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NUTRIENT COMPOSITION OF AMARANTHUS SPECIES AS AFFECTED
BY NITROGEN FERTILIZATION AND HARVEST DATE

A Thesis
Presented for the
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ABSTRACT

A study was conducted to evaluate the proximate composition, carbohydrate, mineral and nitrate content of selected Amaranthus accessions from Latin America, Africa and Asia. The effects of varying levels of nitrogenous fertilizer, accession, and harvest date on these nutrients was assessed and compared. Three rates (0, 150 and 300 kg/ha) of ammonium nitrate were applied to an Etowah silt loam in 1984. Whole leaf tissue samples from six accessions were obtained during the growing season at 25, 35, 45, 55 and 65 days after germination. Grain samples from four accessions were obtained at the end of the growing season. The results from laboratory chemical analyses for proximate composition, carbohydrate, mineral and water soluble NO_3^- concentration were evaluated. Differences in plant growth habit in vegetable vs. grain amaranths accounted for the divergence observed in crude protein response among accessions. Prolonging the vegetative state in vegetable amaranths would likely result in levels of leaf crude protein, and other nutrients, that fluctuate more during the growing season than in the grain types.

While amaranths possess several unique nutritional attributes, early harvesting of tender stems and leaves must be done for maximum nutritional and culinary utility. Early leaf harvests typically had high crude protein and K concentrations, and relatively low fiber content. Water soluble NO_3^- concentration was highest during early growth stages, and declined rapidly thereafter. Overall, levels of leaf NO_3^-

within two accessions did not exceed 239 mmol/kg dry weight for any of the NH_4NO_3 or harvest date treatments.

The proximate and carbohydrate composition of four grain amaranth accessions was not significantly affected by the application of NH_4NO_3 . Grain calcium concentration declined in three accessions as a result of the NH_4NO_3 treatments. Significant differences among accessions in grain composition were observed. Split applications of fertilizer, as practiced in other agronomic crops, may prove more satisfactory as a means of enhancing yields and/or nutritional composition of amaranth grain.

PREFACE

Since the advent of sedentary agriculture, man has exploited some 3,000 plants for food. Of these species, relatively few are utilized worldwide in organized agriculture. At present, a major portion of mankind's dietary needs are met by a mere 20 crops. Clearly, a need for diversifying food supplies exists.

Unfortunately, the trend in agriculture through time has been to concentrate on fewer and fewer staples while increasing genetic uniformity within these crops. The paradigm of crop monoculture brings with it many dangers, particularly its vulnerability to catastrophic failure during times of adverse climatic, economic and unforeseen epidemic conditions. Furthermore, concern about world population growth, acute food shortages and diminishing supplies of nonrenewable natural resources has prompted researchers to question the long term sustainability of agricultural systems founded on this approach.

It is imperative, therefore, that efficient and biologically sound methods for expanding world food supplies be sought. The direct exploitation of plants for food, coupled with an efficient, balanced use of our soil, water and plant genetic resources will play a strategic role in achieving this objective.

The potential contribution of little-known, underexploited plants to expanding the base of world agricultural productivity is excellent. The apparent advantages of staple crops over minor tropical species, such as the amaranth, have often resulted from a disproportionate

allocation of research priority given to them. Indigenous plants often possess equal merit but have been eclipsed by the historical concentration of agricultural research on crops important to the economies of the industrialized nations. For example, the sparse, flinty corn used by native Americans several centuries ago bore little resemblance to modern hybrids. It took scientists several generations of skilled, painstaking work to unlock the dormant wealth of corn's genetic potential. Today it is a major world agricultural commodity, with an annual production of around 300 million metric tons. In this context indigenous plants, particularly tropical species, represent a rich, untapped source of agricultural productivity.

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CHAPTER 1

INTRODUCTION

The genus Amaranthus (family Amaranthaceae) contains about sixty-five species of protein-rich, annual, dicotyledonous herbs of the tropics, subtropics and warm temperate zones. Of these species, genotypes with desirable agronomic traits are utilized as vegetable and/or grain resources, mainly in the underdeveloped countries. There it provides an inexpensive, readily obtainable source of dietary protein for many people. Recent monographs have alluded to the amaranth's popularity as a leafy green vegetable and grain resource in Latin America, Africa and Asia (51, 73, 92, 108, 113, 155). In North America, interest in assessing the vegetable amaranth as a heat tolerant greens crop has increased (19, 89, 90).

Amaranthus possess several attributes that make them important with regard to good nutrition and biologically sound agriculture. Unlike most broad-leaved crops, amaranths possess a C-4 dicarboxylic acid metabolism. Carbon-4 plants have an especially efficient photosynthetic process for reducing and assimilating atmospheric carbon (34, 56). Amaranths, and C-4 plants in general, are also noted for being efficient users of water (66, 67, 102). This unusual type of carbon metabolism enables the amaranth to flourish under sunny, hot and semiarid conditions where other leafy green vegetable and grain crops do not thrive.

Once established, C-4 plants are capable of CO₂ fixation under conditions that can limit and/or inactivate net carbon assimilation in C-3 plants. These qualities point to the amaranth's probable capacity for efficient land and water use, attributes which are essential for a modern crop to effectively utilize diminishing supplies of these resources found in many areas worldwide. Interest has therefore increased in assessing the suitability of the amaranth for development as a versatile, protein-rich food and feed resource.

The National Academy of Sciences, in its monograph Underexploited Plants of Promising Economic Value (100), identified thirty-six plants not used at present in organized agriculture that could be converted to cultivation and further exploited for food, feed and other industrial purposes. Of these thirty-six plants, the amaranth was selected as being of special merit and worthy of research priority.

Later, in a workshop sponsored by the National Academy of Sciences in 1981, a proposal was drafted identifying avenues along which future research on the amaranth should proceed. In the area of agronomy, research on soil fertility and fertilizer needs and the effects of these factors on levels of nutrients and toxic factors in amaranth was strongly recommended.

No previous research has been reported on the amaranth in Tennessee. Warm temperatures, abundant rainfall and sunshine during summer prompted this researcher to feel that conditions in the Tennessee Valley would be ideal for the amaranth. A study was proposed to evaluate the proximate composition, carbohydrate, mineral and nitrate content of

selected Amaranthus accessions from Latin America, Africa and Asia. The effects from varying rates of nitrogenous fertilizer and harvest date on these components were assessed and compared.

CHAPTER 2

REVIEW OF LITERATURE

Botany, Origin, and Distribution of the Amaranth

The genus Amaranthus is endemic to tropical, subtropical and warm temperate regions worldwide. The botany, biosystematics, origin and distribution of the amaranth have been reviewed in work by several authors (57, 110, 131, 134). Generally, Amaranthus species are annual dicotyledonous herbs with alternate, simple and petiolate leaves. The stems are intermediate to large (0.5 to 3.5 m) and may be branched or unbranched, prostrate to erect and succulent (137). The plants, which are often pigmented with brilliant hues of yellow, coral or magenta, have evoked comment from many observers. The five-merous unisexual flowers are monoecious and possess circumscissile utricles. Male flowers consist of several tepals and bracts with three to five stamens. Female flowers consist of bracts, tepals and a spherical ovary containing a single ovule (116, 143). The flowers are crowded into dense terminal or axial diachasial cymes. Grain amaranths,¹ unlike the vegetable types, possess highly developed terminal or axial branched panicles. Amaranth

¹Grain amaranths belong to a minor class of grain-like plants called psuedocereals. Others in this class include buckwheat and quinoa. Unlike corn, sorghum and other grasses, the fruit of psuedocereals, strictly speaking, is not a caryopsis. Normally, the term "grain" is limited to crops in the family Poaceae. Because the term is so entrenched in the amaranth literature, it will be retained herein.

seeds are small, typically 1 mm in diameter and 3 mm in width. Seed shape is lenticular and biconvex. Amaranths are capable of producing over 500 g of seed per plant, a testimony to their enormous fecundity (134). A detailed description of amaranth seed morphology and ultrastructure has been provided by Irving et al. (68, 69).

Originally confined to open, disturbed habitats before man intervened, many amaranths have been widely dispersed outside their native centers. This dispersal may have been partly fortuitous, for at least some of the wild amaranths (pigweeds) are tough, resilient weeds that have followed the footsteps of mankind in his circumnavigations of the globe. Other species were intentionally carried abroad by early explorers and traders for food, trade and medicinal purposes. Today, cultivated and wild species are found in Asia, Africa, Europe, Australia and the Americas (131, 137).

In Southern China and South-East Asia, cultivars of vegetable amaranths, particularly those of Amaranthus tricolor, are highly esteemed as vegetables. These vegetable amaranths have attained considerable horticultural renown (60, 91).

The grain amaranths have ancient historical and cultural origins in the New World. In pre-Columbian America, three species of grain amaranth were domesticated: Amaranthus caudatus, A. cruentus and A. hypochondriacus. Their ethnobotany, origin, distribution and importance to pre-Columbian civilizations have received considerable attention (2, 3, 13, 23, 88, 130, 132, 144). Generally, these species are now cultivated over a wide geographic range, extending from Argentina through the

Andes, from Guatemala through Mexico and the southwestern United States, from Iran and Sri Lanka through India and the Himalayas, and through inner China to Mongolia and East and West Africa (131).

Shortly after the conquest of Mexico by Hernan Cortez in 1519, Amaranthus hypochondriacus, the principal grain species, appeared in various parts of the Old World. Probably brought there by the Spanish as an ornamental, this species underwent a remarkable transcontinental diaspora. Amaranthus hypochondriacus had been domesticated in India and Sri Lanka by the eighteenth century, and it quickly established itself in the local agriculture of these countries. During the nineteenth century the species spread rapidly throughout the Himalayas, and across the Deccan Plain. Today, the amaranth's importance as a grain resource is increasing in these areas (73, 131, 143). But in Latin America, its former élan has been tarnished by the growing popularity of corn. Grain amaranths are still cultivated there, but mainly as doorstep relics in the cool temperate valleys in Argentina, Bolivia, Ecuador and Peru, and in the tropical highlands of Mexico and Guatemala (131).

Physiological Attributes of Carbon Metabolism in the Amaranth

Plant productivity is defined as the total dry matter yield accumulated per unit area (58). Many factors are known to govern the rate, efficiency and quality of dry matter accumulation in crops. Radiant flux, temperature and ambient CO₂ concentration are important, but essentially uncontrollable environmental determinants of crop productivity. Other factors such as the supply of water and inorganic elements,

pests, diseases, etc., may often be controlled by the grower. Much emphasis has therefore been placed by physiologists on identifying suitable crop prototypes that could compete more effectively for limited and/or uncertain supplies of essential growth factors. To accomplish this, it was necessary to take into account the photosynthetic and carbon assimilating characteristics of plants. For it is through this "machinery" that dry matter yield, and hence productivity, is achieved in any environmental setting.

Plants, broadly speaking, are classified as C-3 or C-4 plants depending on their mode of carbon metabolism. The primary carboxylase enzyme of all C-3 plants is ribulose 1,5 bisphosphate (RUBP) carboxylase. This enzyme, along with others of the reductive pentose phosphate pathway, constitutes the sole means of net carbon assimilation in photosynthesis. Most agricultural crops belong to the category of C-3 plants. It has become clear, however, that some plants, for example Amaranthus, possess an additional mechanism for CO₂ uptake. In these plants, the first stable products of CO₂ assimilation are the C-4 dicarboxylic acids, malate and aspartate (56). These plants are therefore classified as C-4 plants as compared to C-3 plants in which the primary product of CO₂ fixation is 3-phosphoglycerate. Numerous detailed monographs on the anatomical, biochemical and ecological features of C-3 and C-4 plants have been published (30, 35, 43, 44, 45, 83).

Two main physiological differences exist between C-3 and C-4 plants. First, plants with a C-4 metabolism possess an additional carboxylating cycle. The "C-4 cycle" serves to transfer CO₂ from the atmosphere to the site of RUBP carboxylase in the bundle sheath cells

(95). The primary carboxylase enzyme of C-4 plants is phosphoenolpyruvate (PEP) carboxylase. Because of the high activity and avid binding of CO_2 by PEP carboxylase, CO_2 uptake in C-4 plants is CO_2 saturated at a relatively low CO_2 concentration in the leaf mesophyll (102). Thus, C-4 plants are capable of net carbon assimilation under conditions that tend to restrict gaseous diffusion between the atmosphere and the mesophyll cell surface. Such conditions are commonly encountered in dry, arid settings. Second, glycolate metabolism in photorespiration is very low in C-4 plants. Carbon-3 plants lack PEP carboxylase, but glycolate metabolism, resulting from the reaction of O_2 with RUBP carboxylase, normally occurs in C-3 and is absent in C-4 plants. Photorespiration is enhanced by several factors such as high mesophyll O_2 concentration, temperatures and radiant flux (95). This wasteful process is capable of dissipating 20-60% of the total carbon assimilated in C-3 plants (102).

The amaranth is a rare example of a dicotyledonous plant possessing a C-4 dicarboxylic acid metabolism. Like other tropical C-4 monocots such as sugar cane, sorghum and burmuda grass, amaranths may attain enormous growth rates in hot, sunny environments. Yet amaranths differ from these grasses because aspartate, rather than malate, is the primary product of net carbon assimilation. The amaranth is therefore classified as a NAD-malic enzyme type C-4 plant. Aspartate is an important precursor in the biosynthesis of several amino acids, for example lysine. Thus, CO_2 metabolism in NAD-malic enzyme species is linked directly to amino acid metabolism (95).

The Amaranth as a Food Resource: Its Chemical Composition
and Nutritive Value

Introduction

In agriculture, the biochemical analysis of plant material to assess its palatability, digestibility, or nutritive value is increasingly used to evaluate new crop varieties or changed methods of husbandry. However, caution has been advised in the evaluation and interpretation of data characterizing plant chemical composition (32, 134). This is particularly evident for plants like the amaranth that express much environmentally induced variability. Data characterizing the chemical composition of amaranth during periods of active growth may vary significantly in response to factors such as climate, plant nutrition, agronomic practices, cultivar, soils and age of plant parts at harvest (134). This is especially true of leaves, which often contain a high proportion of metabolically labile substances. For example, nitrogen in plant tissues may undergo diverse transformations during periods of active vegetative growth. The labile nature of leaf nitrogen makes the evaluation of protein concentration based on total amino-nitrogen estimates problematic. The extent to which the nitrogen complex is distributed in more diverse forms (e.g., nonprotein nitrogen) in contrast to the metabolically quiescent storage protein-N of seeds, is difficult to assess.

Deshpande and Rao (27), using a 1% alkali solution, extracted 48% of the total leaf nitrogen of Amaranthus gangeticus in the protein fraction. About two-thirds of this was nonprotein nitrogen, mostly

amides and peptones. Although a large fraction (52%) of the total leaf nitrogen was unextracted, this study underscores the vexing problems encountered in quantitating precise estimates of leaf protein, and plant metabolites in general, during periods of active crop growth. Emphasis must therefore be placed on the representative, rather than precise, nature of data describing the chemical composition of Amaranthus species.

Leaf Composition

The first recorded vegetable analysis in amaranth was done by Boutin (14). The plant's commercial importance was recognized ostensibly because of its ability to "cut grease" on home cooking utensils. Although the acidic nature of the plant was not recognized until much later, preliminary studies by Boutin (15) found the plants containing large amounts of the neutral salt, KNO_3 . This led to Brosset and Boutin's suggested use of the plant as a fertilizer (16). Later, in what was perhaps the first attempt at experimentation with amaranth, Woo (163) found its leaves and stems rich in nitrates when grown on heavily manured soil.

Watt and Merrill (157) compared the proximate, mineral and vitamin composition of vegetable amaranths with other common leafy green vegetables. Their data were based on analyses of at least eighteen different sets of samples from various species. By this method, a "world average" for these nutrients was computed. Watt and Merrill's data show that amaranths are good dry matter producers. One hundred grams of raw vegetable leaves contained an average of 13.1 g dry matter, which is

somewhat higher than that in spinach and other leafy green vegetables. Protein and fiber were the major components of the dry matter.

Amaranth leaves also contain useful quantities of vitamins and minerals in relation to dietary allowances (36, 40, 106, 160). Specifically, amaranth greens are exceptionally rich in provitamin A, calcium, potassium and iron. Numerous studies (74, 82, 99, 112, 120) have demonstrated the efficacy of amaranth, and dark leafy green vegetables in general, in mitigating the symptoms of human undernourishment. As in most vegetable matter, carbohydrates, fat and B-complex vitamins are in low supply relative to dietary allowances. The protein fraction represents about 27% of the leaf dry matter. This is well reflected by reports elsewhere in the literature (28, 40, 61, 64, 65, 146). Stems reportedly contain half as much protein as the leaves (28, 61).

Amaranth leaves have been utilized as a material in the preparation of leaf protein concentrates (LPC). Leaf protein concentrates are one of several possible new protein products considered for large scale exploitation in developing countries (98, 108). The agronomy, preparation and use of this product is described in some detail by Pirie (122). Exploitation of plant material with large amounts of fiber or nonnutritive water soluble substances is possible, yielding a nutritionally valuable supplement for protein deficient foods.

Amaranth LPC has been analyzed by several researchers (61, 107, 125). Hill and Rawate (61) found that the concentrates recovered 16-36% of the original leaf protein and contained 71-80% protein by mass. Although these researchers pointed to the theoretically high nutritive

value of amaranth LPC, data from live animal feeding studies has been inconclusive (22).

The amino acid composition of amaranth LPC has also been determined (61, 107). These leaf protein concentrates contained biologically useful quantities of the essential amino acid lysine in relation to the FAO/WHO provisional amino acid scoring pattern (41). Threonine invariably was the first limiting amino acid (61). Hegsted et al. (59) suggested that a 100 g portion of fresh leafy vegetables supplies 2-3 g of protein. If this protein, he stated, contained an adequate supply of lysine, it could effect considerable improvement in the biological value of lysine-poor cereal proteins. Thus, amaranth greens or LPC may be useful in supplying lysine deficient foods to enhance their biological utility.

Amaranth species are known to be nitrate accumulators (125, 128, 163). While various environmental gradients can influence the accumulation of nitrates in plant tissues, heavy applications of nitrogenous fertilizers may aggravate this problem (17, 39, 142). Lee et al. (84) found that vegetables grown with added nitrogenous fertilizers contained 3-13 x the amount of nitrate contained in those grown without fertilizer. Although vegetable nitrates alone are nontoxic to mammals, they may be reduced to nitrites (NO_2^-) during postharvest handling or in the mammalian intestinal tract through microbiological activity (121, 162). Nitrite, when absorbed into the bloodstream, binds with haemoglobin to form an oxygen insensitive analog, methhaemoglobin. The intake of leafy green vegetables high in nitrates has been known to incite

methemoglobinemia, or "blue baby" syndrome, after reaction of the nitrate to nitrite (94). The knowledge that dietary nitrates could be converted to mutagenic nitrosamines in the mammalian intestinal tract has prompted added concern over plant nitrate levels.

Weedy amaranths, specifically Amaranthus. spinosis and its relatives, have been noted to accumulate unusual amounts of nitrates (104). Nitrate levels in excess of 0.35% (dry wt basis) in plant material are deemed undesirable for livestock (103). At present, a lethal dose of nitrates for adult humans appears to be in the order of 15-17 mg of nitrate-N per kilogram body weight (18, 84). The usual human dietary intake of nitrate is about 100 mg per day (158). A majority of this nitrate is believed to be of vegetable origin.

Der Marderosian et al. (26) measured nitrate levels in vegetable amaranths over two growing seasons. Levels of nitrates in spinach and chard were also determined for comparison. These researchers found nitrate levels in vegetable amaranths which were comparable to those found in chard and spinach. Nitrate levels in stems were 4-5 x the amount contained in the leaves. They concluded that nitrate levels in amaranth greens do not significantly detract from their nutritional attributes. Researchers in the midsoutheastern United States have also confirmed these results (89, 90).

Grain Composition

The proximate composition of grain from several amaranth species has been summarized by Saunders and Becker (134). These authors also

list average values for these components as compared to the typical composition of conventional grains. Amaranth grain contains a mean of 16% protein (dry wt basis). This figure is somewhat higher than that found in other common cereal species. Senft (139) reported a mean protein concentration of 14.9% ($N \times 5.85$) for four African Amaranthus cruentus samples, 14.8% for five Mexican A. cruentus samples, and 15.3% for four A. hypochondriacus samples.

Generally, fat, fiber and ash concentrations were higher in amaranth, whereas total carbohydrates were lower than in other common grain species. The amylose concentration of amaranth starch is low (9). Seed lipids are high in polyunsaturated fatty acids (7, 9, 115). The essential fatty acid linoleic acid comprises about 50% of this fraction (9, 24, 115).

The amino acid composition of amaranth grain has received considerable attention (9, 10, 24, 31, 80). Amaranth grain is exceptionally rich in lysine in relation to the FAO/WHO provisional amino acid scoring pattern (41). On a mass basis (g lysine/100 g protein) amaranth grain contains considerably more lysine than opaque-2 and floury-2 strains of corn, both of which are high lysine strains (31). Results presented by Abdi and Sahib (1) indicate that amaranth grain lysine concentration is comparable to levels that are found in grain legumes. The grain also contains biologically useful amounts of the sulphur containing amino acids, methionine and cysteine. Leucine is the first limiting amino acid in grain amaranth protein (139).

As most gramineous seed proteins are lysine deficient, but are adequately supplied with leucine, it has been suggested that amaranth grain be used in combination with lysine deficient cereal grains to enhance the biological value of the protein. A 50/50 mixture of corn and amaranth grain has an essential amino acid profile remarkably close to the FAO/WHO provisional scoring pattern (139).

Effects of Fertilization and Harvest Date on the Yield,
Composition, and Nutritive Value
of Vegetable Amaranth

Introduction

Stated in its most elemental terms, agriculture depends on successful crop growth. Yet different criteria are used to measure growth and productivity in crops. While crop yield is often a sufficient quantity standard, it may not provide information about a second important factor in the plant productivity equation: biological utility. Clearly, certain plants are capable of producing enormous amounts of dry matter of low biological usefulness. Unfortunately, the quality of plant produce is less easily defined when expressed as yield. The supply of essential mineral elements to plants during periods of active growth can considerably influence the composition of plant dry matter. The magnitude of these effects depends on the interaction of particular nutrients in physiochemical processes leading to the synthesis of biologically useful plant products. Unquestionably, the current approach to food production is shifting increasingly toward a direct exploitation of plants for human consumption. Developments of this nature will therefore demand

greater understanding of the factors controlling yield, composition and nutritional quality of crop species.

Effects of Nitrogen Fertilization on the
Yield, Composition, and Nutritive Value
of Vegetable Amaranth

The results from several studies have shown that amaranths are efficient nitrogen accumulators (146, 156, 159). These results are not surprising, for nitrogen reduction and assimilation in plants is an energy dependent process, consuming reducing equivalents (H), adenosine triphosphate (ATP) and carbon skeletons derived from anabolic photosynthetic processes. As a C-4 plant, amaranth's photosynthetic capacity is high (37, 67, 86, 105, 119). This attribute, coupled with a lack of photorespiration-induced glycine metabolism, points to the amaranths probable efficiency in the utilization of absorbed N under field conditions. Until recently, research on the effects of nitrogen fertilization in the species has been centered mainly in Asia and Africa (123, 124, 135, 146, 147, 148, 149). In the United States, fertilizer recommendations for amaranths are not readily obtainable. The reliance of extension personnel on recommendations used for spinach and other greens is a common default.

Studies designed to assess the combined effects of nitrogen fertilization and harvest date in leafy green vegetables are desirable, for the labile nature of biochemical constituents in actively growing plants may change or undergo interconversion during a relatively short period of time. Thus, assuming a knowledge of the functional response in a

crop species to varying inputs of these factors, yield and biological utility of plant produce may be optimized through judicious fertilization and timing of harvests. It is also desirable to understand how a crop species behaves, physiologically speaking, when each of two or more treatment factors are considered alone.

Several such studies have been conducted on the vegetable amaranth. In Nigeria, Olufolaji and Tayo (114) evaluated changes in the concentration of the proximate and mineral composition in three cultivars of Amaranthus cruentus at different harvest dates. Plants were grown under controlled greenhouse conditions, without added inorganic nitrogenous fertilizers. Predictably, these researchers found that the concentration of most major and minor elements in the edible plant parts (leaves and succulent stems) declined with age. A notably precipitous decline in nitrogen concentration was observed. Plants harvested five to seventeen weeks after sowing showed no increases with time in nitrogen concentration. Five week old plants showed significant differences in nitrogen concentration among cultivars. Similarly, leaf phosphorus concentration declined with age, but less precipitously than nitrogen. Nine weeks after sowing, phosphorus concentration attained a constant value that changed little during the rest of the growth period. Significant differences in phosphorus concentration among cultivars was observed in nine- and eleven-week-old plants. Conversely, the potassium concentration within cultivars increased from five to seven weeks after sowing. Thereafter, a steady decline in potassium was observed during

the rest of the growth period. Significant differences in potassium concentration among cultivars were also observed.

Studies conducted in West Africa by other researchers, using field grown amaranth plants, have shown similar results (51, 52, 149). For example, Grubben's analysis of four week old plants of Amaranthus cruentus cv. Fotete showed edible parts containing 3.1% nitrogen, 0.61% phosphorus, and 2.03% potassium. Twelve week old plants contained 2.7% nitrogen, 0.39% phosphorus and 4.3% potassium (51, 52).

Several researchers have investigated variations in proximate composition of vegetable amaranths as influenced by seasonal variation and harvest date (8, 29). Bassir and Fanfunso (8), using unfertilized plants, found significant differences in crude protein, fiber, lipid and ash concentration among harvest dates. Seasonal variations (wet season vs. wet-dry transitional) did not exert significant effects on any of the nutrients.¹ Six to eight weeks after sowing, crude protein levels in the growing plants reached a maximum of 32% (dry wt basis). Crude fiber increased during the first eight weeks of growth but remained constant thereafter. Ash concentration increased continuously with age of the plants.

Several workers in Africa conducted studies to evaluate the combined effects of nitrogen fertilization and harvest date on protein

¹It should be noted that amaranths, due to their C-4 metabolism, might respond quite differently to seasonal temperature gradients, as are common in higher latitudes, than moisture gradients, to which they are physiologically better adapted.

and mineral composition in vegetable amaranths. Stafford et al. (146), working with Amaranthus hybridus cv. Green Head, found nitrogen concentration of the edible parts increased significantly in response to nitrogen fertilization. Additions of nitrogen above 75 kg/ha had no significant effect on plant nitrogen concentration. With unfertilized plants, increasing the time before harvest from 45 to 63 days after planting had little effect on crude protein concentration. Most significantly, a large difference in the crude protein concentration between these dates was observed in fertilized plants. At 63 days postplanting, leaves from plants receiving 0 kg/ha nitrogen had 12.3 g protein 100 g dry matter. Amaranths from plots receiving 150 kg/ha and 225 kg/ha nitrogen had 22.9 g and 24.4 g protein 100 g dry matter, respectively. Averaged over a harvest period of 63 days postplanting, crude protein concentration of the edible parts was 14.5 g and 28.1 g 100 g dry matter on the 0 kg/ha and 150 kg/ha nitrogen plots, respectively. This represents a two-fold overall increase in protein concentration.

Reports in literature regarding the effects of nitrogen fertilization on the uptake of mineral ions are quite variable. Some researchers feel that nitrogen fertilization enhances the dilution of mineral elements of plant tissues (5, 141). Additionally, under some conditions nitrogen fertilization is known to interact with potassium and phosphorus nutrition in crops (81, 140, 161). Taylor et al. (149) found that nitrogen rates of 200 kg/ha had no significant effects on potassium uptake in Amaranthus caudatus as compared to the smaller nitrogen increments. Contradicting this view is Schmidt (135), who observed an

increase in K concentration in A. cruentus grown on nitrogen enriched soils. Taylor et al. (149) noted that increasing levels of nitrogen to 100 kg/ha enhanced phosphorus concentration in amaranth over the 0 kg/ha nitrogen control. In both cases, phosphorus and potassium concentration declined with age of the plant. Nitrogen rates had no significant effects on accumulation of calcium, sodium, copper and zinc. In contrast, iron and magnesium uptake was depressed, while manganese and chloride concentration was increased over the range of nitrogen applications. Except for copper and chloride, all elements in A. cruentus increased with age of the plant.

Studies designed to evaluate the yield and nutritional attributes of vegetable amaranths resulting from nitrogen fertilization have been conducted (124, 135, 148). Using Amaranthus gangeticus (syn. A. tri-color), Ramachandra (124) found that increasing nitrogen and phosphorus fertilization enhanced levels of those nutrients in the plants. Ramachandra noted that nitrogen fertilization had beneficial effects on height, dry weight, yield and other physiological growth parameters important to plant productivity. Sundararajan et al. (148) noted significant increases in yield per unit area with A. flavus in response to added nitrogen, phosphorus and potassium.

The application of nitrogenous fertilizers usually results in an increase in the proportion of protoplasm to cell wall constituents (126). The net result is that synthesized carbohydrates are more rapidly converted to proteins and protoplasm, while an overall decrease in

the proportion of indigestible, fibrous matter in plant material occurs. Ramachandra (124) noted that levels of crude fiber in Amaranthus gangeticus varied inversely as a function of nitrogen supply. However, Schmidt (135) observed no significant differences in leaf fiber concentration resulting from N fertilization in A. cruentus. At present, insufficient data is available for making general conclusions about the fiber-nitrogen fertilization interaction in vegetable amaranths.

Up to this point, no assumptions have been made about the interdependence between yields of vegetable amaranths and the biological value of its nutrient components. As noted previously, crop yield cannot always be regarded as a quality standard. Factors such as biological value, palatability and consumer appeal must be evaluated and their merits judged separately. It is desirable, therefore, to ascertain what kind of relationship, if any, exists between these various productivity standards. If the correlation is significant for a nutrient of interest, and a clearly beneficial effect from nitrogen fertilization in the species is assumed, then breeding of species for overall optimum response to adjusted levels of fertilizer inputs can be achieved.

This approach to the yield-utility question was pursued by Mathai et al. (93). These researchers studied the correlation between yield and nutritional attributes in vegetable amaranths. They found a significant correlation between total plant weight in 17 cultivars of Amaranthus caudatus and percent crude protein concentration. However, in A. tricolor, this correlation was not significant. This latter observation concurs with those reported by Makus and Davis (90). These researchers

found that stem tissues of eight accessions of A. tricolor consisted as much as 47% of the amaranth plant as compared to only 10% with spinach. As protein concentration in amaranth stem tissue is low, and high in fiber and nitrates, selection of species for high leaf/stem ratios has been recommended as an important breeding criterion (89). However, in terms of yield response, Mohideen and Muthukrishnan (96) found that leaf/stem ratios were not significantly correlated with yield components in A. tricolor.

Results of pot culture studies conducted by Purushothaman (123) to assess the effects of nitrogen sources and levels on yields on Malaysian amaranths have been reported in literature. Six nitrogen sources were evaluated along with five levels of each respective carrier. The highest plant productivities were obtained with calcium nitrate and sodium nitrate. Fresh and dry weight yields increased to 120 kg/ha nitrogen for all sources except calcium nitrate. In the latter case, a substantial, if not remarkable, increase in yield was obtained at 180 kg/ha. The high uptake of mono- and divalent cations by amaranths could partly explain the unusual effects exerted by calcium nitrate in the species.

The implication that nitrogen fertilization may affect the synthesis and/or accumulation of toxic factors in plant foods has prompted concern from nutritionists and agronomists alike. Briefly, a toxic factor can be defined as any substance occurring in animal foodstuffs which has an undesirable effect on animal growth, metabolism and overall health (85). Two examples of naturally occurring toxic factors in plant foods include nitrate (NO_3^-) and the organic anion oxalic acid. As noted

in the previous chapter, vegetables grown with added nitrogenous fertilizers contained 3-13 x the amount of nitrates contained in those grown without fertilizer (84). Such findings point to a probable difficulty in developing feasible fertilizer practices which enhance growth, yield and general consumer appeal of amaranth without somewhat increasing nitrate concentration levels in the species.

In an experiment conducted over a two year period, Der Marderosian et al. (26) measured nitrate levels in vegetable amaranths grown at two nitrogen fertility levels. Analysis of spinach and chard was also done for comparison. Generally, chard and amaranth contained levels of nitrates which showed relatively small variations during the growing season at both fertility levels. Nitrate levels in unfertilized samples were two to three times below those in samples from fertilized plots. Results from work of Schmidt et al. (136) in Nigeria on Amaranthus cruentus agree with these observations.

Amaranth Grain Yield, Composition, and Nutritive Value as Influenced by Nitrogen Fertilization

Few data are available to assess the effects of nitrogen fertilization on yield and quality attributes of amaranth grain. Although several reviews (9, 134, 150) have appeared dealing with amaranth grain on a molecular, physicochemical level, reports on the effects of agronomic treatments in the species are nearly absent.

Preliminary observations by Kauffman (78) indicate that lodging is a problem in most grain amaranth species. Such findings suggest that nitrogen fertilization is likely to limit, rather than increase yields

in grain amaranths, unless lodging resistant cultivars can be developed. Yield evaluations on numerous accessions of grain amaranths have been reported (12, 20, 76, 77).

Some proponents have claimed that amaranth grain protein concentration is increased with increasing nitrogen fertilization (110). Yet there is little de facto evidence in literature to support this notion. Becker et al. (9) have presented data which in fact contradict this view. These researchers found no significant differences in the protein, crude fiber, fat and ash concentration of grain samples from plots receiving 0 kg/ha and 150 kg/ha nitrogen. Levels of calcium, potassium and iron in the grain were not affected by the agronomic treatments. Magnesium concentration was somewhat depressed, however.

Reported results regarding the effects of population density, nitrogen fertilization and amaranth grain yields are still quite variable. While increasing population densities generally enhanced grain yields, studies directed toward assessing the role played by nitrogen fertilization on enhanced yields in grain amaranths have been inconclusive (4, 33, 55).

CHAPTER 3

MATERIALS AND METHODS

General Information

In 1984, amaranth germplasm was obtained from three different sources: the Rodale Research Center (RRC), Kutztown, PA, the North Central Regional Plant Introduction Station (NCRPIS), Ames, IA and the Redwood City Seed Company (RCSC), Redwood City, CA. Eight accessions were selected for use in this study. The selections were made to maximize diversity of genetic and geographic origin among accessions. Six of the eight accessions were evaluated as "vegetable" amaranths: Amaranthus tricolor (RRC #241), A. dubius (NCRPIS #2014), A. paniculatus (RCSC), A. blitum cv. Horsetooth (RRC #313), A. cruentus cv. Fotete (NCRPIS #1964) and A. cruentus (RRC #1011). The others were "grain" amaranths: A. cruentus (RRC #1011), A. hybridus (RRC #1044) and A. hypochondriacus (RRC #1023). Amaranthus cruentus (RRC #1011 and NCRPIS #1964) had their vegetable and grain composition analyzed simultaneously. All accessions were grown at the Plant Science Field Laboratory, Knoxville, in 1984.

The experimental design used was a randomized complete block with a 3 x 6 x 5 split-split plot factorial arrangement of treatments. The experimental area consisted of twelve blocks and four replications. The treatments consisted of a main plot factor (fertilizer N rate), subplot factor (accessions), and a sub-subplot factor (harvest date). A total of ninety treatment combinations was analyzed (Table 1). Analysis of

Table 1. Factors and levels of each factor comprising the 3 x 6 x 5 split-split plot factorial arrangement of treatments in the vegetable analysis.

Plot factor	Level
Rate of NH_4NO_3 (main)	0 kg/ha (0 kg/ha N) 150 kg/ha (50 kg/ha N) 300 kg/ha (100 kg/ha N)
Amaranth Accession (sub)	6 Accessions
Harvest Date [sub (sub)]	25, 35, 45, 55 and 65 days after germination

grain amaranth accessions was performed similarly, using one sampling date in October 1984 (Table 2). A total of twelve treatment combinations was considered in the grain analysis. All treatments were randomized within a block, with each block and factor receiving a separate randomization. Randomizations were formulated using the Plan Procedure in the Statistical Analysis System (SAS).

Two grain amaranth accessions, Amaranthus hypochondriacus and A. hybridus, were not included in the vegetable analysis. These plants were allowed to mature without any leaf sampling. Plots of A. cruentus NCRPIS #1964 and RRC #1011 contained plants from which leaf and grain samples were collected simultaneously. However, plants with leaf sampling were logged, and only unlogged plants were used for grain sampling.

The experimental area measured 22.6 m x 40.2 m. Individual blocks measured 4.3 m x 12.2 m. A 1.8 m alley separated each block on all sides. Plots containing the amaranth accessions measured 1.5 m x 4.3 m. Within and between-row spacing was 30 cm for all accessions. Two rows in each plot were reserved for sampling. Harvest was scheduled at ten day intervals beginning 25 days after germination. Five sets of four plants each were logged as sample plants. These four plant units comprised the sub-sub plot. Harvests were randomized so that a different set of four plants were sampled at each of the five harvest dates.

Table 2. Factors and levels of each factor comprising the 4 x 3 split plot factorial arrangement of treatments in the grain analysis.

Plot factor	Level
NH_4NO_3 Rate (main)	0 kg/ha (0 kg/ha N) 150 kg/ha (50 kg/ha N) 300 kg/ha (100 kg/ha N)
Amaranth Accession (sub)	4 Accessions with one sampling date in October 1984

Production Methods

Amaranth transplants were grown in the greenhouse in 2.54 cm commercial Speedling[®] flats. Flats were seeded on June 6. In three days, virtually all seeds had germinated. Plants were allowed to grow in the greenhouse and were transferred to the field on June 19. The experimental area, lying on an Etowah silt loam of medium fertility, was ploughed and disced; fertilizer was thoroughly incorporated by discing six days prior to planting. In addition to the NH_4NO_3 treatments, a basal application of 60 kg/ha each of P and K was applied. Irrigation was provided immediately after transplanting and at intervals during the growing season to supplement natural precipitation. As no pest control recommendations for this crop have been established, spraying with insecticides (carbaryl) and fungicides (etridiazole+thiophanate-methyl) was done as needed. A preplant incorporation of chlorpyrifos at 4.4 kg active ingredient/ha was applied for cutworm control. Weed control was achieved through hoeing.

Sampling Methods

Whole leaf samples were collected at 5 ten day intervals. The first samples were taken 25 days after germination, a date arbitrarily chosen when plants were judged to be large enough to yield a sufficient quantity of plant material for the analyses. Six mature, expanded leaves (excluding petiole) were selected at random from each of four plants. Only leaves from the upper portion of the main stem (when there was an identifiable "main" stem) were sampled. This procedure was

modified for Amaranthus blitum cv. Horsetooth, an accession with very small leaves. In this case, 20 leaves were sampled at random from each of four plants.

All samples were bagged, rinsed and dried in a forced-air oven (70 C) for 24 h. The dry material was ground in a Wiley Mill to pass a 20 mesh screen. Ground tissue was placed in air-tight polyvinyl bags and stored in the laboratory until the analyses were run. Grain samples from Amaranthus hypochondriacus, A. hybridus, A. cruentus cv. Fotete and A. cruentus (RRC #1011) were collected when plants attained a relatively dry, mature state. Sampling was done during the second week of October 1984. Six plants were selected at random from subplots and panicles, both terminal and axillary, were cut and bag-bulked. After threshing and cleaning, the grain was washed, dried in a forced-air oven (70 C) for 24 h and stored in air-tight polyvinyl bags. When the analyses were run, a small portion of the clean, dry grain was ground to flour-like fineness in an electric coffee mill.

Sample Preparation and Plant Tissue Analyses

Vegetable Analyses

Total amino-N concentration of the leaf tissue samples was measured on a Technicon Auto Analyzer, after digestion in 18 mol/L H_2SO_4 and 11.9 mol/L H_2O_2 , with the indophenol colorimetric method (151, 154). Crude protein (CP) was estimated by multiplying total amino-N by 6.25. Aliquots of the total amino-N digest were taken to estimate phosphorus and potassium concentration (151). Phosphorus was measured on a Technicon

Auto Analyzer by the vanadomolybdo-phosphoric yellow colorimetric test (21, 70). Potassium concentration was estimated by atomic absorption spectrophotometry on a Perkin Elmer 5000 atomic absorption spectrophotometer.

Neutral detergent fiber (NDF) was used to estimate total cell wall constituents (48), using a Fibertec "System M" apparatus. Water soluble tissue nitrate (NO_3) was extracted by addition of 50 mL boiling deionized water to 500 mg of sample and placed on a mechanical shaker for 30 minutes. After filtering on No. 42 Whatman paper, the eluant was brought to a constant volume. Nitrate concentration of the eluant was measured by ion-selective High Performance Liquid Chromatography (HPLC) on a Dionex 4022i ion exchange chromatograph.

Tissue concentration of crude protein and NDF is expressed as grams per kilogram (g/kg) dry weight. Phosphorus, potassium and nitrate concentration is expressed as millimoles per kilogram (mmol/kg) dry weight.

Grain Analyses

Total amino-N concentration of grain samples was measured as in the vegetable analysis. Grain crude protein concentration was estimated by multiplying total amino-N by 5.85 (9). Phosphorus concentration was measured as in the vegetable analysis. Concentration of potassium, calcium, magnesium and iron were determined by atomic absorption spectrophotometry as in the vegetable analysis, after ashing samples for 4 h in a muffle furnace. Ashed samples were prepared for analysis by methods described by Jackson (70) and Johnson and Ulrich (72). Methods

outlined by the AOAC were used to determine grain crude fiber (7.061-7.065), ether extract (7.055-7.056), and ash (7.009) concentration. Water soluble carbohydrates (WSC) were determined using the procedures of Gaines and Mitchell (47) and Scientific Instruments Corporation (138). Total carbohydrates (TC) and nitrogen-free extract (NFE) were computed by difference, after correction for moisture content.

Concentration of crude protein, ether extract, ash, TC, WSC and NFE are expressed as grams per kilogram (g/kg) dry weight. Mineral concentration is expressed as millimoles per kilogram (mmol/kg) of dry weight.

Data Analysis

A two-way analysis of variance was performed for each accession separately. Data on crude protein, NDF, P, K and NO_3^- for vegetable accessions were subjected to trend analysis, using the method of orthogonal polynomials (49). Single and multiple degree of freedom contrasts were used to partition the variance in crude protein, NDF, P, K and NO_3^- concentration into linear, quadratic and cubic trend components. Linear regression was used to calculate single and multiple factor response functions for main effects and interactions. Linear regression was used to analyze the crude protein NH_4NO_3 x accession (N x A) and harvest x accession (H x A) interaction means after running a three-way analysis of variance on the data. Orthogonal contrasts were used for some within-class mean comparisons. Finally, a three-way analysis of variance

was performed on all data to estimate the general (group) response among accessions. The methods used were similar to those described above.

A two-way analysis of variance was performed on the grain compositional data. Significant main plot (NH_4NO_3) effects were analyzed within rather than among accessions, as suggested by Freund and Littell (46). Duncan's Multiple Range Test (5% level of probability) was used to separate main plot treatment means. Main effect treatment means were ranked and tabulated for comparison. Analyses were run on the ANOVA and GLM programs in SAS (129).

CHAPTER 4

RESULTS AND DISCUSSION OF LABORATORY CHEMICAL ANALYSES:

VEGETABLE AMARANTH

Amaranthus cruentus cv. Fotete NCRPIS #1964

The cultivar Fotete belongs to the "African vegetable type" category, as classified by Daloz (25). Alternately, Kauffman (77) has subsumed this cultivar under the rubric "African grain type," alluding to its use as both a vegetable and grain resource. The accession 1964 was obtained in Benin, West Africa. Grubben (51, 52) states that the cv. Fotete is grown throughout West Africa, wherever moisture and light conditions are favorable. There, it is mainly used as a leafy green vegetable. Various detailed descriptions of its botanical, agronomic and nutritional attributes have been published (25, 54, 62, 76, 77, 78).

Components of variance and associated degrees of freedom for tissue crude protein, NDF, P, K and NO_3^- concentration are presented in Table 3. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatment effects are given in Table 4. Except for P and K, a significant response ($p = 0.01$) was observed for tissue crude protein, NDF and NO_3^- concentration. As might be expected, harvest date exerted a significant ($p = 0.01$) effect on tissue crude protein, NDF, P, K and NO_3^- concentration. Sixty-eight percent of the total variance in crude protein concentration resulted from the harvest date treatments. Except for crude protein, none of the nitrogen x harvest (N x H) interactions was significant.

Table 3. Components of the analysis of variance for tissue crude protein, NDF, P, K and NO_3^- concentration for Amaranthus cruentus cv. Fotete NCRPIS #1964.

Source [†]	Degrees of freedom		Nutrient				
			CP	NDF	P	K	NO_3^-
Nitrogen (N)	2	2 ^{††}	**	**	NS	NS	**
Error A	6	6					
Harvest (H)	4	2	**	**	**	**	**
Nitrogen x Harvest	8	4	*	NS	NS	NS	NS
Error B	36	18					
N Linear	1	1	**	**	NS	NS	**
Deviation from Linear	1	1	NS	NS	NS	NS	NS
H Linear	1	1	**	**	**	**	**
Quadratic	1	1	NS	NS	NS	**	NS
Cubic	1		NS		NS	**	NS
Deviation from Cubic	1		NS				NS
N Linear x H Linear	1	1	**				
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	**	**	NS	NS	**
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	*	**	NS	NS	**

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 4. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus cruentus cv. ⁴Fotete NCRPIS #1964.

Nutrient [†]	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	279	296	304
	35	254	278	284
	45	202	255	271
	55	186	235	261
	65	162	189	221
NDF (g/kg)	25	278	268	251
	45	323	288	254
	65	372	343	292
P (mmol/kg)	25	164	154	138
	35	153	132	125
	45	161	154	131
	55	122	137	123
	65	118	116	120
K (mmol/kg)	25	1176	1244	1201
	35	901	1016	879
	45	941	1002	910
	55	1000	972	883
	65	888	902	777
NO_3^- (mmol/kg) ^{††}	25	91	170	203
	35	68	119	166
	45	3	95	176
	55	2	26	151
	65	*	4	57

[†] Nutrient estimates are means of four observations.

^{††} Percent NO_3^- is obtained by multiplying mmol/kg $\text{NO}_3^- \cdot 6.2 \times 10^{-3}$.

* NO_3^- concentration in plant extract $<5\mu\text{mol/L}$ ($6.2 \times 10^{-2}\%$).

To increase the precision by which differences in response to the NH_4NO_3 treatments could be estimated, linear contrasts were constructed to partition the variance in mean effects from the 0 kg/ha vs. 150 and 300 kg/ha NH_4NO_3 treatments. Likewise, a contrast was made to compare the mean effects resulting from the 150 vs. 300 kg/ha NH_4NO_3 treatments. Results are presented in Table 3. The effects from the application of 150 kg/ha NH_4NO_3 resulted in a significant ($p = 0.01$) response in mean tissue crude protein, NDF and NO_3^- concentration. Significant responses in mean tissue crude protein ($p = 0.05$), NDF and NO_3^- ($p = 0.01$) also resulted from the 150 vs. 300 kg/ha NH_4NO_3 treatment contrast. In some cases (crude protein, NO_3^-) the overall response was positive; that is to say, the NH_4NO_3 treatments enhanced the concentration of these nutrients in the leaf tissue. For NDF the response was negative, hereafter referring to a decreasing concentration of a nutrient in the leaf tissue.

While the increase in tissue crude protein concentration resulted in enhanced nutritional value of the cv. Fotete, the response did not attain the magnitude reported by Stafford et al. (146). These researchers reported a two-fold increase in crude protein concentration in Amaranthus hybridus cv. Green Head when averaged over a period of 63 days. Over approximately the same time interval, the percentage difference in crude protein concentration over the 0 kg/ha NH_4NO_3 control for cv. Fotete was 13.6% and 19.1% for the 150 and 300 kg/ha NH_4NO_3 treatments, respectively (see Chapter 6, p. 103).

To describe more precisely the response of cv. Fotete to the NH_4NO_3 and harvest date treatments, single degree of freedom contrasts were

constructed to partition the variance in leaf nutrient composition in nitrogen (N) linear and harvest (H) linear, quadratic and cubic trend components. Results of the analysis are presented in Table 3. Trend components, when significant, were used to calculate single factor response functions to estimate the effects of harvest date for each NH_4NO_3 rate. Likewise, functions were calculated to estimate the effects of NH_4NO_3 for each harvest date. A multiple, or "response surface" function was also calculated to estimate how each of the two factors simultaneously affected leaf crude protein concentration in cv. Fotete. These functions, their coefficients of determination ($r^2_{yx1 \cdot x2}$ or R^2) and standard errors ($S_{yx1 \cdot x2}$ or $S_{y \cdot x1x2}$) are given in Table 5.

For each NH_4NO_3 rate, tissue crude protein concentration in cv. Fotete declined as a linear function of harvest date. The rate of decline in crude protein over harvest dates was diminished as a function of the increasing NH_4NO_3 dosage. The magnitude of this dose response is clearly seen by comparing the slopes of the equations for each NH_4NO_3 rate presented in Table 5. Similarly, at each harvest date, tissue crude protein concentration was enhanced as a linear function of the NH_4NO_3 dosages. Slopes for the latter set of equations were significant, but the response to NH_4NO_3 varied at each harvest date. Hence, tissue crude protein concentration among NH_4NO_3 levels varied appreciably at each harvest date. Figures 1 and 2 show the general effects resulting from the NH_4NO_3 treatments at each harvest date and the effect of harvest dates for each NH_4NO_3 rate, respectively. A three dimensional view is presented in Figure 3 and shows the simultaneous effects of

Table 5. Calculated response functions and their partial coefficients of determination ($r^2_{y \cdot x_1 \cdot x_2}$) and standard errors ($S_{y \cdot x_1 \cdot x_2}$) for tissue crude protein, NDF, P and K concentration in Amaranthus cruentus cv. Fotete NCRPIS #1964.

Nutrient	^{††} Effect of harvest date for each fertilizer N rate (kg/ha NH_4NO_3)		Effect of NH_4NO_3 rate for each harvest date (days after germination)		$r^2_{y \cdot x_1 \cdot x_2}$		Function		$r^2_{y \cdot x_1 \cdot x_2}$		$S_{y \cdot x_1 \cdot x_2}$	
	0	150	300	Response Surface Function:	0	150	300	Response Surface Function:	0	150	300	Response Surface Function:
Crude protein												
NDF												
P												
K												

[†] Pooled over N levels when N is nonsignificant.

^{*}, ^{**} Slope is significant at the .05 and .01 levels of probability, respectively.

^{††} Units for harvest date (H) are 1 to 5 by 0.1. Each 0.1 increase represents 1 day in each 10 day interval.

[§] NH_4NO_3 units are kg/ha.

^Δ Standard error = $S_{y \cdot x_1 \cdot x_2}$.

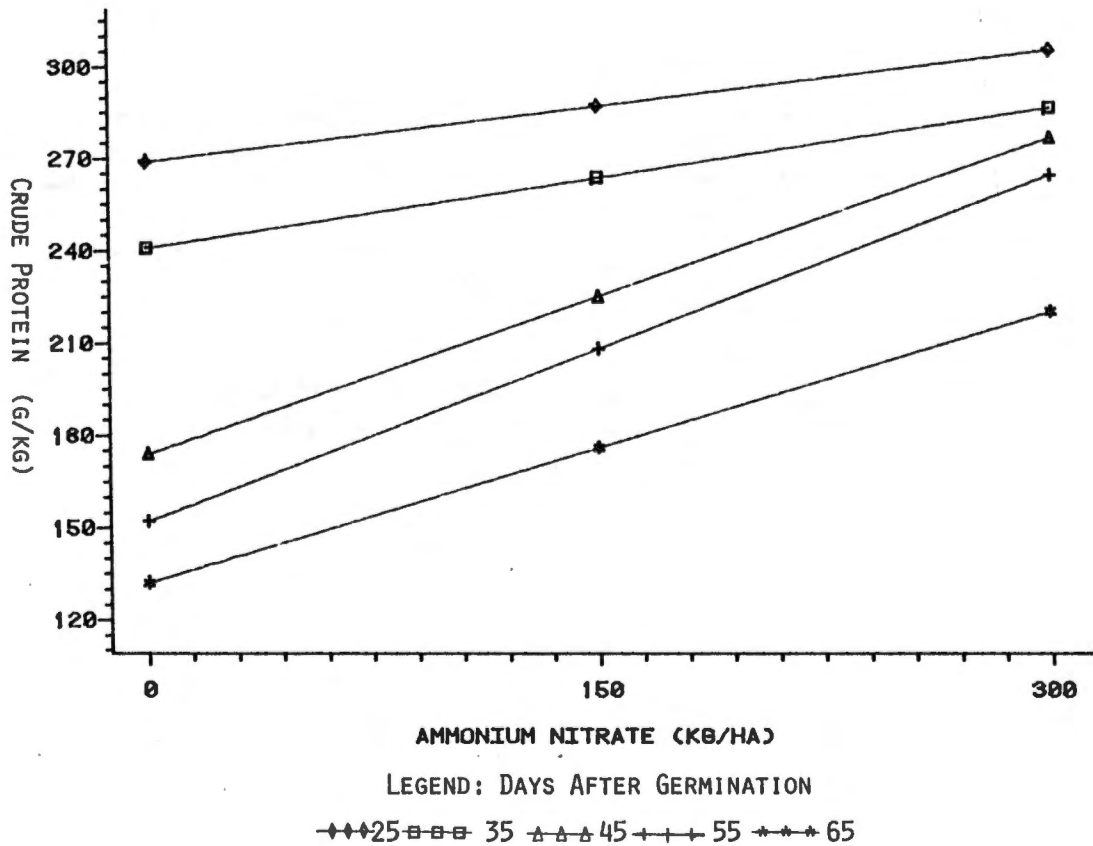


Figure 1. Tissue crude protein response to NH_4NO_3 at each harvest date in *Amaranthus cruentus* cv. Fotete NCRPIS #1964. Points on the lines are predicted values as calculated from the equations in Table 5.

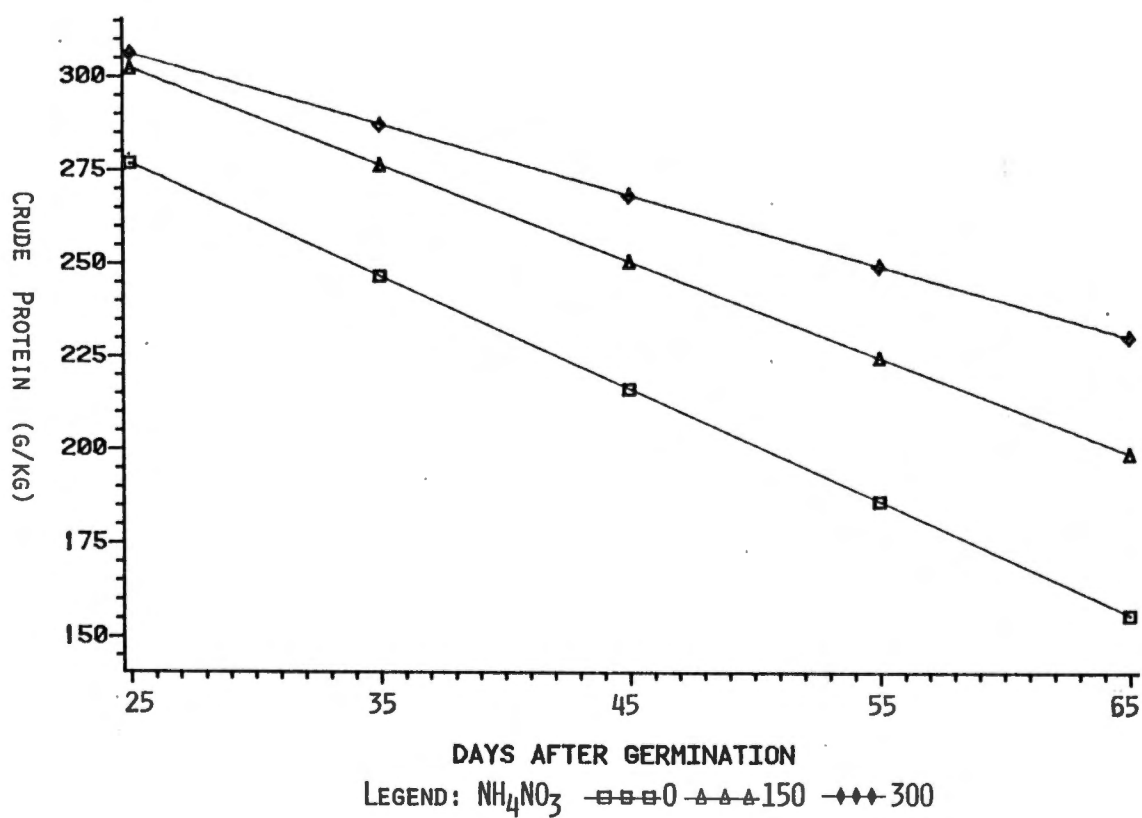


Figure 2. Tissue crude protein response over a harvest interval of 65 days after germination and at 3 levels of NH₄NO₃ in Amaranthus cruentus cv. Fotete NCRPIS #1964. Points on the lines are predicted values as calculated from the equations in Table 5.

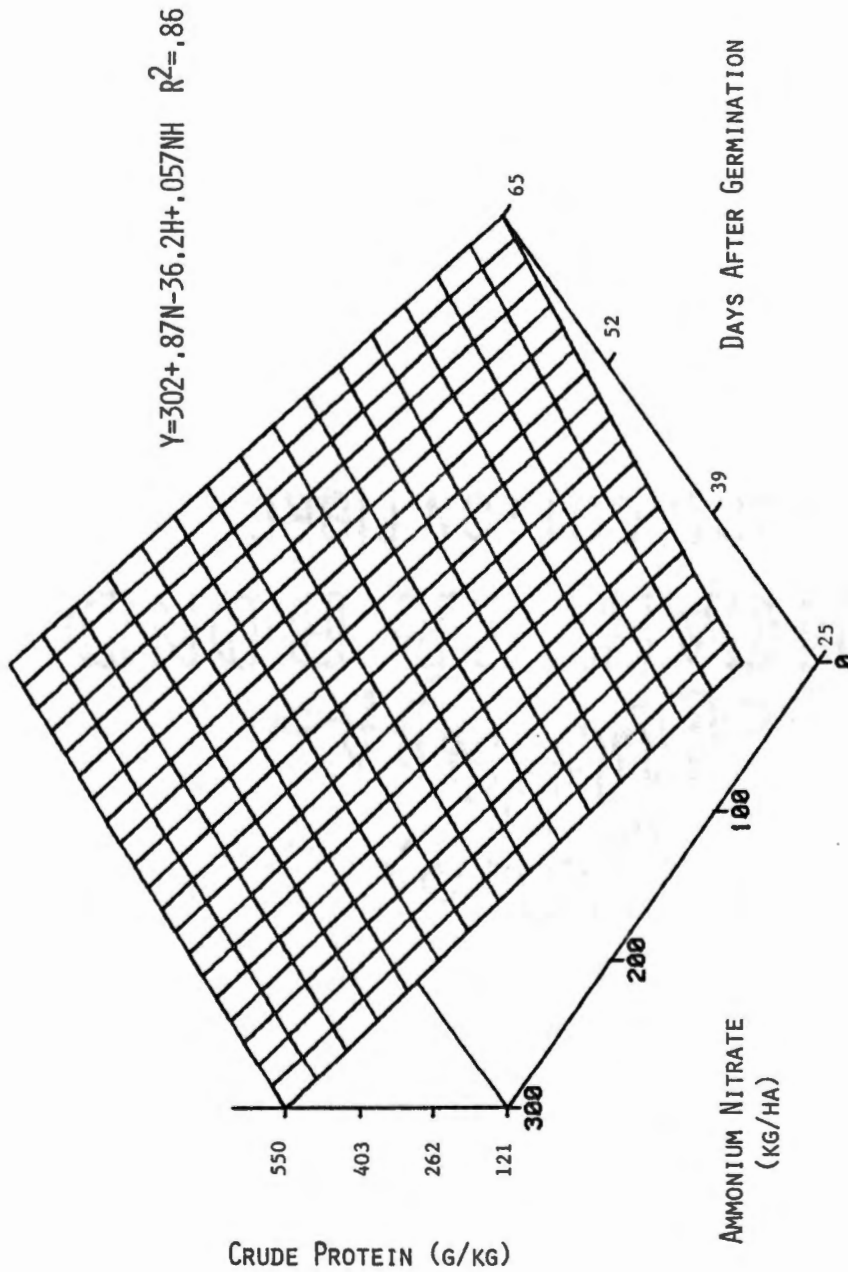


Figure 3. Three dimensional response surface showing the simultaneous effects of the NH_4NO_3 and harvest date treatments on tissue crude protein concentration in *Amaranthus cruentus* cv. Fotete NCRPIS #1964. Points on the surface represent the predicted crude protein response as calculated from the equation in Table 5.

the NH_4NO_3 and harvest date treatments on tissue crude protein concentration in cv. Fotete. The flat, planar aspect of this surface in three dimensional space resulted from the N linear, H linear, and N x H linear interaction terms, significant components of variance for crude protein in cv. Fotete.

Significant ($p = 0.01$) components of variance for tissue NDF, P and K were observed from the harvest date treatments in cv. Fotete. Generally, NDF concentration decreased from the addition of NH_4NO_3 , while the effect of harvest date resulted in enhanced levels of tissue NDF. A significant ($p = 0.01$) linear trend for tissue NDF resulted from both NH_4NO_3 and harvest date treatments. Response functions calculated to estimate the magnitude of this linear trend are presented in Table 5. As observed for crude protein, slope estimates for the regression equations were significant, but the response at each NH_4NO_3 rate and harvest date was different. Partitioning the variance in harvest date for tissue NDF concentration resulted in a significant ($p = 0.01$), negative linear trend. Significant ($p = 0.01$) linear, quadratic and cubic responses resulted from the effect of harvest date on leaf K concentration. Equations calculated to estimate the rate of change in tissue P and K over harvest dates are presented in Table 5. As observed from the data in Table 5, tissue K concentration in cv. Fotete declined precipitously between the first and second harvest dates. Thereafter, the K concentration remained relatively constant.

Data from the analysis of tissue NO_3^- in cv. Fotete are presented in Table 4. Generally, the effect from the increasing NH_4NO_3 fertilization

resulted in enhanced levels of NO_3^- in the leaf tissues. This response was expected. Plants fertilized with 150 kg/ha NH_4NO_3 contained nearly twice the amount of water soluble NO_3^- than that found in unfertilized plants. A more significant facet of this response was the observed variation in NO_3^- concentration in regard to both the NH_4NO_3 and harvest date treatments. As observed in Figure 4, the magnitude of difference in tissue NO_3^- concentration was markedly enhanced as the plants matured. For example, at 45 days after germination, plants fertilized with 150 and 300 kg/ha NH_4NO_3 contained 32 x and 59 x the concentration of tissue NO_3^- as compared to the 0 kg/ha control. Yet among the treatments analyzed, mean tissue NO_3^- concentration did not exceed 203 mmol/kg (1.25% dry wt basis). Comparable effects from N fertilization have been reported for Amaranthus cruentus in West Africa by Schmidt et al. (136). As these workers made no reference to harvest date, a direct comparison of NO_3^- data in this species was not possible. Compared to spinach, chard and other common greens crops under similar management, tissue NO_3^- concentration in cv. Fotete was not unusual (26).

Partitioning of the variance for tissue NO_3^- concentration resulted in significant ($p = 0.01$) linear trend effects from the NH_4NO_3 and harvest date treatments. The individual sample variance in NO_3^- concentration was considerable, though. Analyses performed to compare the NH_4NO_3 treatment means for tissue NO_3^- concentration resulted in significant ($p = 0.01$) differences for both 150 and 300 kg/ha treatment doses. The percentage difference of the total variance in tissue NO_3^- was greater than that observed for crude protein (20.3%). These results indicate

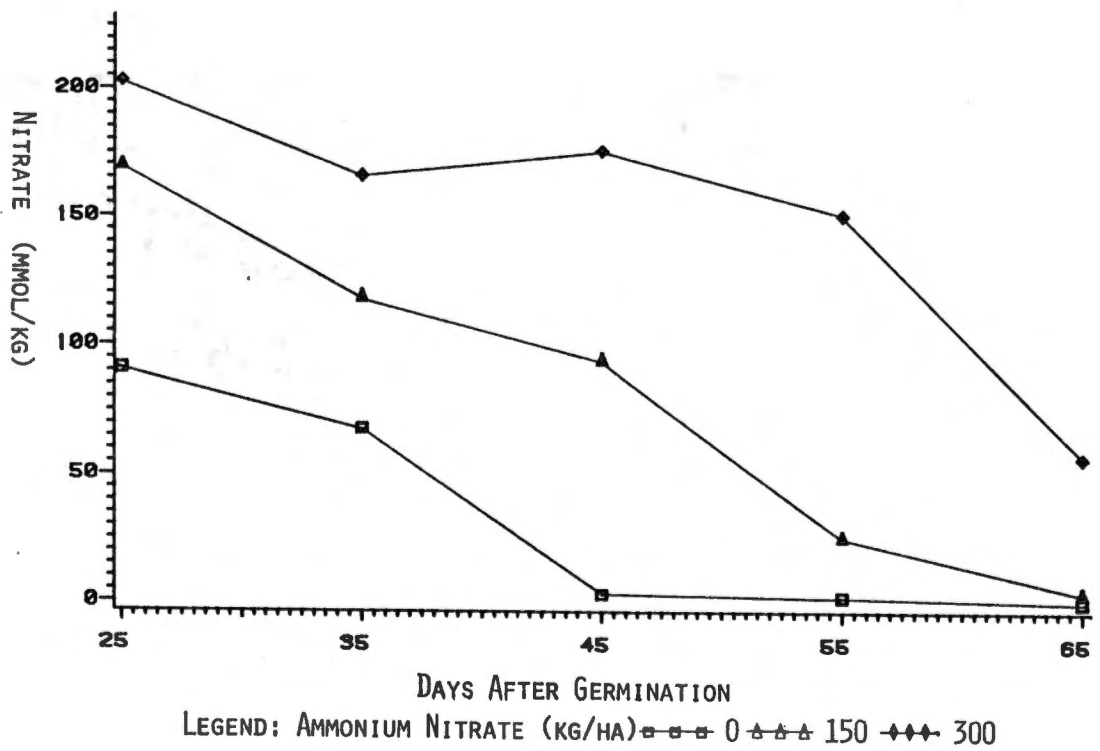


Figure 4. Mean Tissue NO_3^- concentration resulting from the NH_4NO_3 treatments over a harvest interval of 65 days after germination in *Amaranthus cruentus* fotete NCRPIS #1964.

that overall, tissue NO_3^- concentration was enhanced to a greater degree from the NH_4NO_3 as compared to crude protein in cv. Fotete.

Amaranthus dubius NCRPIS #2014

Amaranthus dubius is the only New World tetraploid amaranth with $2x = 2n = 64$ (112). The accession #2014 was obtained in Surinam. The species includes many weedy types, a few of which are cultivated as a greens crop in Latin America, the Caribbean and the Indian subcontinent (137). Its botanical and agronomic characters have been described by numerous workers (25, 42, 62).

Components of variance and associated degrees of freedom for tissue crude protein, NDF, P, K and NO_3^- concentration are presented in Table 6. Observed mean differences for each of the five nutrients as affected by the NH_4NO_3 and harvest date treatments are shown in Table 7. A significant ($p = 0.01$) response in tissue crude protein and NO_3^- concentration resulted from the NH_4NO_3 treatments. However, no response was observed for NDF, P or K. All the nutrients showed a significant ($p = 0.01$) response from the harvest date treatments. A major portion (50.1%) of the variance in crude protein concentration resulted from the harvest date treatments. A significant ($p = 0.01$) $N \times H$ interaction was observed for crude protein and NO_3^- .

As before, analyses were performed to partition the mean effects of the NH_4NO_3 treatments in single degree of freedom contrasts (Table 6). A significant ($p = 0.01$) difference in mean tissue crude protein and NO_3^- concentration resulted from the 150 kg/ha NH_4NO_3 treatment dose. The

Table 6. Components of the analysis of variance for tissue crude protein, NDF, P, K and NO_3^- concentration for Amaranthus dubius NCRPIS #2014.

Source [†]	Degrees of freedom		Nutrient				
			CP	NDF	P	K	NO_3^-
Nitrogen (N)	2	2 ^{††}	**	NS	NS	NS	**
Error A	6	6					
Harvest (H)	4	2	**	**	**	**	**
Nitrogen x Harvest	8	4	**	NS	NS	NS	**
Error B	33	15					
N Linear	1	1	**				**
Deviation from Linear	1	1	*				NS
H Linear	1	1	**	**	**	**	**
Quadratic	1	1	NS	NS	NS	**	**
Cubic	1		**		NS	**	**
Deviation from Cubic	1		**		NS	**	NS
N Linear x H Linear	1	1	**				**
N Linear x H Residual	1	1	*				**
N Linear x H Cubic	1	1	*				NS
N Residual x H Quadratic	1	1	*				NS
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	**				**
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	*				*

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 7. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus dubius NCRPIS #2014.

Nutrient [†]	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	250	267	268
	35	198	242	245
	45	135	208	214
	55	125	211	253
	65	104	130	169
NDF (g/kg)	25	223	246	223
	45	276	243	263
	65	257	273	271
P (mmol/kg)	25	172	164	165
	35	166	155	132
	45	123	149	145
	55	120	150	162
	65	147	128	113
K (mmol/kg)	25	1097	1087	1250
	35	786	770	783
	45	824	812	759
	55	807	755	714
	65	863	845	779
NO_3^- (mmol/kg) ^{††}	25	112	215	239
	35	*	83	142
	45	35	18	63
	55	*	29	112
	65	*	*	*

[†] Nutrient estimates are means of four observations.

^{††} Percent NO_3^- is obtained by multiplying mmol/kg $\text{NO}_3^- \cdot 6.2 \times 10^{-3}$.

* NO_3^- concentration in plant extract $< 5 \mu\text{mol/L}$ ($6.2 \times 10^{-2}\%$).

response from the 300 kg/ha NH_4NO_3 treatment also resulted in a significant ($p = 0.05$) mean difference in tissue concentration for both nutrients. The magnitude of the variance in NO_3^- concentration resulting from the application of NH_4NO_3 was less than that observed for crude protein, but the difference was not large (Chapter 6, p. 108). As the data in Table 7 shows considerable ($>1\%$ dry wt basis), though not excessive, amounts of NO_3^- accumulated in the leaf tissues of Amaranthus dubius plants that were fertilized with NH_4NO_3 . The unfertilized plants also accumulated NO_3^- , but the concentration quickly diminished to undetectable levels as the plants matured.

Further partitioning of variance was performed to investigate the nature of the response in tissue nutrient concentration in Amaranthus dubius resulting from the NH_4NO_3 and harvest date treatments. Results of single degree of freedom trend comparisons are presented in Table 6. A positive linear response in tissue crude protein and NO_3^- concentration was observed from the addition of NH_4NO_3 . However, a significant ($p = 0.05$) N quartic, or residual, effect resulted in the analysis of variance for crude protein. The N residual effect de facto measures the error, or "failure" of A. dubius to response linearly to the NH_4NO_3 treatments at each harvest date. Partitioning of the variance in harvest date resulted in linear, cubic and quartic (residual) responses in crude protein concentration. A similar analysis performed for tissue NO_3^- concentration resulted in linear, quadratic and cubic trend effects from the harvest date treatments. Additional analyses were performed for crude protein to calculate linear response functions in order to

estimate the effects of NH_4NO_3 at each harvest date. The results of these analyses are presented in Table 8. Slope estimates for the NH_4NO_3 response functions were significant for all harvest dates except the first. Hence, the mean tissue crude protein levels resulting from the NH_4NO_3 treatments were not significantly affected at this early harvest date.

In Figure 5, the general tissue crude protein responses to NH_4NO_3 at each harvest date are shown. These regression lines were calculated from their respective equations in Table 8. Figure 6 is a plot of the actual, rather than predicted, response in tissue crude protein concentration at each NH_4NO_3 level over the harvest dates. From these figures, the nature of the N x H interaction term becomes clear. The N linear x H linear term resulted in a different N linear (estimated slope) at each harvest date and a different H linear for each NH_4NO_3 rate (slope estimates for harvest date effects are presented in Table 8). Similarly, a different H cubic effect was observed for each NH_4NO_3 rate. Although the analysis of variance indicated a significant N linear x H quadratic effect, the H quadratic component in the general harvest sum of squares was not significant ($F < 1$). The reason for this anomaly was unclear, and will not be further discussed here.

Generally, tissue crude protein concentration in Amaranthus dubius declined as a quadratic function of harvest date when no NH_4NO_3 was applied. For both the 150 and 300 kg/ha NH_4NO_3 treatments, an unexpected but significant increase in crude protein concentration occurred at the fourth harvest date. Hence, highly significant cubic trend effects resulted in the analysis of variance. At first this response seems

Table 8. Calculated response functions and their partial coefficients of determination ($r^2_{yxl \cdot x2}$) and standard errors ($S_{yxl \cdot x2}$) for tissue crude protein, P and K concentration in *Amaranthus dubius* NCRPIS #2014.

Nutrient	Effect of harvest date for each fertilizer N rate (kg/ha NH_4NO_3)	Function	$r^2_{yxl \cdot x2}$	$S_{yxl \cdot x2}$	Effect of NH_4NO_3 rate for each harvest date (days after germination)	Function	$r^2_{yxl \cdot x2}$	$S_{yxl \cdot x2}$
Crude protein	0	$Y = 314 - 63.9H + .9H^3$	.89	19.9	25	$Y = 244 + .093N$	NS	10.4
	150	$Y = 272 - 9.87H = .68H^3$	.78	25.2	35	$Y = 181 + .236N$	**	21.1
	300	$Y = 249 + 2.7H = .66H^3$	.48	30.6	45	$Y = 106 + .397N$	**	27.7
	Response Surface Function:	$Y = 334 + .262N - 86.8H$ $+ 1.33H^2 - .399NH^3$	$R^2 = .83$	24.5 ^Δ	55	$Y = 68.9 + .637N$	**	25.6
		$+ .262NH^2 = .0359NH^3$			65	$Y = 69.4 + .324N$	**	19.9
P	†	$Y = 170.7 - .819H$	.85	6.3				
K	†	$Y = 1779 - 888H$ $+ 24.5H^2 - 21.1H^3$	.67	87.5				

† Pooled over N levels when N is nonsignificant.

*,** Slope is significant at the .05 and .01 levels of probability, respectively.

NS Slope is nonsignificant.

Δ Standard error = $S_{y \cdot x1x2}$.

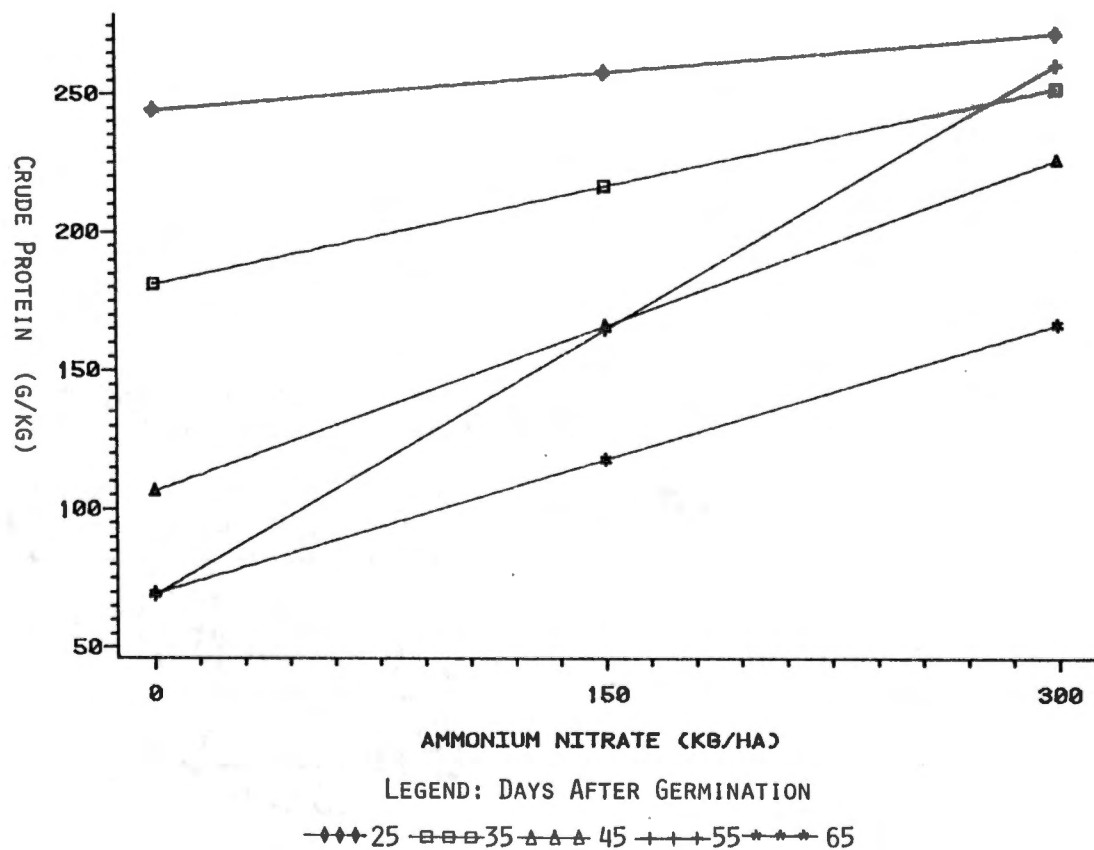


Figure 5. Tissue crude protein response to NH_4NO_3 at each harvest date in Amaranthus dubius NCRPIS #2014. Points on the lines are predicted values as calculated from the equations in Table 8.

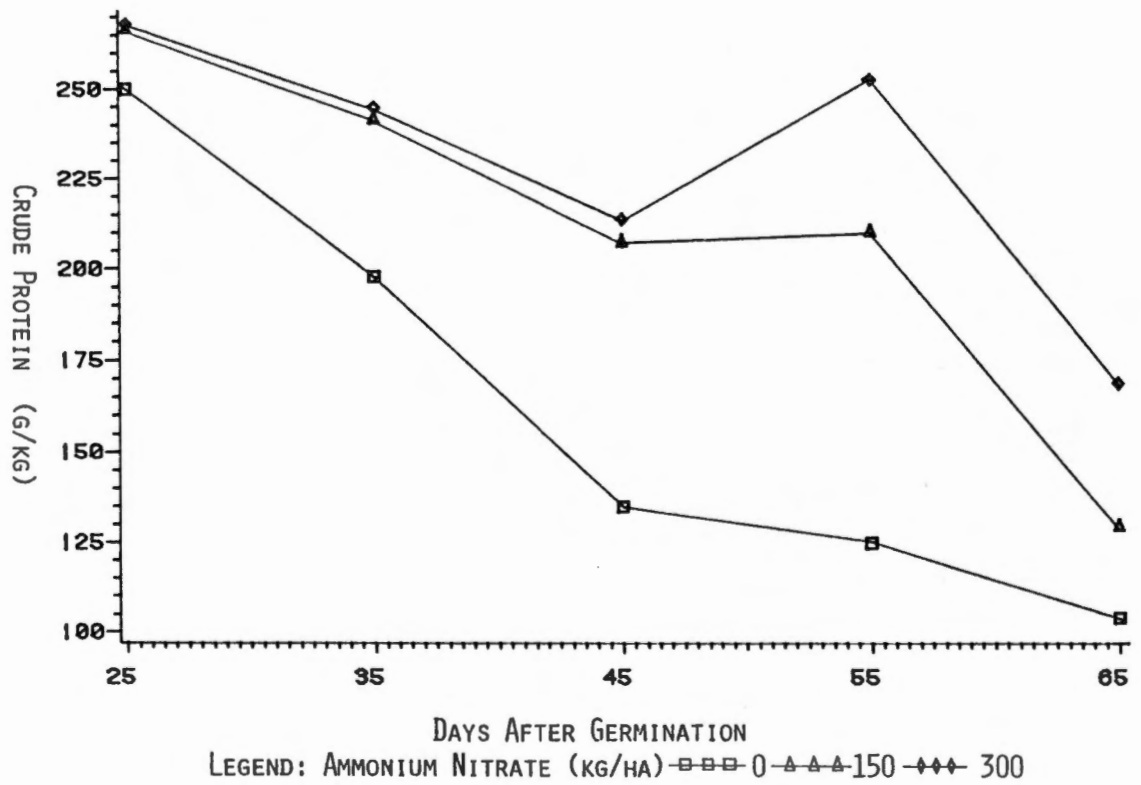


Figure 6. Mean tissue crude protein concentration resulting from the NH_4NO_3 treatments over a harvest interval of 65 days after germination in *Amaranthus dubius* NCRPIS #2014.

paradoxical. However, after a combined response analysis among accessions was performed, a hypothesis was proposed to explain this observed deviation. The combined analyses and their interpretations will be presented in Chapter 6.

Partitioning of the variance in tissue NDF, P, K and NO_3^- concentration from the harvest date treatments resulted in significant trend effects. Analyses performed for NDF indicated an increasing, though somewhat erratic linear trend in concentration over three of the five harvest dates. Leaf tissue P generally declined as a linear function of harvest date, but less precipitously as compared to crude protein, K and NO_3^- . Trend analyses performed for K resulted in significant ($p = 0.01$) linear, quadratic, cubic and quartic (residual) trend effects. Estimated slopes for the harvest response functions are presented in Table 8. Generally, K concentration in the leaf tissue declined steeply between the first and second harvest dates, but thereafter remained relatively constant. Trend analyses performed on the tissue NO_3^- data for Amaranthus dubius resulted in harvest date effects similar to those observed in the crude protein analysis (Table 6).

Amaranthus tricolor RRC #241

Native to South-East Asia, the Amaranthus tricolor complex comprises a large, desirable group of vegetable cultivars. Seven cultivars have been recognized by Daloz (25). This distinction was based on differences in plant habit, leaf color and shape, and stem quality. Unlike

the New World amaranths, the A. tricolor complex is polyploid. The genome reportedly contains a large number of amphidiploid chromosomes (50). The accession #241 belongs to the "white leaf" category (25), a commercially prominent group in South-East Asia.

Components of variance and associated degrees of freedom for tissue crude protein, NDF, P and K are presented in Table 9. Observed mean differences in nutrient concentration resulting from the NH_4NO_3 and harvest date treatments are presented in Table 10. Significant ($p = 0.01$) differences in tissue crude protein and NDF concentration resulted from the NH_4NO_3 treatments. No significant effects from the NH_4NO_3 were observed for P or K. Tissue crude protein, NDF and K showed significant ($p = 0.01$) responses in concentration over harvest dates. The response from the harvest date treatments for P was significant at the 5% level. Again, a major portion (66.6%) of the variance in crude protein concentration resulted from the effects of the harvest date treatments. None of the N x H interactions was significant.

Crude protein and NDF data was subjected to single degree of freedom contrasts in order to provide further insight regarding the differences in effects resulting from the NH_4NO_3 treatments (Table 9). A comparison of the mean differences in tissue crude protein concentration among NH_4NO_3 levels resulted in a significant ($p = 0.01$), positive response at $<150 \text{ kg/ha } \text{NH}_4\text{NO}_3$. Mean tissue crude protein concentration was not significantly enhanced from the addition of $300 \text{ kg/ha } \text{NH}_4\text{NO}_3$. Neutral detergent fiber concentration showed a significant ($p = 0.01$), negative response from the NH_4NO_3 treatments at $<150 \text{ kg/ha } \text{NH}_4\text{NO}_3$.

Table 9. Components of the analysis of variance for tissue crude protein, NDF, P and K concentration for Amaranthus tricolor RRC #241

Source [†]	Degrees of freedom		Nutrient			
			CP	NDF	P	K
Nitrogen (N)	2	2 ^{††}	**	**	NS	NS
Error A	6	6				
Harvest (H)	4	2	**	**	**	**
Nitrogen x Harvest	8	4	NS	NS	NS	NS
Error B	36	18				
N Linear	1	1	**	**		
Deviation from Linear	1	1	NS	*		
H Linear	1	1	**	**	NS	**
Quadratic	1	1	NS	**	**	**
Cubic	1		**		NS	**
Deviation from Cubic	1		NS		NS	**
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	**	**		
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	NS	NS		

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 10. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus tricolor RRC #241.

Nutrient [†]	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	256	270	279
	35	173	204	233
	45	155	217	230
	55	157	197	219
	65	118	167	176
NDF (g/kg)	25	280	240	249
	45	306	282	258
	65	238	212	210
P (mmol/kg)	25	153	154	158
	35	177	179	136
	45	186	167	156
	55	189	178	169
	65	144	134	138
K (mmol/kg)	25	1139	1164	1152
	35	770	743	645
	45	954	871	865
	55	779	697	688
	65	768	704	707

[†] Nutrient estimates are means of four observations.

Overall, crude protein concentration increased, while NDF levels decreased as a linear function of the increasing NH_4NO_3 rates. Response functions calculated to estimate the effects from the NH_4NO_3 in leaf tissue crude protein and NDF concentration are presented in Table 11. The usual pattern of differential response to NH_4NO_3 at each harvest date was observed. Enhanced absorption of NH_4NO_3 seemed to be a result of the advancing maturity of the plants. This effect could have resulted from increasing root activity and/or assimilation of labile tissue NO_3^- (assuming the presence of a tissue NO_3^- pool) during growth.

Analyses performed to partition the variance in tissue crude protein concentration from harvest date treatments resulted in significant, though different trend effects from the NH_4NO_3 and harvest date treatments. Results of regression analyses performed to estimate the response to NH_4NO_3 each harvest date are shown in Figure 7. Crude protein concentration in Amaranthus tricolor declined as a linear and cubic function of harvest date. The observed cubic trend effects resembled those that were reported for A. dubius. Although the magnitude of the cubic effects observed in A. tricolor were small as compared to those observed in A. dubius, results obtained in the combined analyses (Chapter 6) suggest that the differences probably were not due to sampling error. Figure 8 shows the observed mean values for crude protein concentration at each NH_4NO_3 rate. Response functions were calculated to estimate the effects of harvest date for each NH_4NO_3 rate, but these functions were not plotted because of the "smoothing" effects resulting from the regression. However, a three dimensional response surface for

Table 11. Calculated response functions and their partial coefficients of determination ($r^2_{y \cdot x_1 \cdot x_2}$) and standard errors ($S_{y \cdot x_1 \cdot x_2}$) for tissue crude protein, NDF, P and K concentration in Amaranthus tricolor RRC #241.

Nutrient	Effect of harvest date for each fertilizer N rate (kg/ha NH_4NO_3)	Function	$r^2_{y \cdot x_1 \cdot x_2}$	$S_{y \cdot x_1 \cdot x_2}$	Effect of NH_4NO_3 rate for each harvest date (days after germination)	Function	$r^2_{y \cdot x_1 \cdot x_2}$	$S_{y \cdot x_1 \cdot x_2}$
Crude protein	0	$Y = 293 - 51.5H + .74H^3$	.72	28.5	25	$Y = 245 + .117N^*$	.49	10.7
	150	$Y = 280 - 24.2H + .02H^3$	.65	26.2	35	$Y = 144 + .297N^{**}$	.49	26.9
	300	$Y = 290 - 19.7H - .07H^3$	.64	25	45	$Y = 126 + .372N^{**}$	.68	22.7
	Response Surface Function:	$Y = 232 + .277N - 31.8H^2 + .23H^3$	$R^2 = .72$	26.8 $^{\Delta}$	55	$Y = 129 + .31N^{**}$	.53	26
NDF	0	$Y = 306 = 10.4H$	.23	34.1	65	$Y = 92.1 + .29N^{**}$	.55	23.4
	150	$Y = 266 - 7.12H$	.12	35	25	$Y = 287 - 15.2N^{NS}$	.21	26.5
	300	$Y = 268 - 9.81H$	.29	27.2	45	$Y = 330 - 23.6N^*$	.48	22.1
	†	$Y = 167.2 - .247H^2$	.49	19.4	65	$Y = 248 - 14N^{NS}$	.24	22.1
P	†							
K	†	$Y = 1958 - 1151H + 358H^2 - 35.7H^3$	.61	122				

† Pooled over N levels when N is nonsignificant.

*,** Slope is significant at the .05 and .01 levels of probability, respectively.

NS Slope is nonsignificant.

$^{\Delta}$ Standard error = $S_{y \cdot x_1 \cdot x_2}$.

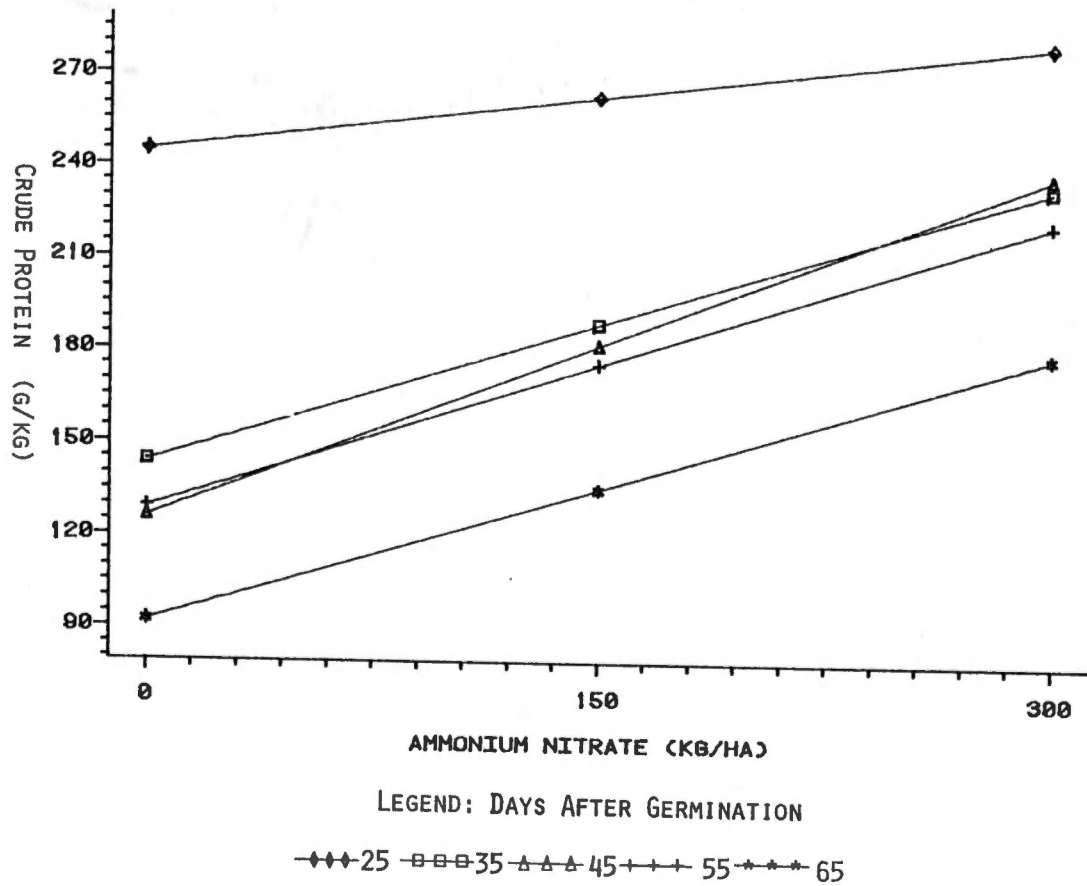


Figure 7. Tissue crude protein response to NH_4NO_3 at each harvest date in Amaranthus tricolor RRC #241. Points on the lines are predicted values as calculated from the equations in Table 11.

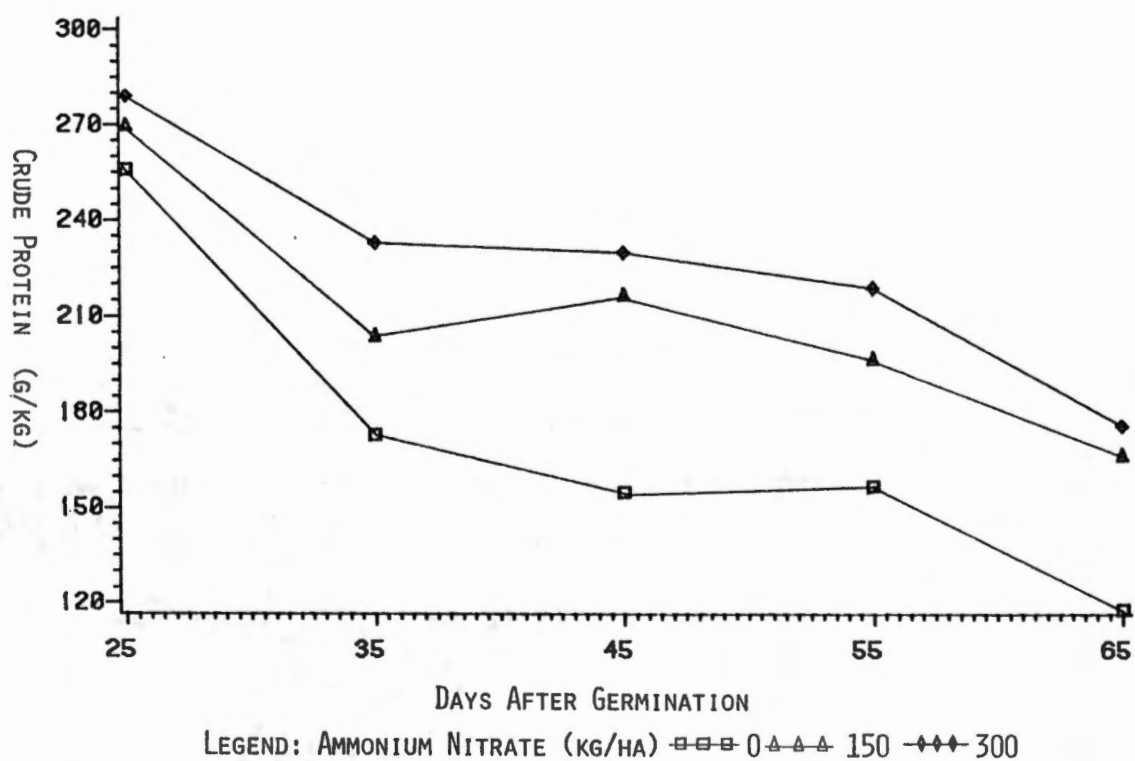


Figure 8. Mean tissue crude protein concentration resulting from the NH_4NO_3 treatments over a harvest interval of 65 days after germination in Amaranthus tricolor RRC #241.

tissue crude protein concentration was constructed in Figure 9. The highly significant NH_4NO_3 and harvest date linear components of variance for crude protein in A. tricolor resulted in the flat, planar conformation of this surface in three dimensional space.

Harvest date treatments also exerted different effects on tissue NDF, P and K concentration in Amaranthus tricolor. In spite of a significant ($p = 0.01$) effect resulting from the harvest dates in the analysis of variance, leaf tissue NDF concentration did not increase as expected over harvest dates. A careful scrutiny of the data revealed no clear trend in NDF concentration over harvest dates in this accession. Analyses performed on the K data resulted in significant ($p = 0.01$) linear, quadratic, cubic and quartic (residual) trend effects. As observed in the other accessions, a precipitous decline in tissue K concentration occurred between the first and second harvest dates. On the other hand, tissue P concentration increased over four of the five harvest dates.

Amaranthus cruentus RRC #1011

The accession #1011 is a diploid ($2x = 2n = 32$) New World grain amaranth and not typically cultivated as a vegetable crop. Daloz (25) states that thinnings of Amaranthus cruentus may serve at early stages for use as a vegetable. When young, the plants form dense terminal leafy whorls or rosettes. This terminal rosette can be picked and yields a mass of mostly leaf material. The accession #1011 was obtained in Mexico, the place of its aboriginal domestication. Biosystematic,

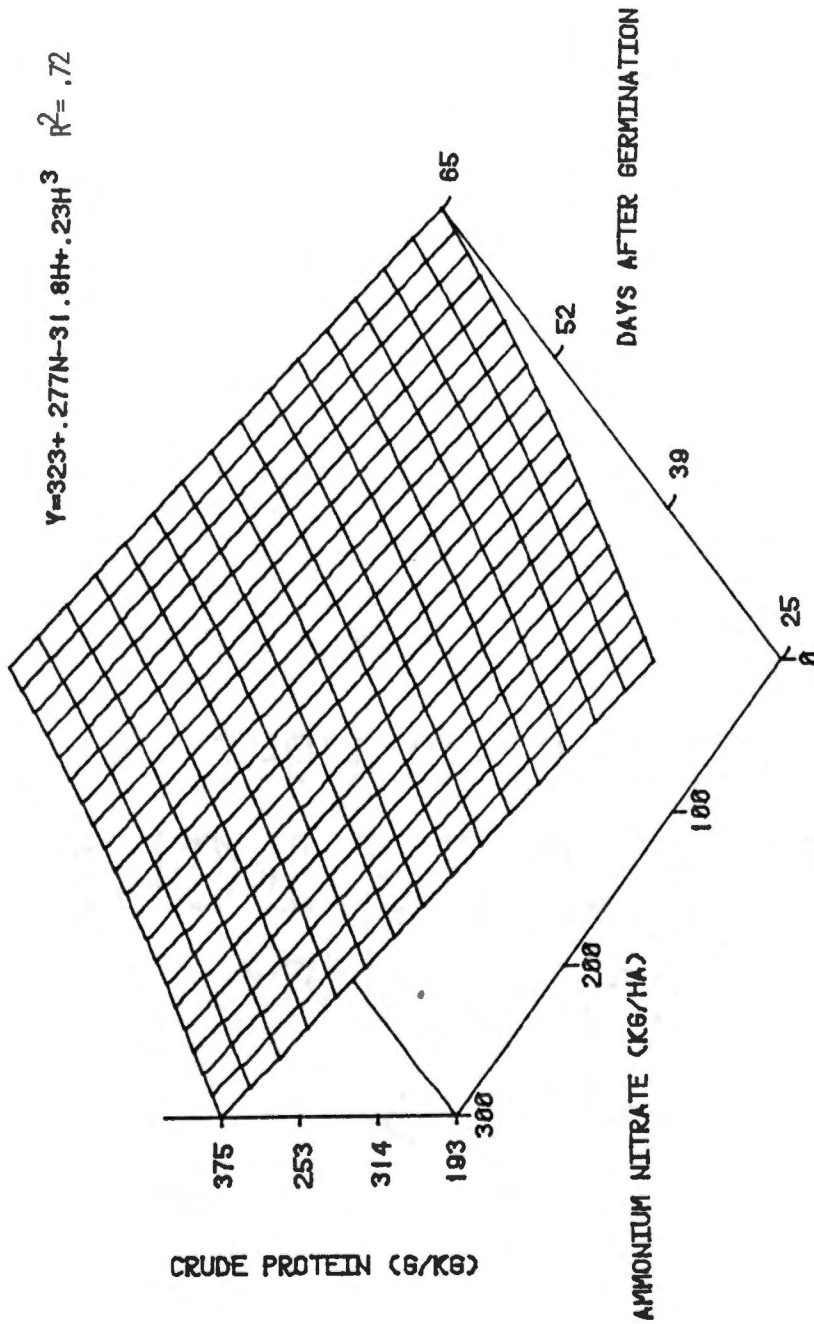


Figure 9. Three dimensional response surface showing the simultaneous effects of the NH_4NO_3 and harvest date treatments on tissue crude protein concentration in *Amaranthus tricolor* RRC #241. Points on the surface represent the predicted crude protein response as calculated from the equation in Table 11.

ethnographic, agronomic and nutritional information for A. cruentus have been reported by various workers (11, 25, 55, 57, 69, 76, 77, 78, 118, 131).

The analysis of variance and associated degrees of freedom for tissue crude protein, NDF, P and K concentration are presented in Table 12. Data describing the mean effects resulting from the NH_4NO_3 and harvest date treatments for each nutrient are presented in Table 13. A significant response in tissue crude protein and NDF concentration resulted from the NH_4NO_3 treatments. No significant effects were observed for P or K. Crude protein concentration was significantly ($p = 0.01$) enhanced as a result of the NH_4NO_3 treatments, while a significant ($p = 0.05$) but negative effect was observed for NDF. Single degree of freedom contrasts performed for crude protein and NDF indicated significant differences in mean tissue concentration for both nutrients resulted from the addition of $<150 \text{ kg/ha } \text{NH}_4\text{NO}_3$. Significant, negative effects resulted in a diminishing tissue crude protein and K concentration over the harvest dates. Once again, a major portion (81.3%) of the variance in crude protein concentration resulted from a decline over the harvest dates. None of the N x H interactions was significant.

Partitioning of the variance in tissue crude protein concentration resulting from the NH_4NO_3 and harvest date treatments was performed in order to describe more precisely the nature of the observed response trends. A significant ($p = 0.01$) linear trend in crude protein concentration was observed in response to the NH_4NO_3 treatments. Crude protein concentration declined significantly as a linear ($p = 0.01$) and

Table 12. Components of the analysis of variance for tissue crude protein, NDF, P and K concentration for Amaranthus cruentus RRC #1011.

Source [†]	Degrees of freedom		Nutrient			
			CP	NDF	P	K
Nitrogen (N)	2	2 ^{††}	**	*	NS	NS
Error A	6	6				
Harvest (H)	4	2	**	NS	NS	**
Nitrogen x Harvest	8	4	NS	NS	NS	NS
Error B	36	18				
N Linear	1	1	**	**		
Deviation from Linear	1	1	NS	NS		
H Linear	1	1	**			**
Quadratic	1	1	*			**
Cubic	1		NS			**
Deviation from Cubic	1		**			NS
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	**	**		
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	NS	NS		

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 13. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus cruentus RRC #1011.

Nutrient [†]	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	262	295	304
	35	248	271	273
	45	152	208	220
	55	145	190	212
	65	125	163	176
NDF (g/kg)	25	286	238	237
	45	272	272	258
	65	266	260	234
P (mmol/kg)	25	155	158	166
	35	162	138	139
	45	144	194	148
	55	164	178	176
	65	136	162	152
K (mmol/kg)	25	1123	1204	1196
	35	902	873	814
	45	956	936	768
	55	979	924	756
	65	936	822	822

[†]Nutrient estimates are means of four observations.

quadratic ($p = 0.05$) function of harvest date. Unlike the vegetable amaranths, fluctuations in tissue crude protein concentration did not occur over any of the harvest dates. The general response in crude protein concentration over harvest dates was quadratic. Equations calculated to estimate the effect of harvest date for each NH_4NO_3 rate are presented in Table 14. These functions were used to plot the response curves shown in Figures 10 and 11. The estimated general response from the NH_4NO_3 treatments at each harvest date are also presented in Table 14. The slope estimates describing the NH_4NO_3 response at each harvest date were significant, and their magnitude generally increased as a function of plant age. Overall, the general response from the NH_4NO_3 among harvest dates was similar to that observed in Amaranthus cruentus cv. Fotete. Due to the significant ($p = 0.01$) H residual effect observed in the analysis of variance in Table 12 a three dimensional response surface was not plotted for A. cruentus RRC #1011.

Partitioning of the variances in tissue NDF, P and K concentration resulted in different trend effects. Tissue NDF and P concentration did not change appreciably over the harvest dates. Significant ($p = 0.01$) linear, quadratic and cubic trend effects were observed for K. The steepest decline in tissue K concentration occurred between the first and second harvest dates.

Table 14. Calculated response functions and their partial coefficients of determination ($r^2_{y \cdot x_1 \cdot x_2}$) and standard errors ($S_{y \cdot x_1 \cdot x_2}$) for tissue crude protein, NDF, P and K concentration in Amaranthus cruentus RRC #1011.

Nutrient	Effect of harvest date for each fertilizer N rate (kg/ha NH_4NO_3)	Function	$r^2_{y \cdot x_1 \cdot x_2}$	$S_{y \cdot x_1 \cdot x_2}$	Effect of NH_4NO_3 rate for each harvest date (days after germination)	Function	$r^2_{y \cdot x_1 \cdot x_2}$	$S_{y \cdot x_1 \cdot x_2}$
Crude protein	0	$Y = 337 - 70.3H + 5.46H^2$	.82	27.8	25	$Y = 244 + .21N^{**}$	.67	13.2
	150	$Y = 348 - 51.4H + 2.82H^2$	.90	17.9	35	$Y = 239 + .125N^{**}$	.42	13.1
	300	$Y = 348 - 45.8H + 2.37H^2$	.84	20.8	45	$Y = 125 + .341N^{**}$	.63	23.1
	Response Surface Function:	$Y = 294 + .253N - 55.8H^2 + 3.35H$	$R^2 = .85$	22.9 Δ	55	$Y = 115 + .336N^{**}$	.57	25.9
K	†	$Y = 1879 - 984H + 303H^2 - 29.4H^3$	.66	87.4	65	$Y = 104 + .254N^{**}$	.54	20.8

†Pooled over N levels when N is nonsignificant.

*,** Slope is significant at the .05 and .01 levels of probability, respectively.

Δ Standard error = $S_{y \cdot x_1 \cdot x_2}$.

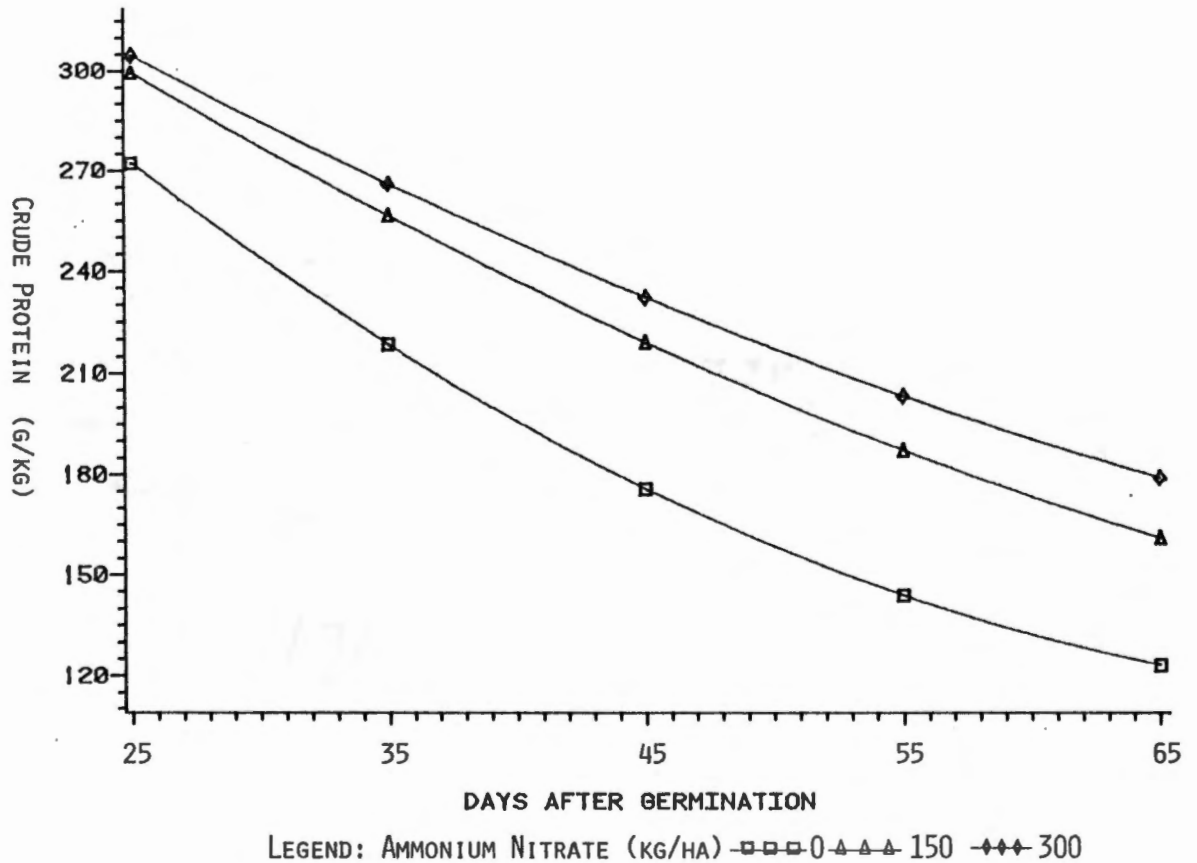


Figure 10. Tissue crude protein response over a harvest interval of 65 days after germination and at three levels of NH_4NO_3 in Amaranthus cruentus RRC #1011. Points on the lines are predicted values as calculated from the equations in Table 14.

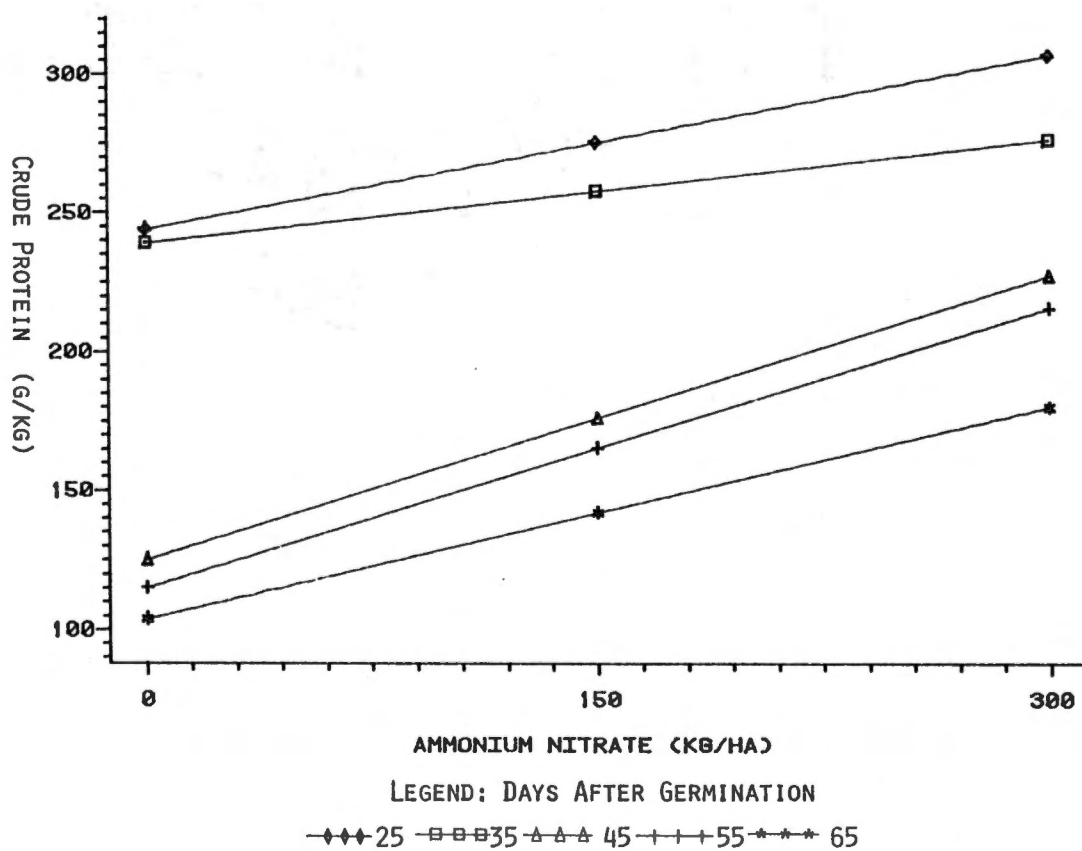


Figure 11. Tissue crude protein response to NH_4NO_3 at each harvest date in *Amaranthus cruentus* RRC #1011. Points on the lines are predicted values as calculated from the equations in Table 14.

Amaranthus paniculatus RCSC

Sauer (131) considers this species to be synonymous with Amaranthus cruentus. Sanchez-Marroquin et al. (127) made similar observations for A. paniculatus in Mexico. This accession, whose exact origin is unknown, resembled A. hybridus, a semiwild progenitor of A. cruentus. Apparently it is utilized as a potherb, but clearly has no relation to the smooth leaved vegetable amaranths of South-East Asia.

Components of variance and associated degrees of freedom for tissue crude protein, NDF, P and K are presented in Table 15. Observed mean differences in tissue nutrient concentration resulting from the effects of the NH_4NO_3 and harvest date treatments are presented in Table 16. Crude protein and P were the only components significantly ($p = 0.01$) affected by the NH_4NO_3 treatments. Tissue crude protein concentration was significantly ($p = 0.01$) enhanced from the addition of <150 kg/ha NH_4NO_3 . Overall, the enhancement in tissue crude protein concentration resulting from the NH_4NO_3 treatments was not strong in Amaranthus paniculatus as compared to the other accessions (from Chapter 6, p. 108). Only 13.4% of the total variance in tissue crude protein concentration resulted from the application of NH_4NO_3 . A significant ($p = 0.01$), negative response was observed in tissue P concentration from the NH_4NO_3 treatments. Whether this resulted from a "dilution" effect, or some other environmental variance, could not be surmised from the data. Significant effects were observed for each of the four tissue components in relation to the harvest date treatments. Harvest date

Table 15. Components of the analysis of variance for tissue crude protein, NDF, P and K concentration for Amaranthus paniculatus RCSC.

Source [†]	Degrees of freedom		Nutrient			
			CP	NDF	P	K
Nitrogen (N)	2	2 ^{††}	**	NS	**	NS
Error A	6	6				
Harvest (H)	4	2	**	*	**	**
Nitrogen x Harvest	8	4	*	NS	NS	NS
Error B	36	18				
N Linear	1	1	**	NS	**	
Deviation from Linear	1	1	NS	NS	NS	
H Linear	1	1	**	NS	**	**
Quadratic	1	1	NS	*	**	*
Cubic	1		NS		NS	
Deviation from Cubic	1		NS		NS	
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	**		**	
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	NS		*	

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 16. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus paniculatus⁴ RCSC.

Nutrient [†]	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	266	284	286
	35	236	259	263
	45	212	234	246
	55	178	195	226
	65	150	183	198
NDF (g/kg)	25	285	289	251
	45	311	311	297
	65	290	289	282
P (mmol/kg)	25	169	168	156
	35	176	150	143
	45	148	146	127
	55	127	122	113
	65	91	90	86
K (mmol/kg)	25	1290	1313	1322
	35	1278	1048	972
	45	1091	1049	1058
	55	1000	1065	1075
	65	982	962	1007

[†] Nutrient estimates are means of four observations.

accounted for 81.8% of the total variance in tissue crude protein. None of the N x H interactions was significant.

Partitioning of the variance in tissue crude protein concentration resulting from the NH_4NO_3 and harvest date treatments led to observed significant ($p = 0.01$) trend effects. Generally, crude protein concentration was enhanced as a linear function of increasing NH_4NO_3 doses. While crude protein concentration declined as a linear function of harvest date, the rate of decline was not greatly affected by the different NH_4NO_3 treatments. The magnitude of difference in the slope estimates from the effects of harvest date at each NH_4NO_3 rate (Table 17) clearly indicate a marginal response. Generally, the slopes estimating the response to NH_4NO_3 at each harvest date were significant (Table 17). The magnitude of response to NH_4NO_3 was observed to increase steadily over the harvest dates. Figures 12 and 13 show two dimensional responses from the effects of the NH_4NO_3 and harvest date treatments. A three dimensional response surface showing the simultaneous effects of the NH_4NO_3 and harvest date treatments appears in Figure 14. A significant linear response from two factors (N and H) in two dimensions resulted in the flat, planar aspect of the response surface depicted in three dimensional space.

Significant differences in tissue NDF, P and K concentration was observed over the harvest dates. Tissue concentration of NDF was significantly ($p = 0.05$) affected in the three harvest date treatments analyzed. Tissue NDF concentration increased at the third harvest date, but declined thereafter. Partitioning of the variance for harvest date

Table 17. Calculated response functions and their partial coefficients of determination ($r^2_{yx1 \cdot x2}$) and standard errors ($S_{yx1 \cdot x2}$) for tissue crude protein, NDF, P and K concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus paniculatus RCSC.

Nutrient	Effect of harvest date for each fertilizer N rate (kg/ha NH_4NO_3)	Function	$r^2_{yx1 \cdot x2}$	$S_{yx1 \cdot x2}$	Effect of NH_4NO_3 rate for each harvest date (days after germination)	Function	$r^2_{yx1 \cdot x2}$	$S_{yx1 \cdot x2}$		
Crude protein	0	$Y = 295 - 29.1H$	.93	11.8	25	$Y = 259 + .099N^{**}$	.40	10.8		
	150	$Y = 310 - 26.5H$	.88	14.6	35	$Y = 225 + .135N^{**}$	.58	10.2		
	300	$Y = 308 - 21.3H$	.89	11.1	45	$Y = 196 + .172N^{**}$	.67	10.7		
P	Response Surface Function:		$Y = 292 - .061N - 33.4H^2 + .0387NH$		$R^2 = .91$		$Y = 151 + .24N^{**}$		.86	8.5
	0	$Y = 164 + 13.6H - 5.67H^2$	.92	9.5	25	$Y = 177 - 6.12N^{NS}$	.12	14.6		
	150	$Y = 168 + 1.37H - 3.32H^2$	.80	14.5	35	$Y = 190 - 1.67N^{**}$	.71	9.5		
	300	$Y = 164 - 6.17H - 1.82H^2$	.84	11.7	45	$Y = 162 - 1.06N^{**}$	.37	12.5		
	Response Surface Function:		$Y = 182 - .86N + 2.9H^2 - 3.6H^{**}$		$R^2 = .85$		$Y = 134 - .675N^{NS}$		.33	8.5
K	†	$Y = 1465 - 202H + 21.9H^2$	.91	49.8	65	$Y = 94.5 - .275N^{NS}$	.03	13.6		

† Pooled over N levels when N is nonsignificant.

* **, Slope is significant at the .05 and .01 levels of probability, respectively.

NS Slope is nonsignificant.

Δ Standard error = $S_{y \cdot x1 \cdot x2}$.

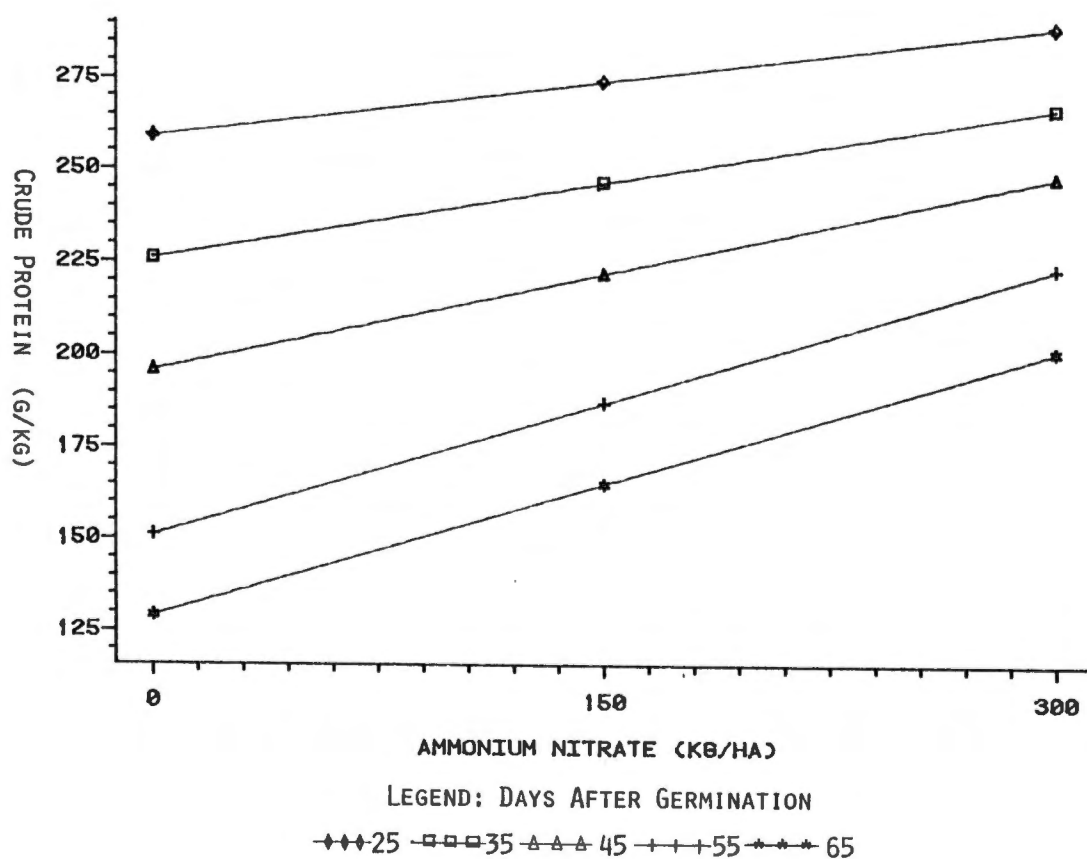


Figure 12. Tissue crude protein response to NH_4NO_3 at each harvest date in *Amaranthus paniculatus* RCSC. Points on the lines are predicted values as calculated from the equations in Table 17.

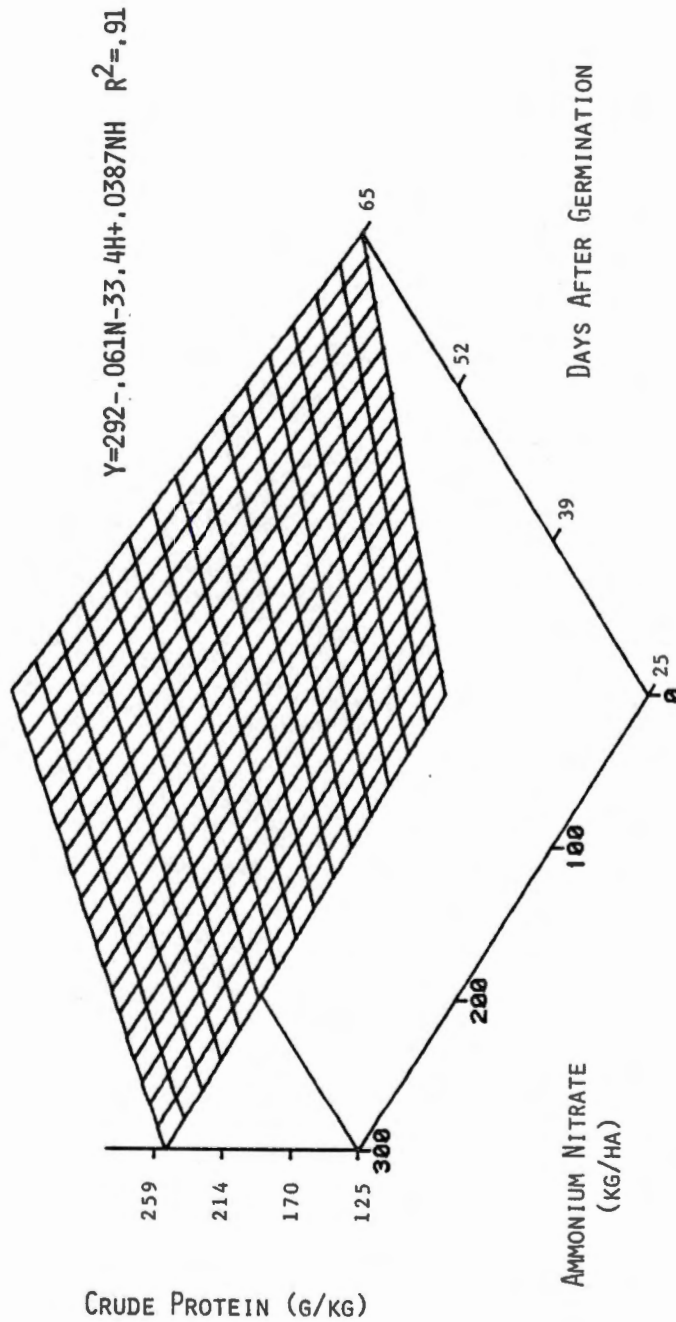


Figure 14. Three dimensional response surface showing the simultaneous effects of the NH_4NO_3 and harvest date treatments on tissue crude protein response in Amaranthus paniculatus RCSC. Points on the surface represent the predicted crude protein response as calculated from the equations in Table 17.

therefore resulted in a predictable, significant quadratic (residual) effect. Partitioning of the variance in tissue P and K concentration resulted in significant linear and quadratic trend effects. The overall effects resulting from the harvest date treatments resulted in a comparatively steep decline in tissue P. The steepest decline in tissue K concentration occurred between the first and third harvests, but thereafter remained relatively constant. Amaranthus paniculatus sustained unusually high levels of tissue K over harvest dates as compared to the other accessions.

Amaranthus blitum cv. Horsetooth RRC #313

Cultivars of Amaranthus blitum have attained horticultural prominence in Asia (60, 91). Unfortunately, its growth habit is regarded by some as being weedy and unattractive. The plants form a caespitose mound of bush-like vegetation about 30 cm deep. This author observed that branching and floral initiation occurred <15 days after germination. The accession RRC #313 was obtained in South-East Asia. Its botanical and agronomic attributes have been described in work by several authors (25, 54, 62).

Components of variance and associated degrees of freedom for tissue crude protein, NDF, P and K are presented in Table 18. Data describing the mean effects resulting from the NH_4NO_3 and harvest date treatments are given in Table 19. Crude protein and NDF were the only components for which an observed significant difference in mean tissue concentration resulted from the NH_4NO_3 treatments. Partitioning of the variance

Table 18. Components of the analysis of variance for tissue crude protein, NDF, P and K concentration for Amaranthus blitum cv. Horsetooth RRC #313.

Source [†]	Degrees of freedom		Nutrient			
			CP	NDF	P	K
Nitrogen (N)	2	2 ^{††}	*	**	NS	NS
Error A	6	6				
Harvest (H)	4	2	**	**	**	**
Nitrogen x Harvest	8	4	NS	**	NS	NS
Error B	36	18				
N Linear	1	1	*	**		
Deviation from Linear	1	1	NS	NS		
H Linear	1	1	**	**	**	**
Quadratic	1	1	**	**	**	**
Cubic	1		*		**	**
Deviation from Cubic	1		NS		NS	*
N Linear x H Linear	1	1	NS	**		
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	*	**		
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	NS	*		

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 19. Observed differences in mean tissue nutrient concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus blitum cv. Horsetooth RRC #313.

Nutrient [†]	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	248	262	260
	35	234	236	260
	45	197	238	263
	55	179	221	243
	65	126	132	173
NDF (g/kg)	25	231	232	238
	45	334	296	239
	65	367	289	204
P (mmol/kg)	25	130	153	142
	35	121	138	120
	45	126	125	126
	55	122	122	126
	65	76	75	72
K (mmol/kg)	25	1116	1075	1108
	35	774	745	824
	45	704	760	833
	55	693	691	742
	65	622	665	766

[†] Nutrient estimates are means of four observations.

in crude protein concentration into single degree of freedom contrasts resulted in a significant ($p = 0.05$) enhancement from the addition of <150 kg/ha NH_4NO_3 . However, the effects from the NH_4NO_3 treatments accounted for only 12.4% of the total variance in crude protein, the weakest response among accessions. Comparison of the mean NDF concentrations resulted in significant differences in tissue NDF levels in response to both the 150 kg/ha and 300 kg/ha NH_4NO_3 treatment doses. All of the components analyzed showed a significant ($p = 0.01$) response to the harvest date treatments. The portion of total variance in tissue crude protein accounted for by harvest date was 63.8%. Except for NDF, none of the components showed significant N x H interaction effects.

Analyses were performed to describe more precisely the observed responses in tissue crude protein and NDF resulting from the NH_4NO_3 treatments. Results from single degree of freedom trend comparisons are presented in Table 18. A significant ($p = 0.05$) positive linear trend in tissue crude protein concentration resulted from the addition of NH_4NO_3 . However, further analysis of these results revealed that the NH_4NO_3 treatments had no significant effect on mean crude protein concentration for three out of the five observed harvest dates (Table 20). Slope estimates for crude protein response at 45 and 55 days after germination were significant, but relatively weak. Overall, the general response to the NH_4NO_3 treatments in cv. Horsetooth was weak and variable. Conversely, the application of NH_4NO_3 resulted in a significant ($p = 0.01$), negative linear trend in tissue NDF concentration. Partitioning of the N x H interaction resulted in a significant ($p = 0.01$) N

Table 20. Calculated response functions and their partial coefficients of determination ($r^2_{yxl \cdot x_2}$) and standard errors ($S_{yxl \cdot x_2}$) for tissue crude protein, NDF, P and K concentration resulting from the NH_4NO_3 and harvest date treatments for Amaranthus blitum cv. Horsetooth RRC #241.

Nutrient	Effect of harvest date for each fertilizer N rate (kg/ha NH_4NO_3)	Function	$r^2_{yxl \cdot x_2}$	$S_{yxl \cdot x_2}$	Effect of NH_4NO_3 rate for each harvest date (days after germination)	Function	$r^2_{yxl \cdot x_2}$	$S_{yxl \cdot x_2}$
Crude protein	0	$Y = 274 - 28.7H + 4.77H^2 - .98H^3$	.50	48.1	25	$Y = 245 + .059NS$	.04	24.1
	150	$Y = 367 - 160H + 63.9H^2 - 8.25H^3$	.82	23.4	35	$Y = 217 + .132NS$	.26	19.7
	300	$Y = 288 - 51.5H + 27.3H^2 - 4.33H^3$	.76	21.3	45	$Y = 166 + .331NS$	.48	30.9
NDF	Response Surface							
	0	$Y = 267 + .215N - 80.2H + 32H^2 = 4.52H^3$	$R^2 = .66$	32.7 ^A	55	$Y = 150 + .32NS$	.41	34.3
	150	$Y = 152 + 87.4H - 8.87H^2$	.78	35.5	65	$Y = 97.3 + .232NS$	.16	48.3
P	Response Surface							
	0	$Y = 175 + 66.2H - 8.65H^2$	.37	42.8	25	$Y = 226 + .04NS$	.07	13
	150	$Y = 225 + 18.4H - 4.5H^2$	.40	23.4	45	$Y = 385 - .475NS$	.69	28.4
K	Response Surface							
	0	$Y = 185 + .224N + 56H - .214NH$	$R^2 = .68$	35.5 ^A	65	$Y = 449 - .815NS$	.69	48.2
	†	$Y = 211 - 104H + 40.9H^2 + 5.09H^3$	.73	14.3				
	†	$Y = 1728 - 852H + 240H^2 - 22.4H^3$	.70	100				

† Pooled over N levels when N is nonsignificant.

*,** Slope is significant at the .05 and .01 levels of probability, respectively.

NS Slope is nonsignificant.

^A Standard error = $S_{y \cdot x_1 x_2}$.

linear x H linear trend effect (for an interpretation of this trend effect, the reader is referred to the explanation presented earlier in this chapter for crude protein in Amaranthus cruentus cv. Fotete or A. dubius).

Partitioning of the variances for harvest date into single degree of freedom trend comparisons resulted in significant ($p = 0.01$), but different effects among the four nutrient components analyzed (Table 20). Tissue crude protein responded by declining as a linear, quadratic and cubic function of harvest date. The magnitude of the observed decline was attenuated from the addition of NH_4NO_3 . Trend effects observed for NDF were significant ($p = 0.01$), positive linear and quadratic (residual) responses, resulting in an overall increase in tissue NDF concentration over the harvest dates. Difference in mean tissue NDF concentration were not significant at 25 days after germination. Thereafter, its concentration generally increased, except for the 300 kg/ha treatment. In this case, a considerable decline in NDF resulted at the fifth harvest date. Harvest date exerted a significant ($p = 0.01$), negative effect on tissue P and K concentration. Significant ($p = 0.01$) harvest linear, quadratic and cubic trend effects were observed for both P and K. In general, the decline in tissue P and K concentration over the harvest dates was relatively steep. A notably precipitous decline in tissue K occurred between the first and second harvest dates.

In Figure 15, the general crude protein responses to NH_4NO_3 at each harvest date as observed in the cv. Horsetooth are presented. Functions

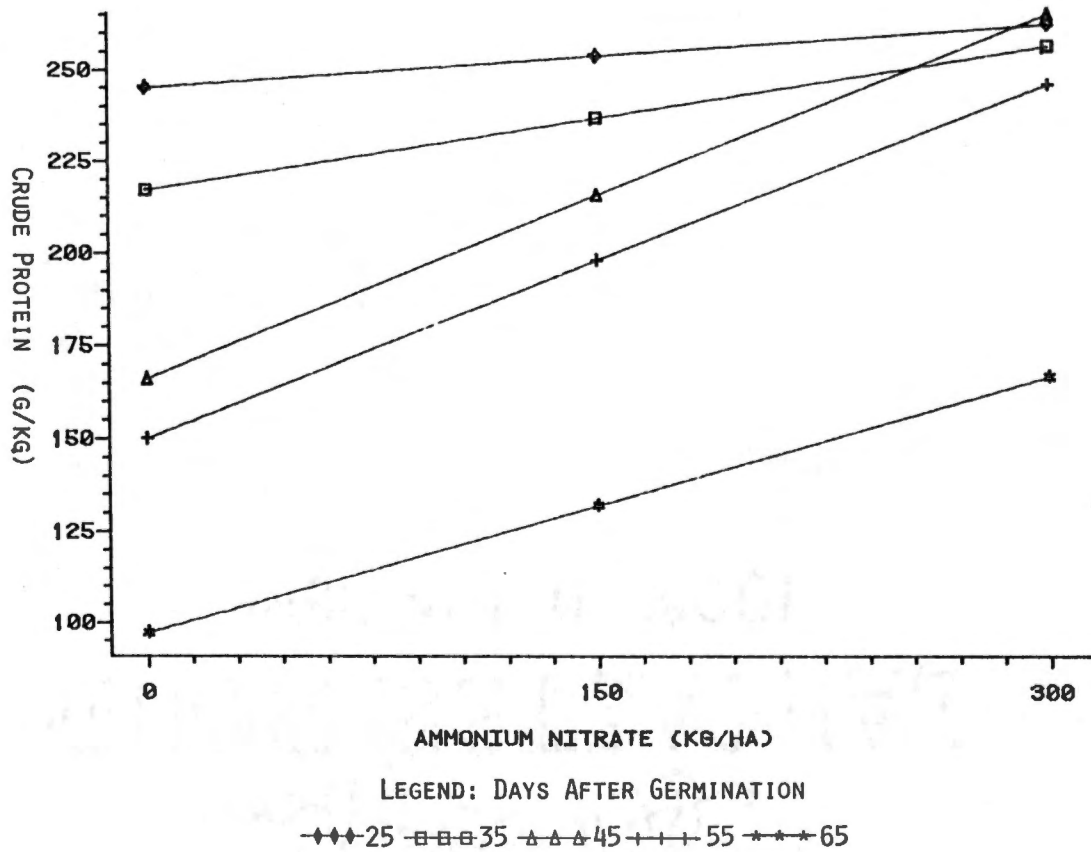


Figure 15. Tissue crude protein response to NH_4NO_3 at each harvest date in *Amaranthus blitum* cv. Horsetooth RRC #313. Points on the lines are predicted values as calculated from the equations in Table 20.

that were used to calculate these regression lines are presented in Table 20. Figure 16 is a plot of the predicted crude protein response at each NH_4NO_3 rate over the harvest dates. From these figures, an interesting relationship can be observed. The addition of NH_4NO_3 enhanced the quadratic and cubic crude protein response appreciably. Compared to these, a visibly quadratic, but attenuated curve resulted from the 0 kg/ha NH_4NO_3 treatment effects. A three dimensional response surface showing the simultaneous effects resulting from the NH_4NO_3 and harvest date treatments appears in Figure 17. The highly significant H quadratic component of variance in tissue crude protein concentration resulted in the "scrolled" conformation of the response surface in three dimensional space.

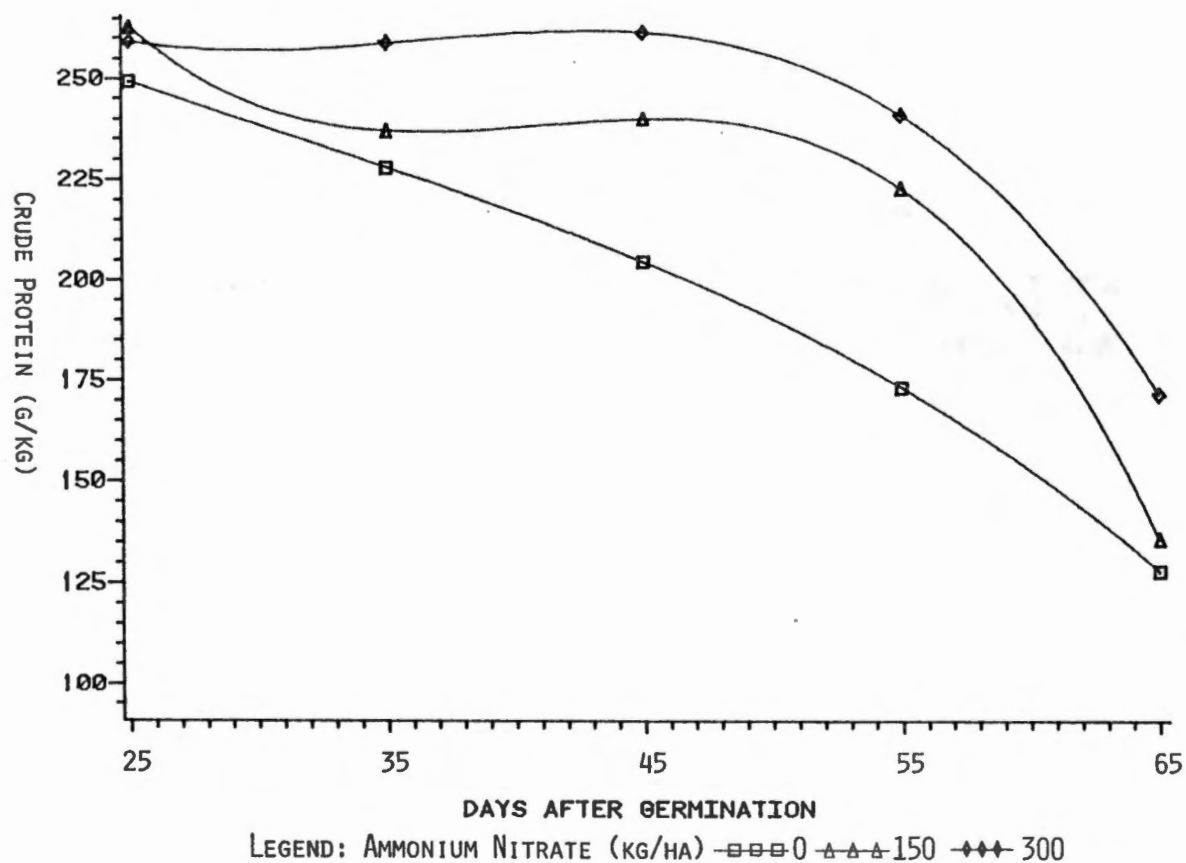


Figure 16. Tissue crude protein response over a harvest interval of 65 days after germination and at three levels of NH_4NO_3 in Amaranthus blitum cv. Horsetooth RRC #313. Points on the lines are predicted values as calculated from the equations in Table 20.

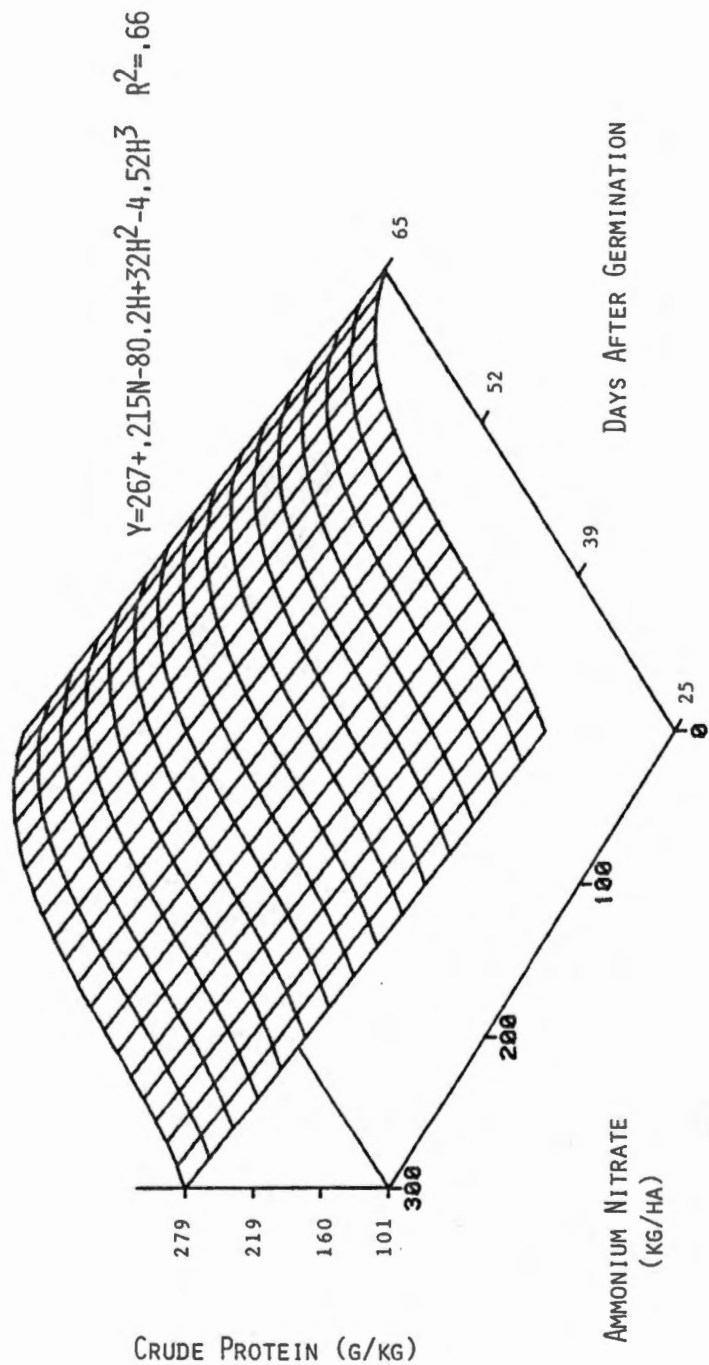


Figure 17. Three dimensional response surface showing the simultaneous effects of the NH_4NO_3 and harvest date treatments on tissue crude protein response in *Amaranthus blitum* cv. Horsetooth RRC #313. Points on the surface represent the predicted crude protein response as calculated from the equations in Table 20.

CHAPTER 5

RESULTS AND DISCUSSION OF LABORATORY CHEMICAL ANALYSES:

GRAIN AMARANTH

Introduction

Laboratory chemical analyses were performed on grain samples obtained from four individual grain amaranth accessions. The objective was to determine the mean differences among accessions and the effects of applied NH_4NO_3 on grain proximate, carbohydrate and mineral composition. Results from the analysis of variance are shown in Table 21. Generally, the proximate, carbohydrate and mineral composition was not significantly affected by the NH_4NO_3 treatments among accessions. Calcium was an exception, and further analyses were performed to investigate the nature of the response. Partitioning of the variance in grain calcium resulted in a significant ($p = 0.01$) linear decline in calcium concentration from the addition of NH_4NO_3 . Additional analyses were performed to determine which among the four accessions was affected. Results from the analysis of variance for each accession are presented in Table 22. Observed mean differences in grain proximate, carbohydrate and mineral composition resulting from the NH_4NO_3 and accession treatments are presented in Tables 23 and 24. Ranked means and results of Duncan's Multiple Range Test (.05 level of probability) are given in Table 25. The results from the analysis of variance in Table 21 indicates that most of the variance in proximate, carbohydrate and mineral composition resulted from differences in mean concentration among

Table 21. Components of the analysis of variance for grain proximate, carbohydrate and mineral composition resulting from the NH_4NO_3 and accession treatments.

Source [†]	Degrees of freedom	g/kg					mmol/kg				
		CP	Ether extract	ASH	Total CH_2O	NFE WSC	P	K	Ca	Mg	Fe
Nitrogen (N)	2	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
Error A	6										
Accession (A)	3	**	**	**	**	**	**	**	**	NS	**
Nitrogen x Accession	6	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
Error B	27										
N Linear	1									**	
Deviation from Linear	1									NS	
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1									**	
150 kg/ha vs. 300 kg/ha NH_4NO_3	1										NS

[†]Error A = block x N; Error B = block x H + block (N x H).

^{††}Degrees of freedom for ether extract, ash, total CH_2O , and NFE.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 22. One-way analysis of variance by accession for grain calcium concentration as influenced by the NH_4NO_3 treatments.

Source	Degrees of freedom	Accession #			
		1023	1964	1011	1044
Nitrogen	2	*	**	**	NS
Nitrogen x Block	9				

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

Table 23. Mean differences in grain crude protein and fiber, ether extract, ash, total CH_2O , NFE and WSC concentration resulting from the NH_4NO_3 and accession treatments.

Nutrient	Accession	Ammonium nitrate (kg/ha)		
		0	150	300
		g/kg		
Crude protein	RRC #82S1023	11.4	11.1	11.9
	NCRPIS #1964	15.6	15.8	16.2
	RRC #82S1011	13.6	13.4	14.0
	RRC #1044	10.8	10.9	12.0
Crude fiber [†]	RRC #82S1023	67.8	56.8	
	NCRPIS #1964	72.2	74.2	
	RRC #82S1011	67.0	61.5	
	RRC #1044	60.2	53.5	
Ether extract [†]	RRC #82S1023	65.1	65.5	
	NCRPIS #1964	66.9	65.9	
	RRC #1011	71.8	69.2	
	RRC #1044	64.7	61.7	
Ash [†]	RRC #82S1023	27.8	27.2	
	NCRPIS #1964	33.2	32.5	
	RRC #1011	28.2	26.2	
	RRC #1044	30.0	30.2	
Total CH_2O [†]	RRC #82S1023	753	752	
	NCRPIS #1964	698	712	
	RRC #1011	732	739	
	RRC #30S1044	767	768	
NFE [†]	RRC #82S1023	719	726	
	NCRPIS #1964	672	669	
	RRC #1011	696	708	
	RRC #1044	738	746	
WSE	RRC #82S1023	13	16.5	18.7
	NCRPIS #1964	14.6	18.7	21.2
	RRC #1011	14.6	8.45	12.1
	RRC #1044	13.4	12.4	11.4

[†] Analysis run for two fertilizer N rates.

Table 24. Mean differences in grain mineral concentration resulting from the NH_4NO_3 and accession treatments.

Nutrient	Accession	Ammonium nitrate (kg/ha)		
		0	150	300
mmol/kg				
Phosphorus	RRC #82S1023	154	182	183
	NCRPIS #1964	217	210	191
	RRC #82S1011	216	234	205
	RRC #1044	235	234	222
Potassium	RRC #82S1023	124	116	110
	NCRPIS #1964	117	126	124
	RRC #82S1011	114	114	115
	RRC #1044	111	113	111
Calcium	RRC #82S1023	49.2	42.8	43.2
	NCRPIS #1964	81	61.5	51.2
	RRC #1011	43.5	35.5	32
	RRC #1044	46.2	47	43.2
Magnesium	RRC #82S1023	133	72	69
	NCRPIS #1964	92.5	92.2	88
	RRC #1011	77.5	74.8	73.8
	RRC #1044	78.8	77.5	75.8
Iron	RRC #82S1023	1.28	1.50	1.23
	NCRPIS #1964	.98	1.40	.95
	RRC #1011	1.58	1.64	2.2
	RRC #30S1044	2.80	3.30	4.5

Table 25. Ranked means and results of Duncan's Multiple Range Test for significant differences in proximate, carboxydrate and mineral composition among four grain amaranth accessions.

g/kg					mmol/kg						
CP	CF	Ether extract	ASH	Total CH ₂ O	NFE	WSC	P	K	Ca	Mg	Fe
(2)159a ^{†,††}	(2)73.2a	(3)70.5	(2)32.9a	(4)76.7a	(4)742a	(2)18.2a	(4)230a	(2)122a	(2)64.6a	(1)91.2a	(4)3.54a
(3)137b	(3)64.2b	(2)66.4	(4)30b	(1)75.2a,b	(1)722b	(1)16.1a,b	(3)218a	(1)116b	(4)45.5b	(2)90.9a	(3)1.82b
(1)114c	(1)62.2b,c	(1)65.3	(1)27.5c	(3)73.6b	(3)702c	(4)12.4b	(2)206a	(3)114b	(1)45.3b	(4)75.3a	(1)1.34b
(4)112c	(4)56.9c	(4)63.2	(3)27.2c	(2)70.5c	(2)671d	(3)11.7b	(1)173b	(4)112b	(3)36.9c	(3)77.3a	(2)1.11b

[†]Numbers in parentheses designate accession as: (1) A. hypochondriacus RRC #82S1023; (2) A. cruentus cv. Fotete NCRPIS #1964; (3) A. cruentus RRC #1011; (4) A. hybridus RRC #1044.

^{††}Means with the same letter are not significantly different at the .05 level of probability.

accessions. Further investigation of these results, therefore, was done separately for each accession.

Amaranthus hypochondriacus RRC #1023

With its center of origin in Mexico, this species has evolved two morphologically distinct types. Kauffman (77) distinguished a "Mexican grain type" and "Nepal grain type" within the species. Biosystematic, ethnographic, agronomic and nutritional attributes of Amaranthus hypochondriacus have been reviewed by various workers (54, 57, 68, 76, 77, 78). The accession #1023 was obtained from Mexico.

Results presented in Table 25 show that crude protein, fiber, fat and mineral (ash) concentration in accession #1023 ranked relatively low (referring hereafter to third ranked). Significant differences in the concentration of these nutrient, as determined by DMRT, are indicated in Table 25. Total CH_2O , NFE, and WSC concentration was relatively high (referring hereafter to second ranked). Mineral compositional analyses for Amaranthus hypochondriacus revealed that grain P concentration was ranked lowest and significantly different from the other accessions. Calcium and Fe concentration was relatively low, while Mg was ranked highest. However, none of the mean differences in Mg concentration among accessions was declared significantly different. A significant ($p = 0.01$) decline in Ca concentration resulted from the NH_4NO_3 treatments, but mean differences between the 150 kg/ha and 300 kg/a treatments were not significant (Table 26).

Table 26. Ranked means and results of Duncan's Multiple Range Test for significant differences in grain calcium concentration resulting from the application of NH_4NO_3 .

Calcium (mmol/kg)			
RRC #82S1023	NCRPIS #1964	RRC #82S1011	RRC #1044
(1)49.3a ^{†,††}	(1)81a	(1)43.5a	(2)47.1a
(3)43.6b	(2)61.4b	(2)35.4b	(1)46.2a
(2)43b	(3)51.4b	(3)32.0b	(3)43.4a

[†]Number in parentheses designates fertilizer N rate as: 1 = 0 kg/ha; 2 = 150 kg/ha; 3 = 300 kg/ha NH_4NO_3 .

^{††}Means with the same letter are not significantly different at the .05 level of probability.

Amaranthus cruentus cv. Fotete NCRPIS #1964

A description of this accession, its origin and uses has been provided in the second section of Chapter 4. Results of DMRT performed on grain compositional data showed that crude protein, fiber, ash and WSC concentration ranked highest in cv. Fotete. Observed mean P concentration was relatively low. Mean concentration of Mg and Fe were relatively high and lowest among accessions, respectively. In general, the ranges in mean concentration for the two nutrients were not large, and few significant differences were detected (except Fe). Mean K and Ca concentration was ranked highest. A significant ($p = 0.01$) decline in mean grain calcium concentration resulted from the application of NH_4NO_3 (Table 26).

Amaranthus cruentus RRC #1011

A description of this accession has been provided in the fifth section of Chapter 4. Results of the DMRT performed on the grain compositional data revealed that mean crude protein, fiber and fat concentration were relatively high, while ash and WSC were ranked lowest in accession #1011. Mean concentration of total CH_2O and NFE were relatively low. Significant differences in mean concentration of these nutrients were observed. Mean concentration of P and Fe were relatively high, whereas the concentration of K and Mg were relatively low and lowest among accessions, respectively. Calcium concentration was ranked lowest among accessions. A significant ($p = 0.01$) decline in calcium concentration resulted from the application of NH_4NO_3 (Table 26).

Amaranthus hybridus RRC #1044

Kauffman (78) has classified this species as a "short, early grain type." Compared to other domesticated grain amaranths, Amaranthus hybridus was diminutive, but variations in plant height within the species were considerable. Personal observation by this author confirmed a certain degree of introgression from wild, locally growing amaranths (pigweeds) in Tennessee. Dark-brown seeded plants were commonly encountered at harvest.

As a likely progenitor of the domesticated Amaranthus cruentus, A. hybridus has been utilized as both a grain and vegetable resource. Little information is available to assess the extent and range of its use. The accession #1044 was obtained in Mexico. Biosystematic, ethnographic, agronomic and nutritional information on this accession have been presented in work by several authors (54, 57, 76, 77, 78, 118, 153).

Results of the DMRT performed on the grain compositional data revealed that crude protein, fiber and fat concentration was ranked lowest in RRC #1044. Ash concentration was relatively high. Mean total CH_2O and NFE were highest among accessions, while WSC concentration was relatively low. Observed significant differences for these nutrients are presented in Table 25. Mean P and Fe concentrations were highest among accessions. An unusually high grain Fe concentration was observed in RRC #1044. Mean K and Mg concentrations were ranked as lowest and relatively low, respectively. Mean calcium concentration was

relatively high. Although a slight decline in grain calcium concentration resulted from the NH_4NO_3 treatments, Amaranthus hybridus was the only accession for which this decline was not significant (Table 26).

CHAPTER 6

COMBINED ANALYSES: RESULTS AND DISCUSSION

Introduction

In the preceding chapter, data from individual amaranth accessions were analyzed separately to investigate the response in leaf nutrient composition from NH_4NO_3 and harvest date treatments. This was done to facilitate comparison of response among accessions. However, additional insight can be gained by analyzing the response among accessions on the generic, rather than specific, level of observation. The accessions utilized in this study were chosen to reflect the broad geographic and genetic spectrum of the genus. Further analyses were performed, therefore, to estimate the magnitude of uniformity and/or divergence in response to the treatment factors for the accessions as a group. Occasional reference to individual accessions will be made for clarity of interpretation.

A three way analysis of variance was performed to partition the variance in leaf nutrient concentration resulting from the NH_4NO_3 , accession and harvest date treatments. Components of variance and associated degrees of freedom for tissue crude protein, NDF, P and K concentration are presented in Table 27. The observed mean differences in tissue crude protein, NDF, P and K concentration for the accession x nitrogen (A x N), nitrogen x harvest (N x H), and accession x harvest (A x H) interactions are presented in Tables 28, 29 and 30,

Table 27. Results of the combined three-way analysis of variance for tissue crude protein, NDF, P and K concentration as influenced by the NH_4NO_3 , accession and harvest date treatments.

Source [†]	Degrees of freedom		Nutrient			
			CP	NDF	P	K
Nitrogen (N)	2	2 ^{††}	**	**	NS	NS
Error A	6	6				
Accession (A)	5	5	**	**	**	**
Nitrogen x Accession	10	10	NS	**	NS	*
Error B	45	45				
Harvest (H)	4	2	**	**	**	**
Nitrogen x Harvest	8	4	**	*	*	*
Harvest x Accession	20	10	**	**	**	**
Nitrogen x Accession x Harvest	40	20	NS	**	NS	NS
Error C	213	104				
N Linear	1	1	**	**		
Deviation from Linear	1	1	NS	NS		
H Linear	1	1	**	**	**	**
Quadratic	1	1	NS	**	**	**
Cubic	1		**		**	**
Deviation from Cubic	1		**		NS	**
0 kg/ha NH_4NO_3 vs. 150 kg/ha and 300 kg/ha NH_4NO_3	1	1	**	**		
150 kg/ha vs. 300 kg/ha NH_4NO_3	1	1	*	**		

[†]Error A = block x N; Error B = block x A + block (N x A); Error C = block x H + block (N x H) + block (A x H) + block (N x A x H).

^{††}Degrees of freedom for NDF.

*,** F statistic significant at the .05 and .01 level of probability, respectively.

NS = F statistic nonsignificant.

Table 28. The general response in mean tissue crude protein, NDF, P and K concentration resulting from the NH_4NO_3 treatments for six vegetable amaranth accessions.

Nutrient	Accession	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	NCRPIS #1964	217	251	268
	NCRPIS #2014	158	212	226
	RRC #241	172	209	227
	RRC82S1011	186	225	237
	RCSC	208	231	243
	RRC #313	197	218	240
NDF (g/kg)	NCRPIS #1964	324	300	266
	NCRPIS #2014	251	254	251
	RRC #241	274	245	239
	RRC82S1011	277	256	242
	RCSC	296	296	277
	RRC #795S313	310	272	227
P (mmol/kg)	NCRPIS #1964	140	138	127
	NCRPIS #2104	145	149	143
	RRC #241	170	158	151
	RRC #82S1011	153	166	156
	RCSC	142	135	125
	RRC #313	115	123	117
K (mmol/kg)	NCRPIS #1964	981	1027	930
	NCRPIS #2014	873	854	860
	RRC #241	882	836	811
	RRC #82S1011	982	952	871
	RCSC	1128	1087	1087
	RRC #313	782	787	854

Table 29. The general response in mean tissue crude protein, NDF, P and K concentration among six vegetable amaranth accessions resulting from the NH_4NO_3 and harvest date treatments.

Nutrient	Days after germination	Ammonium nitrate (kg/ha)		
		0	150	300
Crude protein (g/kg)	25	261	279	285
	35	224	248	260
	45	176	227	241
	55	162	208	236
	65	131	159	185
NDF (g/kg)	25	264	252	241
	45	304	282	262
	65	298	278	249
P (mmol/kg)	25	156	159	154
	35	159	149	132
	45	146	156	139
	55	141	144	144
	65	119	118	113
K (mmol/kg)	25	1159	1181	1200
	35	902	866	819
	45	917	905	865
	55	869	851	810
	65	844	816	809

Table 30. The general response in mean tissue crude protein, NDF, P and K concentration among six vegetable amaranth accessions resulting from the harvest date treatments.

Nutrient	Accession	Days after germination				
		25	35	45	55	65
Crude protein (g/kg)	NCRPIS #1964	293	272	243	227	191
	NCRPIS #2014	261	228	186	196	134
	RRC #241	268	203	201	191	150
	RRC #1011	286	264	193	182	155
	RCSC	278	253	231	199	177
	RRC #313	257	244	233	214	144
NDF (g/kg)	NCRPIS #1964	266		256		275
	NCRPIS #2014	288		282		306
	RRC #241	335		220		287
	RRC #1011	228		256		234
	RCSC	261		267		290
	RRC #313	261		254		287
P (mmol/kg)	NCRPIS #1964	152	136	143	127	118
	NCRPIS #2014	165	151	139	144	130
	RRC #241	155	164	170	172	138
	RRC #1011	160	146	163	172	150
	RCSC	164	156	140	121	89
	RRC #313	142	126	126	123	74
K (mmol/kg)	NCRPIS #1964	1207	932	951	952	855
	NCRPIS #2014	1146	779	798	759	829
	RRC #241	1151	720	896	721	727
	RRC #1011	1174	863	885	891	860
	RCSC	1308	1099	1066	1046	984
	RRC #313	1099	782	765	709	684

respectively. As only two of the six accessions were analyzed for tissue nitrates, a combined analysis for this nutrient was not performed.

Crude Protein

Overall, the combined analysis indicated a significant ($p = 0.01$) enhancement in amaranth leaf crude protein concentration resulted from the application of NH_4NO_3 . Among accessions, significant ($p = 0.05$) differences in mean crude protein concentration were observed. Results of this comparison are shown in Table 31. Pooled over accessions and harvest dates, the portion of total variance in tissue crude protein concentration resulting from the NH_4NO_3 treatments was 16.9%. The effect of the applied NH_4NO_3 resulted in a general enhancement of leaf crude protein concentration. This enhancement increased in magnitude as a function of leaf age of harvest. The significant, positive response to NH_4NO_3 in amaranth was expected, and should not be construed as a major finding in this study. However, the magnitude of the response to NH_4NO_3 among accessions varied considerably. For example, in Amaranthus dubius the proportion of the variance in tissue crude protein concentration resulting from the NH_4NO_3 treatments (26.4%) was greater than that observed in the other accessions (Table 32). The weakest overall response was observed in A. paniculatus and A. dubius cv. Horsetooth (13.4% and 12.5% of the total crude protein variance accounted for by the NH_4NO_3 treatments, respectively). On the other hand, the overall

Table 31. The overall mean tissue crude protein, NDF, P and K concentration among the six vegetable amaranth accessions.

Nutrient	Accession	Mean [†] concentration
Crude protein (g/kg)	NCRPIS #1964	245a
	RCSC	228b
	RRC #313	218b,c
	RRC #1011	216c
	RRC #241	203d
	NCRPIS #2014	198d
NDF (g/kg)	NCRPIS #1964	296a
	RCSC	290a
	RRC #313	270b
	RRC #1011	257c
	NCRPIS #2014	255c
	RRC #241	253c
P (mmol/kg)	RRC #241	160a
	RRC #1011	158a
	NCRPIS #2104	145b
	NCRPIS #1964	135c
	RCSC	134c
	RRC #313	118d
K (mmol/kg)	RCSC	1101a
	NCRPIS #1964	979b
	RRC #1011	934c
	NCRPIS #2014	844d
	RRC #241	843d
	RRC #313	808d

[†]Means within nutrients followed by the same letter are not significantly different as determined by DMRT (.05 level of probability).

Table 32. The percentage of variance in tissue crude protein and NO_3^- concentration resulting from the NH_4NO_3 , harvest date, and nitrogen x harvest interaction effects. Variances are ranked by magnitude.

Source	Accession	CP	NO_3^-
Nitrogen	NCRPIS #2014	26.4	20.3
	NCRPIS #1964	24.7	42.6
	RRC #241	24.6	
	RRC #1011	15.0	
	RCSC	13.4	
	RRC #313	12.5	
Harvest	RCSC	81.9	
	RRC #1011	81.3	
	NCRPIS #1964	68.0	39.2
	RRC #241	66.6	
	RRC #313	63.8	
	NCRPIS #2014	56.1	61.4
Nitrogen x Harvest	NCRPIS #2014	7.78	13.4
	NCRPIS #1964	4.29	7.68
	RRC #313	4.21	
	RRC #241	2.97	
	RCSC	1.81	
	RRC #1011	1.52	

mean crude protein concentration in A. dubius ranked lowest among accessions, while that of A. paniculatus and A. blitum cv. Horsetooth ranked relatively high. Averaged over a harvest period of 55 days, the difference in crude protein concentration obtained by the addition of 150 kg/ha NH_4NO_3 was 25.5% of the 0 kg/ha control (Table 28). This falls considerably short of the two-fold increase in crude protein reported by Stafford et al. (146) at the same N fertilization rate using Amaranthus hybridus cv. Green Head (East Africa). Subbiah and Ramanathan (147), in work reported from South India using A. flavus (= A. dubius¹), observed a much smaller response in crude protein concentration over five (0-80 kg/ha N) levels of nitrogenous fertilizer. These workers reported a mean crude protein difference of 23.1% between 0 and 80 kg/ha N treatments. This estimate is in closer agreement with the results for A. dubius observed in this study.

To more precisely describe the nature of the variance in tissue crude protein concentration among accessions, single and multiple degree of freedom contrasts were constructed to partition the variance in NH_4NO_3 , harvest date, N x A, H x A interactions into linear, quadratic and cubic trend components. Results of the analysis are presented in Table 33. Generally, tissue crude protein concentration among accessions was enhanced as a linear function of the NH_4NO_3 treatment doses.

¹Amaranthus flavus has also been classified as A. hypochondriacus. Identification of Subbiah and Ramanathan's A. flavus Co. 1 as A. dubius was made from photographs of the same cultivar appearing in a paper by Kamalanathan et al. (75).

Table 33. Components of the analysis of variance performed for the Nitrogen x Accession (N x A) and Harvest x Accession (H x A) interaction effects.

Source [†]	Degrees of freedom	Nutrient				
		CP	NDF	P	K	NO ₃ ⁻
Accession (A) x Nitrogen (N)	17					
Accession	5	**	**			
A x N Linear	6	**	**			
A x N Deviation from Linear	6	NS	NS			
Accession (A) x Harvest (H)	29					
Accession	5	**	**	**	**	
A x H Linear	6	**	**	**	**	
A x H Quadratic	6	*	**	**	**	
A x H Cubic	6	**		**	**	
A x H Deviation from Cubic	6	*		NS	**	
A x H Quadratic [(#1011, #313 vs. #1964, #241, #2014, RCSC)] x H _Q	6					
	1	**				
A x H Cubic [(#241, #2104, #313 vs. #1964, #1011, RCSC)] x H _C	6					
	1	**				

[†]Computed by GLM by setting model = A x N, A x H and partitioning the components of variance for all interaction effects.

*,** F statistic significant at the .05 and .01 levels of probability, respectively.

NS = F statistic nonsignificant.

The strength of the response was variable, depending on plant maturity and accession. For example, the significant ($p = 0.05$) N deviation from linear observed for Amaranthus dubius suggests that the response to NH_4NO_3 in this species may be nonlinear. Further investigations using a different set of N treatments would be needed to estimate this response. Similarly, the strength of linear response to the NH_4NO_3 varied as a function of leaf age at harvest. For most accessions, response to NH_4NO_3 was relatively weak before 35 days after germination. Hence, differences in tissue crude protein among N levels usually did not vary appreciably at the early stages of harvest.

Except for Amaranthus dubius, deviations from a linear response to NH_4NO_3 were not significant. This is reflected in the nonsignificant A x N interaction in the analysis of variance. The magnitude of linear response among accessions can be observed from partitioning the A x N interaction into multiple degree of freedom contrasts as shown in Table 34. Interpretation of these contrasts was made as follows. Of the total variance in tissue crude protein concentration, 34.1% of this resulted from differences in magnitude of the linear response to NH_4NO_3 among accessions. Differences in magnitude of linear response can be observed in Figure 18. Nearly 62% of the variation resulted from a positive, linear response in tissue crude protein concentration among accessions. The deviation from linear response was nonsignificant. Response functions were calculated to estimate the pooled harvest date response resulting from the NH_4NO_3 treatments among accessions. This was done to facilitate comparisons of the general NH_4NO_3 response among

Table 34. The percentage of variance in tissue crude protein and NO_3^- concentration resulting from the NH_4NO_3 , harvest date and Nitrogen x Harvest (N x H) interaction effects.

Source	% of Variance accounted for by individual nutrients for each component			
	CP	NDF	P	K
A x N				
Accession	31.4	43.9		
A x N Linear	61.9	54.4		
A x N Deviation from Linear	3.95	1.66		
Total Variance	99.9	99.9		
A x H				
Accession	12.8	40.1	41.4	36.2
A x H Linear	79.7	39.5	43.9	37.4
A x H Quadratic	2.46	20.4	7.55	13.6
A x H Cubic	3.00		6.20	7.54
A x H Deviation from cubic	1.94		.94	5.22
Total Variance	99.9	100	99.9	99.9

accessions. These equations, together with a plot of their predicted response values, are presented in Figure 18.

Partitioning of the variance for harvest date among accessions resulted in significant ($p = 0.01$) linear, cubic and quartic (residual) trend components. Generally, tissue crude protein concentration among accessions declined as a linear function of harvest date. The linear component accounted for 97.8% of the explained variance in crude protein concentration over harvest dates. A small (<2%) though significant ($p = 0.01$) variance resulted from cubic and quartic trend effects. The cubic and quartic variances were restricted to the indeterminate-like vegetable amaranths such as Amaranthus dubius, A. tricolor and A. blitum. Among the determinate grain types, this cubic response was not observed. An understanding of the nature of this differential response can be gained by comparing the growth habits of vegetable amaranths vs. grain amaranths. Typically, vegetable amaranths possess an indeterminate-like growth habit. That is to say, prolonged meristematic activity results in continuous production of new, succulent growth, with floral buds arising directly in the leaf axils (25, 62). Regenerative growth is therefore possible and may occur continuously throughout the season. Hence, ratooning of this crop is a recommended practice (38, 51, 79, 97). The active regeneration of plant parts in vegetable amaranths probably results in a remobilization of plant N and/or enhanced uptake of NO_3^- and other nutrients during periods of regrowth. As a consequence, plant leaf protein levels are likely to fluctuate in a significant, though unpredictable way. Such fluctuations could result in cubic

and quartic trend effects, as was observed in some of the vegetable amaranths studied. On the other hand, grain amaranths are mainly determinate. These plants have a main stem axis that does not remain indefinitely vegetative, but terminates in a large, branched inflorescence. Continuous plant regrowth during the season normally does not occur. In such plants, leaf nutrient concentration is likely to follow a more predictable pattern of change over time. Field crops such as corn and wheat, both of which are botanically determinate, fall into this category. Hence the absence of any "cubic" phenomenon in the coarse leaved grain amaranths. Leaf crude protein decline was typically an uninterrupted linear or quadratic function of time. Interestingly, Subbiah and Ramanathan (147) observed similar fluctuations in crude protein concentration in A. flavus, a vegetable type. These authors rather vaguely ascribed this "paradoxical" effect to a difference in dry matter yields at different harvest dates. Results obtained from this study would suggest that a difference in growth habit rather than dry matter accumulation as the source of this unexpected variation in crude protein concentration.

Partitioning of the variance for the A x H interaction into multiple degree of freedom contrasts resulted in five significant variance components (Table 32). Among accessions, 12.8% of the variance in tissue crude protein concentration resulted from differences in magnitude of linear decline over the harvest dates. Differences in linear decline can be observed from the graph of the general harvest response presented in Figure 19. Approximately 80% of the variance in tissue crude protein

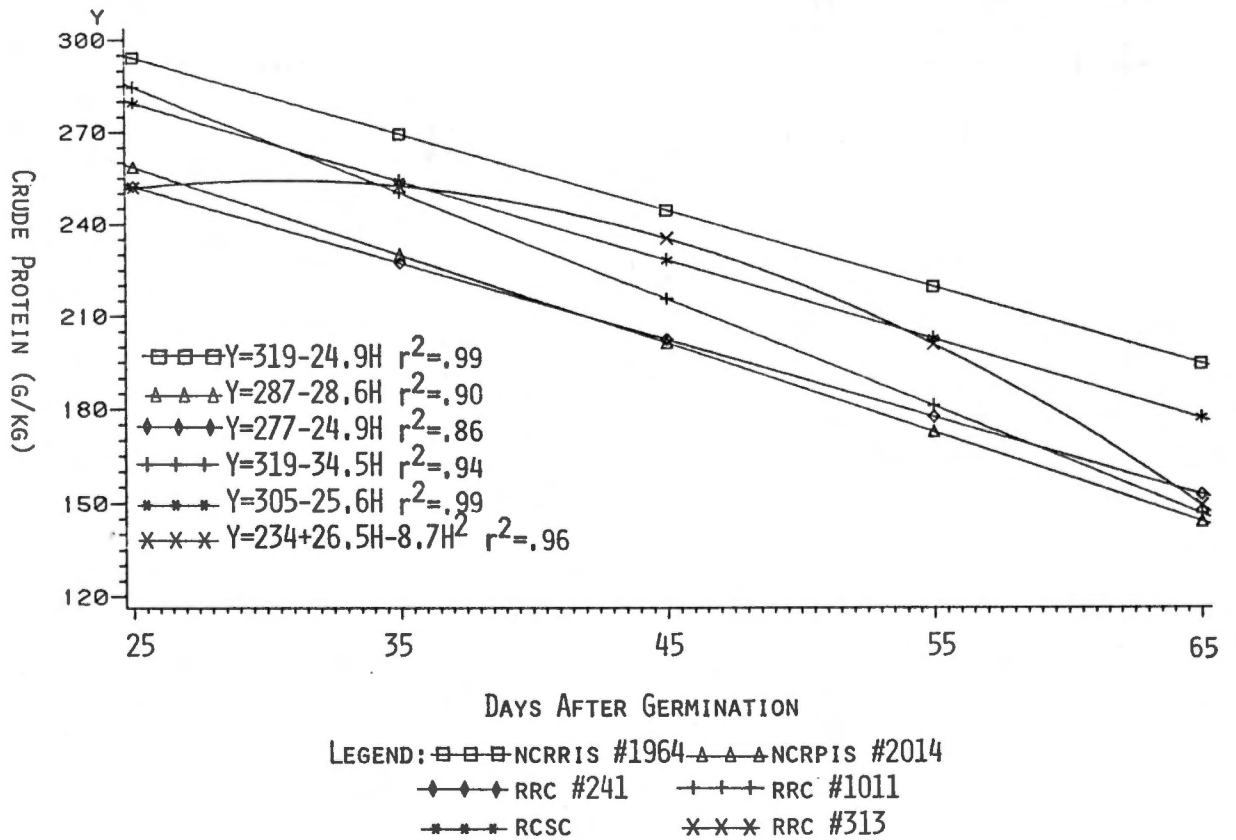


Figure 19. The general tissue crude protein response over a harvest interval of 65 days after germination among the six vegetable amaranth accessions.

concentration among accessions was a function of linear decline over the harvest dates. The A x H quadratic component was significant ($p = 0.05$) but small as compared to the linear response. Further partitioning of this variance revealed a significant ($p = 0.01$) difference in the mean quadratic response of Amaranthus cruentus #1011 and A. blitum cv. Horsetooth vs. the others. Similarly, partitioning the variance of the A x H cubic effect resulted in a significant ($p = 0.01$) difference in the mean cubic response of A. tricolor, A. dubius and A. blitum cv. Horsetooth (indeterminate-like amaranths) vs. the others (determinate grain types). Response functions were calculated to estimate the pooled NH_4NO_3 response over the harvest dates among accessions. Slope estimates for these general functions, and a plot of their predicted response values, appear in Figure 19.

Neutral Detergent Fiber

Among the accessions studied, a significant ($p = 0.01$) decline in tissue NDF concentration resulted from the effects of the NH_4NO_3 treatments. Significant mean differences in tissue NDF concentration were observed among accessions. Results of this comparison are presented in Table 31. Pooled over accessions and harvest dates, the portion of total variance in tissue NDF concentration resulting from the NH_4NO_3 treatments was 15.7%.

Addition of NH_4NO_3 generally resulted in a decline in the concentration of tissue NDF, but the magnitude of decline among accessions was not uniform. In two accessions, Amaranthus dubius and A. paniculatus,

no significant differences in tissue NDF concentration resulted from the addition of NH_4NO_3 . These general results are confirmed by partitioning the variance for the A x N interaction (Table 32). Variability in the magnitude of response among accessions was considerable (43.9% of the N x A variance), as was noted above. Leaf NDF concentration, when significantly affected, declined as a linear function of the increasing NH_4NO_3 treatment doses (54.4% of the N x A variance). These results generally support Ramachandra's (124) observation of an inverse relationship between concentration of plant fiber in A. gangeticus and N supply. The results presented herein suggest that this relationship may extend to other species of Amaranthus.

Partitioning of the tissue NDF variance for harvest date resulted in significant ($p = 0.01$) H linear and quadratic (residual) trend components. Overall, the response from tissue NDF over harvest dates was not well defined. For example, in three accessions, Amaranthus cruentus cv. Fotete, A. dubius and A. blitum cv. Horsetooth, a significant linear increase in tissue NDF concentration occurred over a harvest interval of 65 days after germination. But NDF concentration in A. cruentus #1011 and A. paniculatus changed little over time. An actual decline in tissue NDF concentration occurred over the analyzed harvest dates in A. tricolor. As no clearcut distinction in response among accessions was observed, further interpretation of these results was not made.

Phosphorus

Tissue P concentration was affected very little as a result of the NH_4NO_3 treatments. Results from the combined analyses indicated no overall differences in P concentration resulted from the NH_4NO_3 treatments. Among accessions, significant ($p = 0.05$) differences in mean tissue P concentration were observed (Table 31, p. 107). Partitioning the variance in tissue P concentration over harvest dates resulted in significant ($p = 0.01$) H linear and quadratic trend components. Partitioning the variance for the A x H interaction revealed the 41.4% of the variance in tissue P concentration resulted from differences in magnitude of P decline among accessions. Forty-four percent of that variability in magnitude resulted from a linear decline in tissue P concentration over the harvest dates. A small, though significant ($p = 0.01$) portion of the variance in tissue P concentration resulted from quadratic and cubic trend effects. Overall, tissue P concentration in amaranth declined over the harvest dates, but less precipitously than crude protein, K or NO_3^- .

Potassium

No overall significant differences in tissue K concentration resulted from the NH_4NO_3 treatments. Among accessions, significant differences in mean tissue K concentration were observed (Table 31). On the other hand, decline in tissue K concentration over harvest dates was often precipitous, mainly at the early stages of harvest. Partitioning the harvest date variance in tissue K concentration resulted in

significant ($p = 0.01$) linear, quadratic, cubic and quartic (residual) trend effects. Partitioning of the A x H interaction variance further revealed that 36.2% of the variance in tissue K concentration resulted from differences in magnitude of decline in tissue K over the harvest dates. The A x H linear component accounted for 37.4% of the total A x H variance. The A x H quadratic effect accounted for 13.6% of the A x H variance, while the cubic and quartic effects accounted for 7.55% and 5.22% of the A x H variance, respectively. A consideration of the observed results for tissue K concentration in the individual amaranth accessions will help with the interpretation of these trend effects.

Compared to tissue crude protein, decline in K concentration departed considerably from a linear trend. This is indicated by the relatively smaller (79.7% vs. 37.5% A x H linear variance for K vis à vis crude protein. The A x H quadratic effect was greater for tissue K than for crude protein (13.6% vs. 2.46%). Difference in magnitude of the A x H quadratic effect indicate a fundamental difference in the curvature of the regression lines for K vis à vis crude protein. Individual response functions calculated for K vs. harvest dates presented in Chapter 4 clearly point to this difference. The A x H cubic effect resulted mainly from fluctuations in tissue K concentration observed at various harvest dates.

CHAPTER 7

GENERAL RESULTS AND CONCLUSIONS

General Discussion and Results

Response in leaf tissue nutrient composition of selected amaranth accessions as influenced by the application of nitrogenous fertilizer and harvest date was studied. Three rates (0, 150 and 300 kg/ha) of NH_4NO_3 were used to determine their effects on tissue crude protein, NDF, P, K and water soluble NO_3^- concentration in selected amaranth accessions. Grain samples were obtained from four of the selected accessions to determine the effects of NH_4NO_3 treatments and accession on grain proximate composition, carbohydrate and mineral composition. Five harvest dates (25, 35, 45, 55 and 65 days after germination) were used to determine the effects of plant maturity on leaf nutrient composition in six amaranth accessions. The response among accessions was compared and assessed.

Results of laboratory chemical analyses of vegetable tissue from six amaranth accessions indicated that increased leaf crude protein concentration from the application of NH_4NO_3 resulted in enhanced nutritional value of the amaranths. Among six accessions, significant differences in mean crude protein concentration were observed. Generally, tissue crude protein concentration was enhanced as a linear function of increasing NH_4NO_3 doses. Strength of the response varied, depending mainly on age of the plant at harvest. For example, slope estimates for crude protein response vs. N linear were typically smaller in magnitude

before 35 days after germination. Overall, there was a trend toward enhanced N absorption as the plants matured. The crude protein response observed in Amaranthus dubius appeared to be enhanced as a quadratic function of the increasing NH_4NO_3 doses. This was indicated by the significant N quadratic (residual) trend effect.

Results of analyses performed to assess the effect of harvest date for each NH_4NO_3 rate indicated that crude protein concentration in the indeterminate-like amaranths (Amaranthus dubius, A. tricolor and A. blitum cv. Horsetooth) declined as a linear, quadratic or cubic function of harvest date. In some instances, a significant quartic (residual) component was observed. On the other hand, tissue crude protein concentration in the determinate grain amaranths (A. cruentus cv. Fotete, A. cruentus #1011, and A. paniculatus) declined as a linear or quadratic function of harvest date. The cubic response was not observed in this group. A significant difference in the mean cubic response of A. dubius, A. tricolor and A. blitum cv. Horsetooth vs. the other accessions was observed. Likewise, a significant difference in the mean quadratic response of A. cruentus #1011 and A. blitum cv. Horsetooth vs. the other accessions was observed. It was concluded that differences in plant growth habit (indeterminate-like for vegetable types vs. determinate for grain types) accounted for the divergence in response among accessions. Continuous regrowth during the season for vegetable amaranths would probably result in levels of leaf nutrients that fluctuate in a significant, though unpredictable way. Attempts to accurately predict responses of leaf nutrient concentrations as a function of

fertilizer inputs and plant maturity at harvest are likely to be more difficult in these cultivars.

A general decline in tissue NDF concentration resulted from the addition of NH_4NO_3 , but the magnitude of decline among accessions was not uniform. Concentration of tissue NDF was not significantly affected by the NH_4NO_3 treatments in two accessions, Amaranthus dubius and A. paniculatus. Tissue NDF concentration, when significantly affected, declined as a linear function of the NH_4NO_3 treatments doses. Among accessions, significant differences in mean tissue NDF concentration were observed. A linear inverse relationship between leaf fiber concentration and N supply was significant among four amaranth accessions.

Results of analyses performed to estimate the effect of harvest date on leaf NDF concentration resulted in significant linear and quadratic (residual) trend components. Overall, the response of tissue NDF concentration at three of the five harvest dates was not well defined. As no clearcut distinctions in response among cultivars was seen, no conclusions about the effect of harvest date on tissue NDF concentration were made.

Except in Amaranthus paniculatus, tissue P concentration was not significantly affected by the NH_4NO_3 treatments. Among accessions, significant differences in mean tissue P concentration were observed. Analyses performed to estimate the effect of harvest date on tissue P concentration revealed that P concentration in leaf tissue typically declined as a linear, quadratic or cubic function of harvest date.

Overall, tissue P concentration declined less precipitously than crude protein, K or NO_3^- .

Similarly, tissue K concentration was not significantly affected by the NH_4NO_3 treatments. Among accessions, significant differences in mean tissue K concentration were observed. Analyses performed to estimate the effect of harvest date on tissue K concentration revealed that K concentration in amaranth leaf tissue declined as a linear, quadratic and cubic function of harvest date. The largest single decline in K concentration occurred between 25 and 35 days after germination. Thereafter, tissue K concentration remained relatively constant. Overall, the concentration of K in amaranth leaf tissue fluctuated over harvest dates to a greater extent than crude protein, NDF, P or NO_3^- . These results suggest that early harvests (<35 days after germination) of amaranth leaves is necessary if maximum benefit of their high K concentration are to be gained.

Results of laboratory chemical analyses of leaf tissue from two amaranth accessions indicated that water soluble NO_3^- concentration was enhanced approximately two-fold from application of NH_4NO_3 over the 0 kg/ha NH_4NO_3 control. The response to NH_4NO_3 was linear, while tissue NO_3^- concentration declined as a linear, quadratic or cubic function of harvest date. The rate of NO_3^- decline was often precipitous. Very low or no detectable levels of NO_3^- were observed at later harvest dates. Individual sample variability was often considerable. This fact points to a probable difficulty in accurately estimating amaranth NO_3^- concentration as a function of N supply or plant maturity. Overall, levels of

water soluble NO_3^- did not exceed 239 mmol/kg dry weight for any of the NH_4NO_3 or harvest date treatments. These NO_3^- levels are comparable to those found in other common leafy green vegetables such as spinach, beet and chard when grown under similar management.

Conclusions and Recommendations

There are some overall conclusions and recommendations that can be made from the results of this study. First, vegetable amaranths grow relatively quickly, reaching a nutritional and culinary peak relatively early in their growth cycle. Successive harvesting of tender stems and leaves should be done as soon as plants are large enough to recover from the effects of defoliation. When harvested young, amaranth leaves are rich in protein, relatively low in fiber, and contain adequate supplies of minerals and vitamins. Although estimating plant yield was not an objective of this study, some general observations about productivity among the different accessions were made. Amaranthus dubius and A. tricolor seemed to possess higher leaf/stem ratios as compared to the others, and produced a large mass of edible material in a short period of time. These qualities would contribute to their greater overall productivity. In addition, high leaf/stem ratios would result in an enhancement of the nutritional quality of the edible material, as most of the nutrients are concentrated in the leaves rather than stems. The other accessions yielded a comparatively larger mass of stem tissue and therefore could not be recommended by this author for market or

commercial use. Low leaf/stem ratios should not detract from their usefulness in home gardens and similar situations, however.

Generally, nitrogenous fertilizers can be expected to enhance the nutritional quality of amaranth greens. The concentration of tissue NO_3^- will likewise be enhanced under these conditions. Due to the variability in data for tissue NO_3^- concentrations, predicting how much tissue NO_3^- will result from a given N fertilizer rate or harvest date would be difficult.

Second, amaranths are heat loving, and their use in southern gardens and agriculture should be encouraged during summer months when other greens crops cannot be grown. The grain amaranth's adaptation to the warm, humid climate of the southeast appears to be good. Several problems were noted, however. Specifically, damage from the tarnished plant bug (Lygus lineolaris) was observed, but the extent of the damage was not estimated. Grain amaranths seemed to be somewhat less susceptible to stem rot fungi (Fusarium and Phytophthora spp.) as compared to the vegetable types. Root and stem boring insect larvae infested plants in several plots of a late maturing Nepalese grain amaranth (not analyzed in this study), causing extensive premature lodging. Generally, lodging in the grain types was a severe problem in plots treated with NH_4NO_3 .

Third, the grain amaranths' reputed tolerance to environmental stresses, high lysine content of the grain, and efficient carbon metabolism make it a desirable prototype of a crop that could effectively utilize marginal lands in semiarid situations such as are found in the

southwestern United States, Africa and South Central Asia. Further studies are needed to define the physiological response of the grain amaranth to these environments. Studies aimed at partitioning the dry matter of vegetable and grain amaranths into biologically useful materials also need to be encouraged. The successes obtained from such work performed in other crops, e.g., wheat and rice, may prove to be a fruitful avenue for research in the development of modern, competitive, cultivars of amaranth.

Fourth, vegetable amaranths should be ratooned rather than single cropped, as this encourages the plants to maintain a vegetative state over a longer period of time. Floral initiation results in the production of a minimum of leaf material and a maximum of stems and flowers. This author feels that the overall effect of ratooning may stimulate the uptake of mineral nutrients in vegetable amaranths. This would have the overall effect of enhancing their nutritional value over a longer period of time than conventional once-over harvests.

Fifth, the results of laboratory chemical analyses indicated that grain crude protein content in four grain amaranth accessions was not significantly enhanced by addition of NH_4NO_3 . This suggests that changed methods of fertilizer application may provide a more satisfactory route for future investigations of this type. For example, split applications of nitrogenous fertilizers, as is practiced with other agronomic crops, should be evaluated as a means of increasing yields and/or nutritional composition of the grain.

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