	7.7	12.9

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

[b] Numbers in parentheses represent percent of original population.

Table 19. Effect of N availability upon yield components in the buffalo gourd.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Final Populations[b]	- - - - - - -	-	Plants m^{-2}	-	- - - - - - -	
REP 4	34 (63)	37 (74)	35 (71)	30 (65)	27 (60)	32.6 (67)
REP 5	36 (69)	44 (80)	38 (72)	25 (53)	26 (54)	33.8 (66)
Mean[c]	35.0 (66)	40.5 (77)	36.5 (72)	27.5 (59)	26.5 (57)	33.2 (66)
Individual Root Fresh Weights	- - - - - - - -	-	g $root^{-1}$	-	- - - - - - - -	
REP 4	44.6	59.4	57.3	42.1	44.5	49.6
REP 5	58.9	63.2	61.8	61.4	47.1	58.5
Mean	51.8	61.3	59.6	51.8	45.8	54.0
Individual Root Dry Weights						
REP 4	10.9	14.5	13.8	9.6	9.9	11.7
REP 5	14.1	14.7	12.6	12.6	7.6	12.3
Mean	12.5	14.6	13.2	11.1	8.8	12.0

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

[b] Includes all surviving roots, both healthy and diseased. Numbers in parentheses represent percent of original population.

[c] $LSD_{.05}$ = 6.2

Table 20. ANOVA of yield components in the buffalo gourd as influenced by N fertilization.

Source	df	SS	MS	F	P
Final Populations					
Total	9	333.6	37.1	--	--
Treatments	4	289.6	72.4	7.16	< .05
Blocks	1	3.6	3.6	0.34	NS
Error	4	40.4	10.1	--	--
Individual Root Fresh Weights					
Total	9	632.1	70.2	--	--
Treatments	4	322.9	80.7	2.90	NS
Blocks	1	198.0	198.0	7.12	NS
Error	4	111.2	27.79	--	--
Individual Root Dry Weights					
Total	9	52.6	5.8	--	--
Treatments	4	39.6	9.9	3.25	NS
Blocks	1	0.8	0.8	0.26	NS
Error	4	12.1	3.0	--	--

the difference all but disappears when converted to dry weights because of the higher percent dry matter of block 4.

Final populations (total survivors) were related to N treatments in a quadratic fashion (Figure 4). A treatment effect existed, but only the N_2 and N_3 means in the array were significantly different from one another, as judged by the LSD (Table 19). The percent survivors also follow one another in a quadratic manner, suggesting that the intermediate treatment levels (N_1 and N_2) somehow assisted the plants in resisting the root rot pathogen. Optimal nitrogen nutrition has been shown to effect crop response to pathogens in many crop species, sometimes adversely, sometimes beneficially (Goh and Haynes, 1986), but nothing is known about the interaction of nutrient status with disease agents of the buffalo gourd.

Table 21 reveals that the yields per unit area, whether expressed as fresh weight, dry weight, starch, or total carbohydrate, relate to the N treatments in a quadratic manner. Also, all of these yield parameters are significantly affected by the treatments (Table 22). Most importantly, the N_0 and N_1 treatments are significantly different in every parameter. The N_1 means are not significantly different from the N_2 means for either starch or total carbohydrate, but for fresh weight and dry weight yields these treatments produced distinctly different means. In all

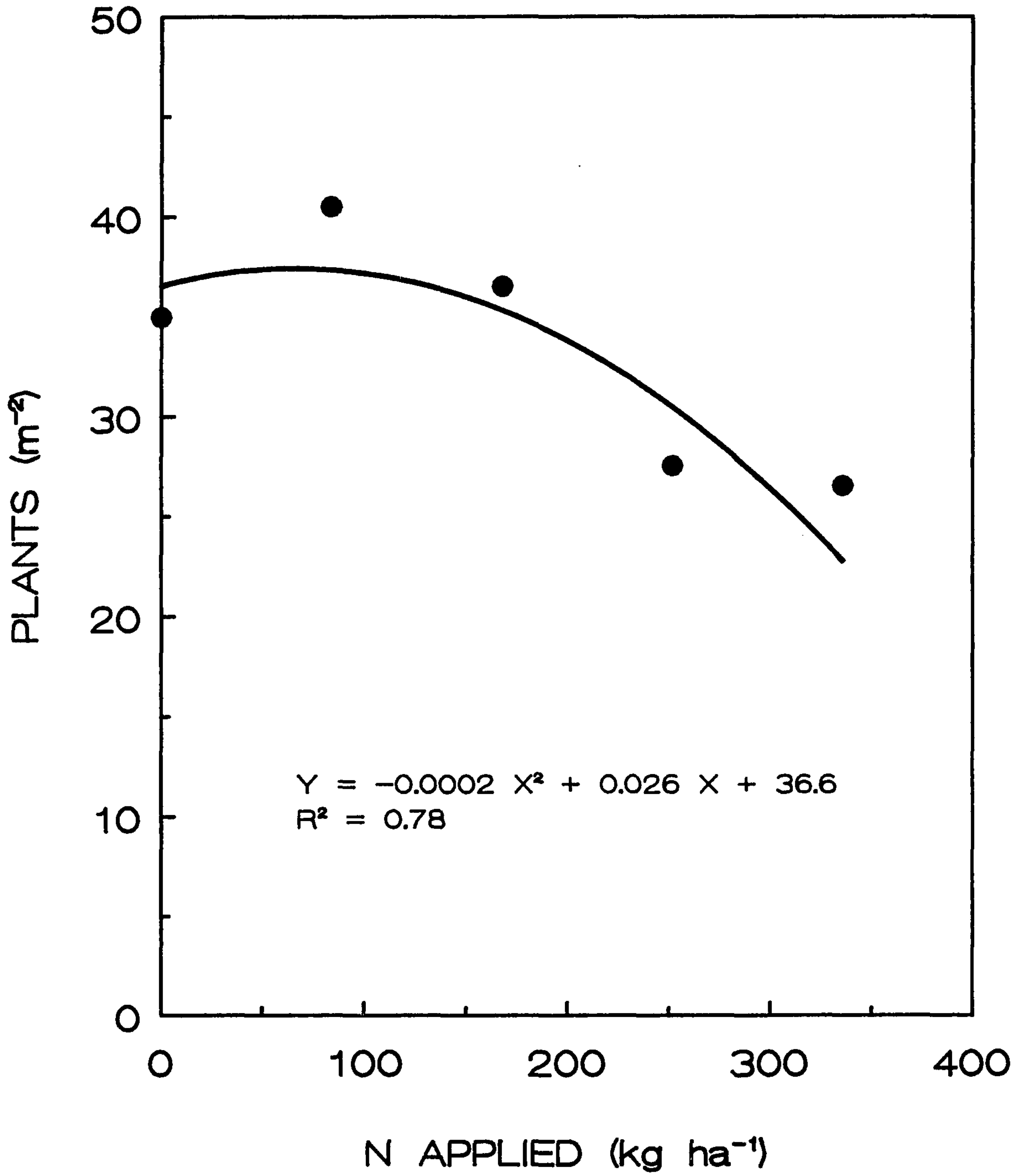

Figure 4. Effect of N upon end-of-season plant populations of the buffalo gourd.

Table 21. Effect of N availability upon potential yield of roots and carbohydrate in the buffalo gourd.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Fresh Weight Yields	- - - - - - - - -	- - - -	Mg ha^{-1}	- - - -	- - - - - - - - -	
REP 4	14.9	21.9	19.7	12.6	11.7	16.2
REP 5	20.9	27.4	23.4	15.0	12.2	19.8
Mean[b]	17.9	24.7	21.6	13.8	12.0	18.0
Dry Weight Yields						
REP 4	3.7	5.3	4.8	2.8	2.6	3.8
REP 5	5.0	6.4	4.7	3.1	1.9	4.2
Mean[c]	4.3	5.9	4.8	3.0	2.3	4.0
Starch						
REP 4	1.7	2.4	2.3	1.1	1.2	1.7
REP 5	2.0	2.9	1.9	1.2	0.6	1.7
Mean[d]	1.8	2.6	2.1	1.1	0.9	1.7
Total Carbohydrate						
REP 4	2.1	2.9	2.8	1.6	1.4	2.1
REP 5	3.1	4.0	2.8	1.7	1.0	2.5
Mean[e]	2.6	3.5	2.8	1.7	1.2	2.3

[a] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.
[b] LSD(.05) = 3.1
[c] LSD(.05) = 1.1
[d] LSD(.05) = 0.6
[e] LSD(.05) = 0.9

Table 22. ANOVA of various yield parameters of the buffalo gourd as influenced by N fertilization.

Source	df	SS	MS	F	P
Total Fresh Weight Yields					
Total	9	2.6618	.2957	--	--
Treatments	4	2.2324	.5581	21.71	< .01
Blocks	1	0.3263	.3263	12.66	< .05
Error	4	0.1030	.0257	--	--
Total Dry Weight Yields					
Total	9	.1825	.0202	--	--
Treatments	4	.1643	.0291	8.31	< .05
Blocks	1	.0040	.0040	1.26	NS
Error	4	.0142	.0035	--	--
Starch					
Total	9	.0439	.0048	--	--
Treatments	4	.0402	.0101	10.94	< .05
Blocks	1	.000012	.000012	0.01	NS
Error	4	.0036	.0009	--	--
Total Carbohydrate					
Total	9	.0768	.0085	--	--
Treatments	4	.0646	.0162	7.04	< .05
Blocks	1	.0038	.0038	1.62	NS
Error	4	.0091	.0023	--	--

yield parameters, major declines occur as N is progressively increased through the third and fourth levels (see also Figure 5).

These data suggest that buffalo gourd has the capability of producing 24.7 Mg ha^{-1} of roots on a fresh weight basis, 5.9 Mg ha^{-1} of root dry matter, 2.6 Mg ha^{-1} starch, and 3.5 Mg ha^{-1} of total (readily fermentable) carbohydrate. This additional carbohydrate in the form of sugar could become very important should this species ever be used as a feedstock for ethanol production.

Another important fact can be gleaned from the data in Table 21: pre-season soil testing would be essential, should this species find a niche as a root crop--if the producer plans upon maximizing yield. The sharp rise in yield from N_0 to N_1 followed by a subsequent decline from N_1 to N_2 indicates that buffalo gourd is very sensitive to N inputs. Without knowledge of the amount of residual soil N at the time of planting, the grower could very easily either overfertilize and consequently reduce yields or fail to apply enough to maximize yields.

The relationship of petiole $[NO_3^-]$ to yield can be seen in Figure 6. It was only possible to correlate the July samples with end-of-season yields because this was the only sample period in which petiole NO_3^- values for the harvested blocks (blocks 4 and 5) existed. This appears to be a

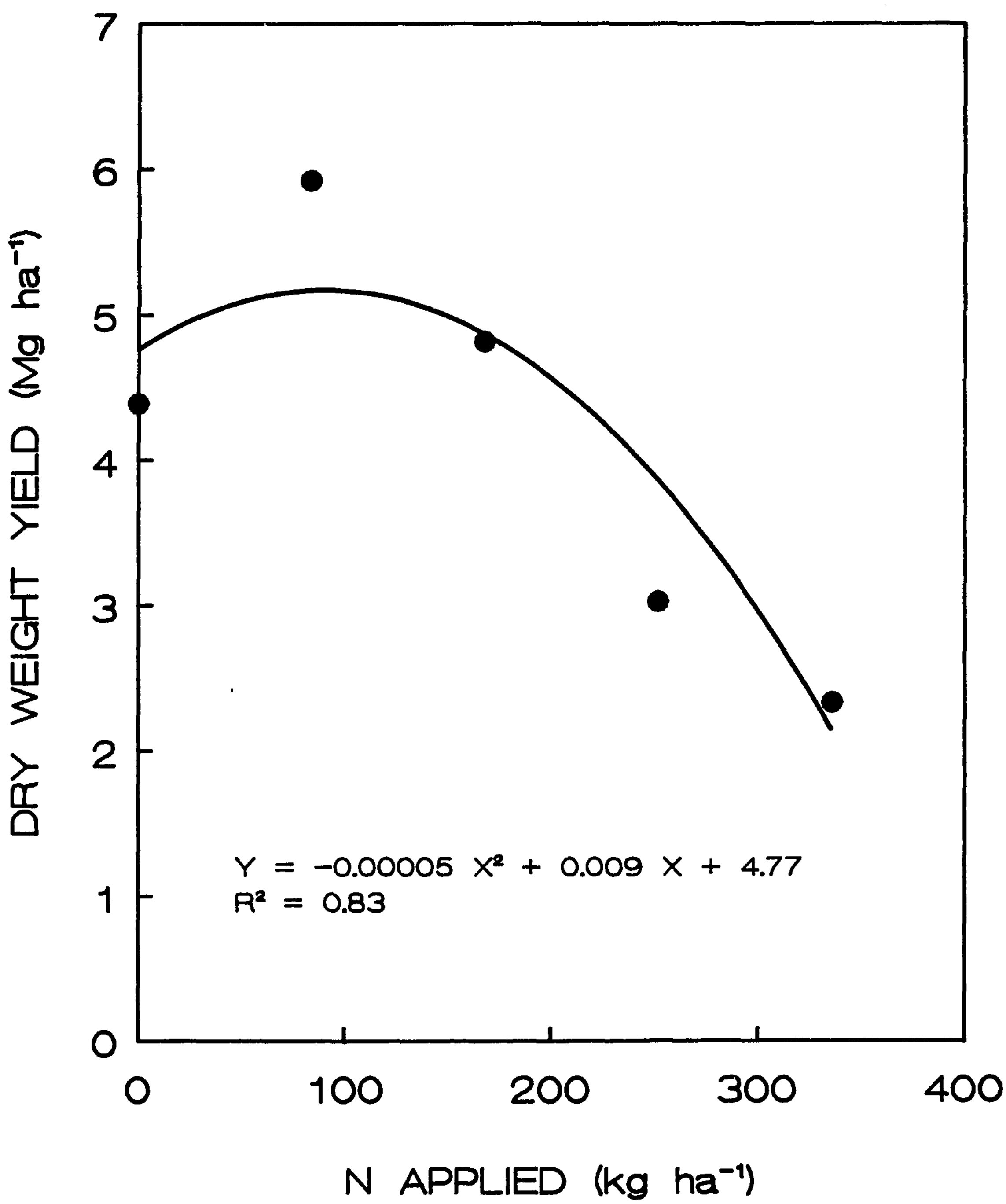

Figure 5. Effect of N upon end-of-season root dry matter production of the buffalo gourd.

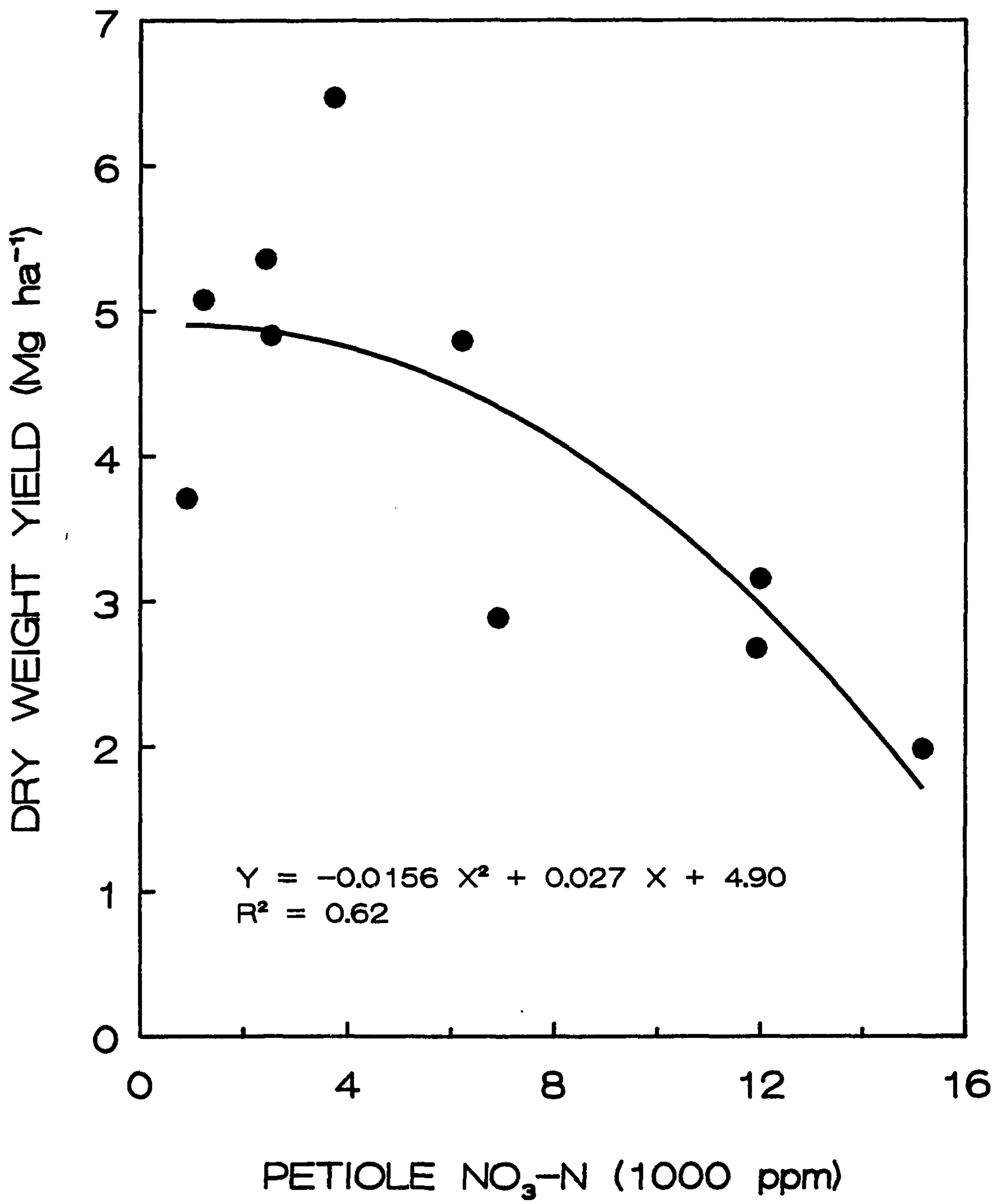

Figure 6. The relationship between petiole NO_3^- concentration and root dry matter production of buffalo gourd.

favorable time to infer a relationship between the plant's N nutrient status and yield, for the soil N availability was still high enough to result in large concentrations within the petiole and major differences between all treatments (refer to Table 8). Once again, a quadratic function describes the relationship most accurately, although the relationship is not a very reliable one, due to the relatively small number of data points used in the determination of the curve.

Correlations Among Yield and Yield Components

In this study many linear relationships were found when the various aspects of yield were correlated with one another (Table 23).

Final populations strongly influenced yield on a per area basis, as measured by fresh weights, dry weights, and total carbohydrate. Past studies have already demonstrated the importance of plant populations as a factor affecting yield in the buffalo gourd (Nelson et al., 1983). Most of these studies have shown a linear relationship to prevail with populations in the range of 100,000-400,000 plants ha^{-1}. However, when populations were increased to 676,000 plants ha^{-1}, a quadratic function was the most appropriate. That yield is linearly related to populations in this study can be further corroborated by comparing the grand means of the

Table 23. Linear correlations between yield and yield components and between the components themselves in the buffalo gourd.[a]

	Yield Components			Yield/Unit Area		
	Initial Population	Individual Root Fresh Weight	Individual Root Dry Weight	Total Root Fresh Weight	Total Root Dry Weight	Total Carbohydrate
Final Population[b]	$.830^{01}$	$.556^{10}$	$.733^{02}$	$.920^{01}$	$.938^{01}$	$.942^{01}$
Individual Root Fresh Weight	--	--	$.838^{01}$	$.831^{01}$	--	--
Individual Root Dry Weight	--	--	--	--	$.918^{01}$	$.896^{01}$
Total Root Dry Weight	--	--	--	--	--	$.993^{01}$

[a] Superscripts represent significance level for the correlation coefficients; df = 8.

[b] Includes all plants, both healthy and diseased.

actual yield and final population of healthy plants (Table 18) to the grand means of the potential yield and final population of total survivors (Tables 19 and 21). In this case one finds an increase of 39.5% in fresh weight yields accompanied by a similar increase of 42.5% in the plant population. Identical increases in both variables are indicative of a linear relationship.

In correlating population with individual root weight, a paradoxical result was obtained: increasing the population resulted in an increase in the size (weight) of the root as well. This seems contradictory to accepted theory, which assumes the size of the plant (or plant part) is reduced as the population per unit area is increased. This manifestation of intraspecies competition is documented in both ecological field studies as well as agronomic ones (Silvertown, 1982). Furthermore, two separate studies with buffalo gourd have shown the size of the root to decrease (as measured by crown diameter) as the population density became greater (Nelson et al., 1983; Scheerens et al., 1983). The explanation for this seemingly unusual effect lies in the N nutrition of the plant. Both populations and root size were related quadratically to the N treatments. As N increased through the intermediate levels (optimum), so did populations and root size. Experiments in which the effect of population on size is studied usually keep other variables constant, whereas in this study the soil N was varied. The increase in available N through optimal levels counteracted the normal competition effect of increasing populations by preventing N from becoming a limiting factor in the plant's development.

The other major component of yield, root weight, also displayed a strong linear relationship with yield, both individual root fresh weight and individual root dry weight correlating well with total fresh weight yields and total dry weight yields, respectively.

The only other relationship worthy of discussion is that of individual root fresh weight versus individual root dry weight, if only because the degree to which the correlation coefficient deviates from 1 is a reflection of the treatment effect. If the percent dry matter did not change from sample to sample, then the r value would closely approximate 1 (allowing small deviations due to experimental error), because any change in the root weight on a fresh weight basis would be matched by an identical change in the dry weight of the root. In this particular study, N was shown to have an effect upon the percent dry matter within the root, hence was the environmental factor responsible for the lowering of the r value between these two variables to less than the theoretical value of 1.

Partitioning of Dry Matter

Table 24 reports changes that occur in total dry matter production and its partitioning as greater quantities of N become available to the plant, while Table 25 displays the ANOVA. Root dry matter production per unit area has already been discussed; suffice it to say that all the means are essentially distinct except for N_3 from N_4. With the canopy a linear relationship best describes the change in dry matter production as the level of N is changed ($r = .990$). Total dry matter production appears to be described best by a quadratic equation, but the only significant difference in means (as judged by LSD) is between N_0 and N_1. The harvest index, a parameter not subjected to statistical scrutiny, reveals that a somewhat greater proportion of the dry matter produced is channeled into the root at the optimal level of N fertilizer. This proportion gradually declines as N assumes the role of a luxury input. To sum up, increasing the quantity of N to the plant beyond the level of optimum root production causes more of the photosynthate to be partitioned into the top growth at the expense of root growth.

Water Use Efficiency

Another means of expressing yield (and of special interest in production areas where water is scarce) is by "water use efficiency" (WUE), or quantity of harvestable material produced per unit of water consumed. The addition

Table 24. Dry matter partitioning in the buffalo gourd at the time of harvest.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	Mean
Roots			Mg ha^{-1}			
REP 4	3.7	5.3	4.8	2.8	2.6	3.8
REP 5	5.0	6.4	4.7	3.1	1.9	4.2
Mean[b]	4.3	5.9	4.8	3.0	2.3	4.0
Canopy						
REP 4	6.4	6.6	7.3	6.8	8.4	7.1
REP 5	5.9	6.8	7.8	9.3	8.4	7.6
Mean[c]	6.1	6.7	7.5	8.1	8.4	7.4
Total						
REP 4	10.1	12.0	12.1	9.7	11.1	11.0
REP 5	11.0	13.3	12.5	12.5	10.4	11.9
Mean[d]	10.5	12.6	12.3	11.1	10.7	11.5
Harvest Index[e]						
REP 4	.36	.44	.39	.29	.24	.34
REP 5	.46	.48	.38	.25	.18	.35
Mean	.41	.46	.39	.27	.21	.35

[a] 0-336 kg ha^{-1} N in increments of 84 kg ha^{-1}.
[b] $LSD_{.05}$ = 1.1.
[c] N treatment vs. canopy dry weight; $r = .990$, $p < .01$.
[d] $LSD_{.05}$ = 1.7.
[e] HI = root weight/root weight + canopy weight.

Table 25. ANOVA of total dry matter production in the buffalo gourd at time of harvest.

Source	df	SS	MS	F	P
Total	9	12.812	1.423	--	--
Treatments	4	7.378	1.844	9.15	< .05
Blocks	1	2.212	2.212	2.74	NS
Error	4	3.222	0.805	--	--

of N has been shown to increase the WUE of many crop species (Goh and Haynes, 1986). Conversely, if water is limiting, then plants cannot take advantage of any additional applied N (Goh and Haynes, 1986).

The WUEs for the various N treatments are shown in Table 26. No statistical analyses were performed on these data, but the WUEs for the fresh weight yields should show significance in the same manner as the fresh weight yields themselves. The logic bolstering this assumption is that the CWU was essentially the same for each treatment surveyed; therefore, each fresh weight yield was divided by the same figure (grand mean of CWU), giving a set of quotients which bear the same relationship to one another as the yields themselves.

These values are not nearly as impressive as the WUEs achieved in another study designed to ascertain the CWU of this feral species (Nelson et al., 1989). This two-year study found WUEs for the optimal irrigation regimes to range from 3.77-4.79 kg m^{-3} (fresh roots). By contrast, the best WUE in this study was 3.0 kg m^{-3}. Furthermore, the fresh weight yields obtained by Nelson et al. were comparable to the higher yielding plots of this experiment, demonstrating that judicious water management can make this species look promising as an arid lands starch crop.

Table 26. How water use efficiency in the buffalo gourd is influenced by soil N availability.[a]

	Treatments[b]					
	N_0	N_1	N_2	N_3	N_4	Mean
Fresh Weight Root Yields	- - - - - - - - Kg m^{-3} H_2O - - - - - - - -					
REP 4	1.8	2.6	2.4	1.5	1.4	1.9
REP 5	2.5	3.3	2.8	1.8	1.4	2.4
Mean	2.2	3.0	2.6	1.7	1.4	2.2
Total Dry Matter[c]						
Rep 4	1.2	1.4	1.4	1.1	1.3	1.3
REP 5	1.3	1.6	1.5	1.5	1.2	1.4
Mean	1.3	1.5	1.5	1.3	1.3	1.4

[a] Consumptive use grand mean of 819.7 mm was used for all calculations.

[b] 0-336 kg ha^{-1} N in 84 kg ha^{-1} increments.

[c] Roots + canopy.

Unlike most crop species, plants from desert environs typically exhibit relatively low WUEs (McLaughlin, 1985). Many of these species survive prolonged periods of water stress by reducing transpiration via closing or partially closing their stomates, through sunken stomates, or by means of a protective pubescence. As a consequence of increasing the diffusive resistance of their stomates a large portion of the time, they also fix less carbon. In fact, they never really evolved efficient metabolic machinery; even if water is readily available, they simply squander it while their sluggish metabolic machinery putters along, fixing meager amounts of CO_2.

Contained in the lower half of Table 26 are the WUEs for total dry matter production ("net primary production" or "NPP"). These were calculated in order to determine how the buffalo gourd compares to other xerophytes and domesticated crop species in terms of efficiency of NPP. Many xerophytes display WUEs in the range of 0.4-0.6 g l^{-1}, whereas most crop species range from 1.2-2.2 g l^{-1} (McLaughlin, 1985). As can be seen from this table, buffalo gourd is exceptional as a xerophyte in its ability to fix CO_2 (grand mean = 1.4 g l^{-1}), falling into the range exhibited by crop species.

Nitrogen Removal from the Soil System

The amount of N applied at the beginning of the season figured prominently in the total amount withdrawn from the soil system by the end of the season and in efficiency of fertilizer use (Table 27). No analysis was performed on the amounts removed by the harvested roots, but it appears, once again, as if it would be best described by a quadratic equation. Amounts of N sequestered in the canopy were linearly related to the rate of N fertilizer application, as were the amounts of N taken up by the combination of roots and top growth. The efficiency at which the applied fertilizer was used by the crop was also linear in nature, but with a negative slope instead, dropping from 50.4% at the lowest application rate down to 33.3% at the N_4 level(336 kg ha^{-1} N). Most experiments in which N recovery has been monitored range from 30-70%, depending upon soil type, environmental conditions, and method and rate of application (Goh and Haynes, 1986).

The question about the source of the N used by the N_0 plants needs to be addressed. These N_0 plots removed approximately 241 kg N during the season, yet calculations based upon the average amount found in the soil profile preseason show that only 120 kg N could have come from the residual NO_3^-. Where did the remainder come from? Certainly some was supplied through mineralization of organic matter. However, most soils range from .08-.40% N by weight, with only 1-3% of this being liberated annually through mineralization (Haynes, 1986). A bulk density of 1.61 g cm^{-3} gives a

Table 27. Seasonal removal of soil N by the buffalo gourd.

	Treatments[a]					
	N_0	N_1	N_2	N_3	N_4	r
	Kg ha^{-1}					
Root N						
Rep 4	35	66	55	45	41	
Rep 5	46	58	54	43	30	
Mean	40	62	55	44	36	--[b]
Canopy N						
Rep 4	204	220	254	213	293	
Rep 5	196	221	264	334	341	
Mean	200	221	259	273	317	.988[c]
Total N						
Rep 4	239	286	309	258	334	
Rep 5	242	279	318	376	370	
Mean	241	283	313	317	352	.972[d]
	%					
Fertilizer Efficiency	--	50.4	43.5	30.4	33.3	-.899[e]

[a] 0-336 kg ha^{-1} N in increments of 84 kg ha^{-1}.

[b] Correlation not determined.

[c] N treatment vs. amount of N removed by canopy ($p < .01$).

[d] N treatment vs. total amount of N removed ($p < .01$).

[e] N treatment vs. efficiency of fertilizer use ($p < .10$).

total weight of 4,830,000 kg ha^{-1} in the upper 30 cm of the soil profile. Assuming 0.5% organic matter for desert soils and a generous 3% N in the organic matter, one arrives at a value of 724.5 kg N ha^{-1} contained in the organic matter fraction. Even at the maximum rate of mineralization (3% yr^{-1}), this provides only 21.7 kg N ha^{-1} towards the 121 kg ha^{-1} deficit. Some might have been supplied by the irrigation water, but most of the remainder of the necessary N must have come from residual NO_3^- located at a depth in the profile greater than 120 cm. This is conceivable, when one considers that only two irrigations penetrated past 120 cm (not exceeding 180 cm). Therefore, most NO_3^- residing deeper than 120 cm would not have been leached away but would have been available for uptake by the plants. As has already been reported in Table 11, a substantial fraction of the plant´s CWU comes from profile depths greater than 120 cm (about 17%), thus allowing it to take advantage of this lower level NO_3^-.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Not only does science begin in wonder, it also ends in wonder.

Abraham Maslow

Careful inspection of the data has yielded the following conclusions and opened up a number of avenues to explore in future experimental efforts.

1. Varying amounts of available N had little or no effect upon the emergence and early growth and development of the buffalo gourd grown under high populations, as measured by vine length, dry matter accumulation, and LAI.
2. Later in the season differences in growth were strikingly apparent, as measured by LAI and canopy dry matter production. No overt signs of N deficiency were ever found in the N_0 treatments. It would have been interesting to quantify chlorophyll content and monitor carbon exchange ratios to discover whether or not photosynthesis was compromised. One wonders whether root production in the N_0 treatments was lowered because suboptimal N affected photosynthesis rates through less chlorophyll production, or whether the decline in yield was simply due

to not being able to produce the optimum leaf area for maximum production of photosynthate.

3. More extensive monitoring is required before definitive conclusions are reached about CWU, but it appears as if N has little effect upon how much H_2O this plant uses. Larger amounts of canopy per area of ground appeared to result in less transpiration per unit area of leaf tissue. Mild stress may have occurred more frequently at the higher N levels, thus somewhat limiting photosynthesis, hence DM production. Future fertility work should incorporate different irrigation regimens so as to be able to look for N x H_2O interactions.

4. A strong link existed throughout the season between the level of N fertilizer applied and the [NO_3^-] within the petioles. The age of the petiole exerted its greatest effect upon [NO_3^-] at the time of the first sampling. Apparently the older petioles will only act as strong sinks for NO_3^- when it is present in great excess. It appears feasible to assess the plant's N nutritional status through periodic analysis of petiole NO_3^- content, as indicated by the high correlations obtained during all samplings, but the almost identical values existing between the N_0 and N_1 treatments of the last sampling make

predicting differences in yield at such a late date in the season extremely uncertain. The reliability of the relationship between petiole NO_3^- content and yield (defined as a quadratic function) is still not adequately ascertained because of the low number of data points used in correlating the July samples with yield. Future work should also focus on establishing what sort of relationship exists for older petioles in addition to the ones already surveyed. Also, once a deficiency is uncovered, how late in the season can it be corrected? This would involve periodic supplemental applications to the N_0 plots in order to determine when a deficiency can be remedied and when it cannot.

5. Increasing amounts of available N stimulated the production of top growth at the expense of root yield and percent dry matter accumulation of the root. The relationship between nitrogen and root yield needs to be further refined by conducting tests utilizing more treatments. Buffalo gourd appears to be different from other root crops such as sugarbeet in that it manifests a peak response and rapid decline at lower levels of N. Given such a rapid increase and decline over such a narrow range of soil N, it would be of interest (and perhaps of practical significance too)

to view the yield response at smaller increments of N.

6. Populations should be modified in future experiments, even though it has already been demonstrated that yield is maximized at extremely high populations (Nelson et al., 1983). Financially speaking, it does not seem feasible to attempt to maximize root yield when such an expensive input to the production process (about 30 kg seed ha^{-1}) is required. The increase in production would probably not warrant the added expense of such large quantities of seed. Another factor to consider is that timely (and perhaps frequent) applications of small amounts of N should maximize the size of individual roots and partially compensate for the reduced populations. In addition, the larger diameter roots naturally resulting from the reduction of plants per unit area would facilitate harvesting with conventional equipment such as the potato combine. These future studies should probably make use of population densities of 15,000-100,000 plants ha^{-1}, as densities at the lower end of this range have already demonstrated root yields of 35-50% of those attained through high density plantings (Hogan and Bemis, 1983).

7. Increased quantities of N seemed to have very little effect on the chemical composition of the canopy. One parameter which was not measured was percent dry matter. SLWs, a type of indirect measure of leaf dry matter concentration, did not vary in a predictable manner, so it would appear that changes in dry matter content are due to factors other than the relative availability of N.
8. Nitrogen availability affected the chemical composition of the root in a variety of ways. Heavier applications of N fertilizer caused a reduction in the dry matter content of the root but simultaneously elevated the organic N content. Both of these parameters correlated strongly with the N treatments. In this study, no attempt was made to characterize the organic N fraction, so it is unknown as to whether the increase represents greater amounts of protein, free amino acids, or other N carriers such as ureides. From a physiological standpoint, it would be interesting to know whether additional protein is being stored in the root or if this increase in N compounds represents a temporary "logjam" of amino acids or other carrier molecules waiting to be transported to other organs. The relationship between root carbohydrate production and

N fertility remains an ambiguous one. Total carbohydrate showed a tendency to drop as N was elevated, but this relationship was only borne out in the <u>average</u> of the replicates. When considered by itself, the starch content fluctuated randomly but correlated well (-.831) with the amount of sugar present. The sugar content in both blocks was much higher than anticipated and may have been a response provoked by the root rot pathogen. Further research is needed to determine conclusively whether or not N can influence the levels of the various carbohydrates of the root.

9. The various plant organs were specific in their ability to concentrate organic N compounds, NO_3^-, and other minerals. Only with NO_3^- did the ranges of values overlap at all.

10. The optimum level of N fertilization resulted in the removal of 283 kg ha^{-1} N from the soil system, thus requiring somewhat more than this amount to be added annually for yields to remain consistently high, depending upon the expected contribution of N from mineralization and losses due to leaching. Efficiencies of fertilizer N uptake were in the range of 33-51%. Undoubtedly, these could be increased through

split applications, which should be a factor studied if any additional fertility studies are undertaken.

11. Based upon the results of this study and subsequent studies focusing on CWU and response to differential N fertilization, it appears feasible to integrate the buffalo gourd into arid lands irrigated agriculture as a root crop. CWU in the range of 487-649 mm has been shown to be sufficient to maximize yields of the relatively unimproved germplasm currently available (Nelson et al., 1989). Additionally, the studies alluded to above have obtained fresh weight yields of 23-28 Mg ha^{-1}, starch yields of 2.6-3.4 Mg ha^{-1}, and an estimated ethanol yield of 2400 l ha^{-1} (assuming a conversion factor of 51.2% for total carbohydrate into ethanol and a total carbohydrate yield of 3.5 Mg ha^{-1}, produced by the N_1 treatment). The potential ethanol yield seems especially promising. Sweet potato, a long-established root crop, has been reported to produce only 1776 l ha^{-1} (Hill, 1989), so perhaps the real future of buffalo gourd will be as an energy crop when the economics of biofuels become more attractive.

REFERENCES

Abbott, J. L. and J. M. Nelson. 1983. Phosphorus fertilization and sugar yields of fall planted sugar beets. Agronomy Journal 75:185-188.

Alcorn, S. M., J. D. Mihail, and T. V. Orum. 1986. What! Our new crops are sick? Journal of Arid Environments 11:111-114.

Anderson, F. N. and G. A. Peterson. 1988. Effect of incrementing nitrogen application on sucrose yield of sugarbeet. Agronomy Journal 80:709-712.

Archer, John. 1985. Crop Nutrition and Fertilizer Use. Farming Press, Ipswich, U.K.

Beevers, Leonard. 1976. Nitrogen Metabolism in Plants. Edward Arnold, London, U.K.

Bemis, W. P., J. W. Berry, C. W. Weber, and T. W. Whitaker. 1978 . The buffalo gourd: A new potential horticultural crop. Hortscience 13:235-240.

Bemis, W. P., L. D. Curtis, C. W. Weber, and J. Berry. 1978. The feral buffalo gourd, Cucurbita foetidissima. Economic Botany 32:87-95.

Berry, J. W., J. C. Scheerens, and W. P. Bemis. 1978. Buffalo gourd roots: Chemical composition and seasonal changes in starch content. Journal of Agricultural and Food Chemistry 26:354-356.

Berry, J. W., C. W. Weber, M. L. Dreher, and W. P. Bemis. 1976. Chemical composition of buffalo gourd, a potential food source. Journal of Food Science 41:465-566.

Bidwell, R. G. S. 1974. Plant Physiology. MacMillan, New York, NY.

Bourke, R. M. 1985. Influence of nitrogen and potassium fertilizer on growth of sweet potato (Ipomoea batatas) in Papua New Guinea. Field Crops Research 12:363-375.

Brill, W. J. 1980. Nitrogen fixation. p. 53-57. In P. S. Carlson (ed.) The Biology of Crop Productivity. Academic Press, New York, NY.

Callow, J. A. 1976. Plant lectins. p. 221-235. In H. Smith (ed.) Commentaries in Plant Science. Vol. 1. Pergamon Press, Oxford, U.K.

Canter, Larry W. 1986. Environmental Impacts of Agricultural Production Activities. Lewis Publishers, Chelsea, MI.

Chew, Victor. 1976. Comparing treatment means: A compendium. Hortscience 11:348-357.

Clegg, K. M. 1956. The application of the anthrone reagent to the estimation of starch in cereals. Journal of the Science of Food and Agriculture 7:40-44.

Constantin, R. J., L. G. Jones, H. L. Hammett, T. P. Hernandez, and C. G. Kahlich. 1984. The response of three sweet potato cultivars to varying levels of nitrogen. Journal of the American Society for Horticultural Science 109:610-614.

Curtis, L. C. 1946. The possibilities of using species of perennial cucurbits as a source of vegetable fats and protein. The Chemurgic Digest 5(13):221-224.

De Veaux, J. S. and E. B. Schultz, Jr. 1985. Development of the buffalo gourd (Cucurbita foetidissima) as a semiaridland starch and oil crop. Economic Botany 39:454-473.

Dittmer, H. J. and B. P. Talley. 1964. Gross morphology of taproots of desert cucurbits. Botanical Gazette 125:121-126.

Donahue, Roy L., Raymond W. Miller, and John C. Shikluna. 1977. Soils: An Introduction to Soils and Plant Growth. 4th ed. Prentice-Hall, Englewood Cliffs, NJ.

Dossey, B. F., W. P. Bemis, and J. C. Scheerens. 1981. Genetic control of gynoecy in the buffalo gourd. The Journal of Heredity 72:355-356.

Dreher, M. L., J. C. Scheerens, C. W. Weber, and J. W. Berry. 1981. Nutritional evaluation of buffalo gourd root starch. Nutrition Reports International 23:1-8.

Gathman, A. C. and W. P. Bemis. 1983. Heritability of fatty acid composition of buffalo gourd seed oil. The Journal of Heredity 74:199.

Giles, J. F., J. O. Reuss, and A. E. Ludwick. 1975. Prediction of nitrogen status of sugarbeets by soil analysis. Agronomy Journal 67:454-459.

Goh, K. M. and R. J. Haynes. 1986. Nitrogen and agronomic practice. p. 379-442. In R. J. Haynes (ed.) Mineral Nitrogen in the Plant-Soil System. Academic Press, Orlando, FL.

Halvorson, A. D. and G. P. Hartman. 1980. Response of several sugarbeet cultivars to N fertilization: Yield and crown tissue production. Agronomy Journal 72:665-669.

Halvorson, A. D. and G. P. Hartman. 1988. Nitrogen needs of sugarbeet produced with reduced-tillage systems. Agronomy Journal 80:719-722.

Hammett, L. K., C. H. Miller, W. H. Swallow, and L. Harden. 1984. Influence of N source, N rate, and K rate on the yield and mineral concentration of sweet potato. Journal of the American Society for Horticultural Science 109:294-298.

Harlan, Jack R. 1976. The plants and animals that nourish man. Scientific American 235(3):89-97.

Harper, F. 1983. Principles of Arable Crop Production. Grenada Press, London, U.K.

Harpstead, Milo I., Francis D. Hole, and William Bennett. 1988. Soil Science Simplified. Iowa State University Press, Ames, IA.

Haynes, R. J. 1986. Uptake and assimilation of mineral nitrogen by plants. p. 303-358. In R. J. Haynes (ed.) Mineral Nitrogen in the Plant-Soil System. Academic Press, Orlando, FL.

Hill, Walter A. 1989. Sweet potato. p. 179-189. In D. L. Plucknett and H. B. Sprague (ed.) Detecting Mineral Nutrient Deficiencies in Tropical and Temperate Crops. Westview Press, Boulder, CO.

Hogan, L. and W. P. Bemis. 1983. Buffalo gourd and jojoba: Potential new crops for arid lands. Advances in Agronomy 36:317-349.

Jeffrey, David W. 1987. Soil-Plant Relationships: An Ecological Approach. Timber Press, Portland, OR.

Johnson, G. V., J. L. Stroehlein, and J. L. Abbott. 1978. Critical tissue levels for predicting nitrogen needs of sugarbeets at Mesa, Arizona. Journal of the American Society of Sugar Beet Technologists 20:65-72.

Joslyn, M. A. 1970. Ash content and ashing procedures. p. 109-141. In M. A. Joslyn (ed.) Methods in Food Analysis: Physical, Chemical, and Instrumental Methods of Analysis. 2nd ed. Academic Press, New York, NY.

Kim, J. Y., M. C. Acock, J. O. Garner, Jr., C. C. Singletary, and M. L. Salin. 1985. Effect of nitrogen fertilization on alcohol soluble carbohydrate content in sweet potato roots. Hortscience 20:434-435.

Kúc, Joseph. 1982. Phytoalexins from the Solanaceae. p. 81-100. In J. A. Bailey and J. W. Mansfield (ed.) Phytoalexins. Blackie and Son, Glasgow, U.K.

Lee, G. S., G. Dunn, and W. R. Schmehl. 1987. Effect of date of planting and nitrogen fertilization on growth components of sugarbeet. Journal of the American Society of Sugar Beet Technologists 24:81-99.

Lehninger, Albert L. 1982. Principles of Biochemistry. Worth Publishers, New York, NY.

Leonard, O. A., W. S. Anderson, and M. Geiger. 1949. Field studies on the mineral nutrition of the sweet potato. Proceedings of the American Society for Horticultural Science 53:387-392.

Lewis, Owen A. M. 1986. Plants and Nitrogen. Edward Arnold, London, U.K.

Lippincott, W. T., Alfred B. Garrett, and Frank Henry Verhoek. 1977. Chemistry: A Study of Matter. 3rd ed. John Wiley and Sons, New York, NY.

Marschner, Horst. 1986. Mineral Nutrition of Higher Plants. Academic Press, London, U.K.

McLaughlin, S. P. 1985. Economic prospects for new crops in the southwestern United States. Economic Botany 39:472-481.

Moraghan, J. T. 1987. Nitrogen fertilizer effects on uptake and partitioning of chloride in sugarbeet plants. Agronomy Journal 79:1054-1057.

Mulrean, E. and T. Russell. 1982. Summary of diseases detected through the Cotton Research Center Diagnostic Laboratory, July 1, 1982 to December 28, 1982. (Unpublished report).

Nelson, J. M. and J. C. Scheerens. 1986. Water use and production of buffalo gourd and coyote gourd: A greenhouse study. Abstract. Journal of Arizona-Nevada Academy of Science (Proceedings Supplement) 21:13.

Nelson, J. M., J. C. Scheerens, J. W. Berry, and W. P. Bemis. 1983. Effect of plant population and planting date on root and starch production of buffalo gourd grown as an annual. Journal of the American Society for Horticultural Science 108:198-201.

Nelson, J. M., J. C. Scheerens, D. A. Bucks, and J. W. Berry. 1989. Irrigation effects on water use, production of tap roots, and starch of buffalo gourd. Agronomy Journal 81:439-442.

Nelson, J. M., J. C. Scheerens, T. L. McGriff, and A. C. Gathman. 1988. Irrigation and plant spacing effects on seed production of buffalo and coyote gourds. Agronomy Journal 80:60-65.

Pathak, M. D. and R. C. Saxena. 1976. Insect resistance in crop plants. p. 61-83. In H. Smith (ed.) Commentaries in Plant Science. Vol. 2. Pergamon Press, Oxford, U.K.

Rosemeyer, M. E., J. K. Brown, and M. R. Nelson. 1986. Five viruses isolated from field-grown buffalo gourd (_Cucurbita foetidissima_), a potential crop for arid lands. Plant Disease 70:405-409.

Rosemeyer, M. E., B. H. Wells, and A. Zaid. 1982. Diseases of the buffalo gourd, _Cucurbita foetidissima_, in Arizona. Phytopathology 72:955.

Rosenthal, Gerald A. 1982. Plant Nonprotein Amino and Imino Acids: Biological, Biochemical, and Toxicological Properties. Academic Press, New York, NY.

Scheerens, J. C., W. P. Bemis, M. L. Dreher, and J. W. Berry. 1978. Phenotypic variation in fruit and seed characteristics of buffalo gourd. Journal of the American Oil Chemists´ Society 55:523-525.

Scheerens, J. C. and J. W. Berry. 1986. Buffalo gourd: Composition and functionality of potential food ingredients. Cereal Foods World 31:183-192.

Scheerens, J. C., M. L. Dreher, W. P. Bemis, and J. W. Berry. 1983. Buffalo gourd root starch, part III: Effects of plant age and spacing upon the physico-chemical and rheological properties. Starch/Stärke 35:193-198.

Scheerens, J. C., M. J. Kopplin, I. R. Abbas, J. M. Nelson, A. C. Gathman, and J. W. Berry. 1987. Feasibility of enzymatic hydrolysis and alcoholic fermentation of starch contained in buffalo gourd (Cucurbita foetidissima) roots. Biotechnology and Bioengineering 29:436-444.

Scheerens, J. C., T. L. McGriff, A. E. Ralowicz, M. H. Wilkins, and J. M. Nelson. 1989. Variation in seed yield components of buffalo gourd (Cucurbita foetidissima HBK) grown as an annual. Euphytica 40:55-62.

Shahani, H. S., F. G. Dollear, K. S. Markley, and J. R. Quimby. 1951. The buffalo gourd, a potential oilseed crop of the southwestern drylands. Journal of the American Oil Chemists´ Society 28:90.

Silvertown, Jonathan W. 1982. Introduction to Plant Population Ecology. Longman Group, New York, NY.

Sprent, Janet I. 1987. The Ecology of the Nitrogen Cycle. Cambridge University Press, Cambridge, U.K.

Steel, Robert G. D. and James H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York, NY.

Ulrich, Albert and F. Jackson Hills. 1989. Sugarbeet. p. 225-241. In D. L. Plucknett and H. B. Sprague (ed.) Detecting Mineral Nutrient Deficiencies in Tropical and Temperate Crops. Westview Press, Boulder, CO.

Vasconcellos, J. A., W. P. Bemis, J. W. Berry, and C. W. Weber. 1981. The buffalo gourd, Cucurbita foetidissima HBK, as a source of edible oil. p. 55-68. In E. H. Pryde, L. H. Princen, and K. D. Mukherjee (ed.) American Oil Chemists´ Society, Champaign, IL.

Vidhyasekaran, P. 1988. Physiology of Disease Resistance in Plants. Vol. 2. CRC Press, Boca Raton, FL.

Walworth, J. L. and M. E. Summer. 1988. Foliar analysis: A review. p. 193-232. In B. Tinker and A. Läuchli (ed.) Advances in Plant Nutrition. Vol. 3. Praeger Publishers, New York, NY.

Wiersma, D. and B. R. Christie. 1987. Water and agricultural productivity. p. 3-56. In B. R. Christie (ed.) CRC Handbook of Plant Science in Agriculture. Vol. 2. CRC Press, Boca Raton, FL.

Yemm, E. W. and A. J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. The Biochemical Journal 157:508-514.