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ENVIRONMENTAL MODIFICATION OF YIELD AND FOOD COMPOSITION OF COWPEA AND LEAF LETTUCE

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) and leaf lettuce (*Lactuca sativa* L.) are candidate species to provide legume protein and starch or serve as a salad base, respectively, for a nutritionally balanced and psychologically satisfying vegetarian diet in CELSS. Greenhouse-grown cowpeas (cv. IT84E-124) were harvested according to several different strategies. Total edible yield (34 gDW plant^{-1}) was equal for vegetative and reproductive harvest strategies, but the vegetative product could be harvested 47% sooner and from smaller plants. Yield efficiency was 2.9 to 4.4 times greater for the vegetative than for a reproductive or mixed harvest strategy. Leaf carbohydrate content increased with leaf age (32-43% of DW), but was greatest in the seed (56%). Protein content of older leaves was similar to that of seeds (31%), while that of young leaves was greatest (43%). Fat content of cowpea leaves (5%) and seeds (1%) was quite low, allowing great flexibility for cowpea in formulating healthy diets. Hydroponic leaf lettuce grew best under CO_2 enrichment and PPF enhancement. High CO_2 (1500 μl l^{-1}) enhanced leaf number 69% relative to ambient CO_2. Leaf protein content reached 36% with NH_4^+ + NO_3^- nutrition, and starch and free sugar content were as high as 7 and 8.4% of DW, respectively, for high PPF/CO_2 enriched environments.

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As members of the CELSS Food Production group, we have been interested in candidate species selection since the beginning of the CELSS program. First and foremost in selecting plants for CELSS is the question of how they contribute to human nutrition (Fig. 1). Energy content and nutritional composition of the harvestable part, as well as processing requirements, are the most important nutritional use criteria. Other nutritional criteria are listed in Figure 2.

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Cultural criteria also are important, especially proportion and yield of edible biomass. Long juvenile periods and dormancy requirements would be very undesirable, but suitability for soilless culture would be very desirable. Given these selection criteria and a scoring system with weighting factors, we evaluated and ranked 115 world food crops for candidacy in the CELSS program. The 36 species listed in Figure 3 are part of a "generous" diet scenario selected from the original list. They tend to be fast-growing herbaceous annuals which, in appropriate proportions, provide a nutritionally balanced vegetarian diet with some variety. The more species used to compose a vegetarian diet, the less risk of deficiency or toxicity. Legume, root and tuber, salad, sugar, cereal, leaf and flower, fruit, and stimulant crops all were part of the generous diet scenario. However, early emphasis in the CELSS program of defining conditions for optimum productivity precluded the opportunity for initially working with a variety of species. With only a handful of candidate species presently under development, nutritional criteria take on exaggerated importance (Fig. 4). In this "modest" diet scenario, only 5 categories of crops are represented: legumes and cereals for complementary protein, tuber crops mainly for complex carbohydrate and calories, cooked vegetables, and raw salad vegetables. The first category of legumes has higher fat content, the second category lower fat content. The low-fat legumes and cereal grains also provide substantial complex carbohydrate. One can argue whether the vitamins, minerals, and fiber provided by the vegetables alternatively could be provided by stored supplements, but fresh vegetables and salad greens definitely are preferred for a psychologically satisfying, nutritionally balanced diet. These analyses and interpretations are contained in NASA Contractor Report 166324, entitled "Nutritional and Cultural Aspects of

Plant Species Selection for a Controlled Ecological Life Support System" (Fig. 5).

NIH has set an eventual goal for a prudent diet for Americans that would consist of no more than 15% of total Calories as protein, 65% as complex carbohydrate, and 20% as fat (Fig. 6). Presently, about 50% of our daily Calories are from fat, but only a few percent fat (as particular fatty acids) is essential for normal growth, development, and maintenance of the human body. The prudent diet will involve 4-5 times more starch foods than protein, and 7 times more starch than fat. On a dry weight basis, much of the carbohydrate in the present candidate species is in a digestible form, except for that in lettuce (Fig. 7). Soybean presently is the main protein source, but since it contains 2-3 times more protein than the NIH desired amount, soybean would have to be mixed with other species to lower overall protein content, raise carbohydrate, and keep fat about where it is in the formulated diet. Water content, of course, determines the absolute food content of the parts that are consumed. This is not a limitation for soybean or wheat, but is somewhat for the other three species. Water content is not a serious limitation for potato and sweet potato because the solid parts are so rich in edible carbohydrate. The main nutritional value of lettuce, besides providing vitamins, minerals, and fiber, appears to be as a water source, plus the fact that it is pleasant to eat, and is a dietary enhancement food. Leaf lettuce is the species we have emphasized in our environmental optimization program for CELSS at Purdue University, primarily because the senior author is interested in its use as a model crop to maximize photosynthetic productivity of leafy vegetable crops, and also because we initially were operating under the assumption of a generous diet scenario. That is gradually developing, and with the recent grass roots demand for a "Salad Machine" on Space Station, lettuce will play an important role in a "modest" diet scenario (Fig. 8).

Leaf lettuce has a short production cycle, a promising yield rate to build upon, an excellent harvest index, a minor nutritional contribution, air revitalization capacity throughout production, adaptability for many forms of hydroponics, and tolerance for NH_4^+ nitrogen during vegetative development (Fig. 9). Actually, the biggest selling point of lettuce is its positive psychological impact. Being around something green in an otherwise austere institutional environment is pleasing to humans (Fig. 10). Furthermore, lettuce has ornamental value. It can be bolted by long photoperiods, gibberellic acid, or heat stress; it even can be decorated for Christmas, and then eaten after the holiday season. We have decorated edible Christmas trees with *Zea mays* (popcorn), *Vaccinium* (cranberry), and *Carambola* (Starfruit), all candidate edible ornaments (Fig. 11).

Conditions found conducive to productivity rates of at least 60 gDW of edible biomass m^{-2} day^{-1} for a responsive cultivar of leaf lettuce are summarized in Fig. 12. All of these conditions are important, but CO_2 enrichment, nitrogen (level and form), and radiation enhancement during critical periods of exponential growth are drivers. We intend to double this figure with judicious canopy management and use of growth-stimulating agents during the early lag phase of seedling development.

We have been successful in growing this salad vegetable with high rates of productivity, but recently we have asked whether optimizing conditions alter the quality of the product. Lettuce is not nutritionally rich, but as a model leafy vegetable crop, we want to know if environmental modification affects the levels of important nutritional components of leaves in a favorable way.

We generate the edible leaf biomass in a 100 ft^2 walk-in growth room equipped with fluorescent and incandescent lighting fixtures (Fig. 13). Within the chamber is a table supporting recirculating hydroponics systems. Troughs mounted on the table are constructed from vinyl downspouts of rain guttering (Fig. 14). These slotted units are nursery troughs into which seeds are sown. Cloth wicks lining the slots keep the seedlings moist. Lids over the nursery troughs keep seedlings dimly illuminated and humid for the first 2 days of germination (Fig. 15). After uncovering, the seedlings are left in the nursery troughs for an additional 4 days until they are transplanted (Fig. 16). The cloth wicks are taken out of the nursery, pulled apart, and polyester wicks are prepared for individual seedlings. The exposed seedling roots are kept moist with Shur-Wipes misted with water. A forceps is used to gently lift a hanging seedling, and it is carefully placed within a slitted Ethafoam plug along with the wick (Fig. 17). The seedlings and wicks mounted in plugs are then floated in a tray containing dilute nutrient solution until they are all transplanted to holes in the troughs at once. The transplanted seedlings have only the cotyledons and rudimentary true leaves on the day of transplant (Fig. 18). From then (day 6) until day 12, when environmental optimization treatments are initiated, the plants develop one leaf per day. On day 12, light treatments begin, CO_2 enrichment is initiated, and various N treatments are applied in nutrient solution (Fig. 19). By day 18, the plants are in rapid exponential growth (Fig. 20), and they are harvested on day 21 (Fig. 21). At this time the entire foliar canopy is closed. Until day 21 when plants are harvested, the plants are in rapid exponential growth (Fig. 22). As the plants are harvested, they are quick-killed with microwave radiation just prior to oven drying.

Proximate and growth analyses were performed on lettuce leaves grown under various optimizing environments. Figure 23 shows the combined effect of light level, CO_2 concentration, and different species and levels of N on leaf number after 21 days of growth. CO_2 enrichment clearly stimulates leaf development, and for all combinations of light and CO_2, NH_4^+ and NO_3^- together tend to enhance leaf number. As far as photosynthetic productivity is concerned, leaf dry weight of lettuce was lowest when light of 330 μmol m^{-2} s^{-1} and ambient CO_2 were used, and was highest when light of 800 μmol m^{-2} s^{-1} plus 1500 ppm CO_2 were used (Fig. 24). High CO_2 alone was more effective than high light alone in stimulating photosynthetic productivity. In terms of the quality of the product, as a point of reference, field-grown, loose-leaf, dark-green lettuce of the 'Grand Rapids' type has 22% protein, 58% carbohydrate distributed between cellulose and starch, 5% fat, including chlorophyll, and 15% ash (Fig. 25). For chamber-grown, hydroponic lettuce, protein content of the leaves in all cases was greater than 22% (Fig. 26). Its content relative to total dry weight tended to be greater for ambient CO_2, regardless of light level. That seems logical because high CO_2 would favor accumulation of carbohydrate *per se*, rather than protein. It also makes sense that protein tends to accumulate when NH_4^+ is included in the nutrient solution, especially double-strength NH_4^+. If total carbohydrate of leaf lettuce is about 58%, then most of it is in the form of non-digestible cellulose, because lettuce normally makes no more than a few percent starch, as opposed to alfalfa leaf, which might be 45% starch. As you would expect, conditions favoring high photosynthetic rates resulted in the greatest accumulation of leaf starch, and in all cases NH_4^+ nitrogen reduced starch content, by siphoning off carbon skeletons to support protein synthesis at the expense of carbohydrate (Fig. 27). The highest starch content we achieved was about 7%. One really interesting finding was that free sugar content was consistently as high or higher than starch content regardless of environmental regime (Fig. 28). Sugar contents of 8-10% were common in freshly harvested hydroponic lettuce, whereas leaf lettuce off the shelf of the grocery store or out of the garden last summer had essentially zero free sugar. Leaf sugar evidently represents a very labile carbon pool that is easily respired away. Microwave quick-kill of enzymes immediately upon harvest apparently preserved free sugar as well as starch in our experimental material. The only fresh tissues that tasted sweet, however, were young leaves of plants that had been grown with single-strength nitrogen (i.e., 15 mM) as $NH_4^+ + NO_3^-$. Incidentally, NH_4^+-treated plants also had the least bitter principle. Sugar and starch together brought total edible carbohydrate to about 15%, still leaving more than 40% as cellulose and other wall polysaccharides. Fat content of controlled environment lettuce was consistently lower than the 5% average of field-grown lettuce (Fig. 29). Components of the solar spectrum may stimulate membrane lipid and chlorophyll synthesis more than do fluorescent and incandescent radiation. Field-grown lettuce has about 15% ash. Most of our controlled-environment lettuce was in that range or a little higher (Fig. 30). 15 to 20% inorganic content seems like a rich source of minerals. Perhaps lettuce is a good source of mineral water!

The results of these proximate analyses demonstrate that the protein and bioavailable carbohydrate composition of leaf lettuce can indeed be modified by environmental and nutritional manipulation. Lettuce will not be a rich Calorie source for CELSS, but as a model leafy vegetable crop the results of this study demonstrate that nutritional value can be improved by certain optimizing environments. The principles we have learned with lettuce in this regard should transfer readily to other, more nutritious crops with edible foliage.

One such promising new candidate species is used as a staple food along with sorghum in certain drought and heat-stricken countries of Central Africa. It is cowpea, or black-eyed pea (Fig. 31). The pods themselves are edible when the seeds are immature, but if the seeds mature you have a dry bean with considerable shelf life. Furthermore, the leaves of this legume are edible either as a raw salad green or as a cooked vegetable. The foliar canopy is "aggressive", and when we have grown it hydroponically along with other legumes in the growth chamber, it tends to choke out all competitors (Fig. 32). Can the same plant produce both seeds and leaves for human consumption? We have obtained additional cowpea lines from a breeder in Niger and continue the screen. Using a promising determinate line, we compared biomass distribution among plant parts as a function of various harvest scenarios (Fig. 33). All parts of a cowpea plant are edible, although not necessarily at the same time. Seeds were harvested either once at 75 days in the greenhouse, or leaves were stripped at 15-day intervals. We compared these with a mixed-harvest scenario of young leaves stripped periodically and the seeds still harvested at 75 days. Total biomass was greatest for the seed-harvest scenario, and least for the mixed scenario. However, at 75 days it is not a good assumption that all leaf, stem, and pod tissues are edible, or even palatable, without sophisticated food processing procedures. The mixed harvest was lowest because the leaves were harvested when they were still expanding, and had not yet contributed to the photosynthetic productivity of the plant. But this is the way subsistence cultures in Africa do it. They are concerned about immediate nutrition and palatability, and don't think about photosynthetic productivity. This shows up in total edible yield, which is equal for seed and vegetative harvests but is reduced 30% for the mixed harvest (Fig. 34). Given other important yield considerations such as harvest index and time to harvest, yield rates and efficiencies were found to be greatest for the vegetative harvest, by far. We think the mixed harvest strategy can be made competitive with the vegetative strategy by allowing leaves to expand fully and contribute something to plant biomass production before harvest, and we plan to test this. We also have subjected cowpea leaves and seeds to proximate analysis, and find that protein content of mature leaves is the same as that of seed protein on a dry weight basis (Fig. 35). Protein content of expanding leaves is 43% greater. The amino acid composition of cowpea seed protein is known and is comparable to that of soybean protein. I am not aware of the quality of cowpea leaf protein, however, but we plan to have amino acid composition done on leaf samples once we get cowpea into hydroponics and CO_2 enrichment in the growth chamber. We also don't have starch analyses on cowpea leaves or seeds yet, but presumably much more total seed carbohydrate (e.g., starch) is potentially bioavailable than is leaf carbohydrate (e.g., cellulose). However, cowpea leaves are much less succulent than lettuce leaves, and it is likely that starch makes up a much higher proportion of cowpea leaf biomass. Fat and ash are about what one would expect for distribution between leaves and seeds.

Future efforts with cowpea will emphasize effects of modified controlled environments on the quality as well as quantity of vegetative and reproductive parts.

CANDIDATE SPECIES SELECTION CRITERIA

Criterion Number	Nutritional use Criteria
1	Energy concentration
2	Nutritional composition
3	Palatability
4	Serving size and frequency
5	Processing requirements
6	Use flexibility
7	Storage stability
8	Toxicity
9	Human use experience

CANDIDATE SPECIES SELECTION CRITERIA

Criterion Number	Cultural Criteria
10	Proportion of edible biomass
11	Yield of edible biomass
12	Continuous vs. determinate harvestability
13	Growth habit & morphology
14	Environmental Tolerance
15	Photoperiodic & temperature requirements
16	Symbiotic requirements & restirctions
17	Carbon dioxide-light intensity response
18	Suitability for soilless culture
19	Disease resistance
20	Familiarity with species
21	Pollination & propagation

PLANT SPECIES RECOMMENDED FOR THE "GENEROUS" DIET SCENARIO

LEGUMINOUS CROPS:

- DRY BEAN
- SNAP BEAN
- CHICK PEA
- SHELL PEA
- SUGAR PEA
- PEANUT
- SOYBEAN

ROOT & TUBER CROPS:

- GARDEN BEET
- CARROT
- POTATO
- SWEET POTATO
- TARO

SALAD CROPS:

- CELERY
- LEAF LETTUCE
- ONION
- TOMATO

SUGAR CROPS:

- SUGAR BEET
- SUGAR CANE

CEREAL GRAIN CROPS:

- BARLEY
- CORN
- OATS
- RICE
- RYE
- WHEAT

LEAF AND FLOWER CROPS:

- BROCCOLI
- CHINESE CABBAGE
- HEAD CABBAGE
- CAULIFLOWER
- CHARD
- KALE
- SPINACH

FRUIT CROPS:

- BANANA
- GRAPE
- STRAWBERRY
- CANTALOUPE

STIMULANT CROP:

- TEA

CROP SPECIES TO SATISFY A "MODEST" (MINIMUM) DIET SCENARIO

SOYBEAN AND/OR PEANUT
DRY BEAN OR COWPEA OR GARDEN PEA
WHEAT AND/OR RICE
POTATO
CHARD AND/OR CABBAGE
TOMATO AND LETTUCE

Hoff, J.E., J.M. Howe, and C.A. Mitchell. 1982. Nutritional and cultural aspects of plant species selection for a controlled ecological life support system. NASA Contractor report 166324, 122 pp.

DAILY ALLOWANCE FOR A 180-LB MALE FOLLOWING A PRUDENT DIET.

DIETARY CONSTITUENT	CALORIC DISTRIBUTION (%)	G·LB DESIRABLE BODY WT^{-1}·DAY^{-1}	G·DAY^{-1}·PERSON^{-1}
PROTEIN	15	0.66	118
CARBOHYDRATE	65	2.85	513
FAT	20	0.39	70

FOOD COMPOSITION OF PRESENT CANDIDATE CROP SPECIES FOR CELSS.

SPECIES	COMPOSITION (% OF DRY WEIGHT)			WATER CONTENT (%)
	CARBOHYDRATE	PROTEIN	LIPID	
WHEAT	82	14	2	13
SOYBEAN	38	38	20	10
POTATO	85	10	0.5	80
SWEET POTATO	89	6	1.4	71
LEAF LETTUCE	58	22	5	94

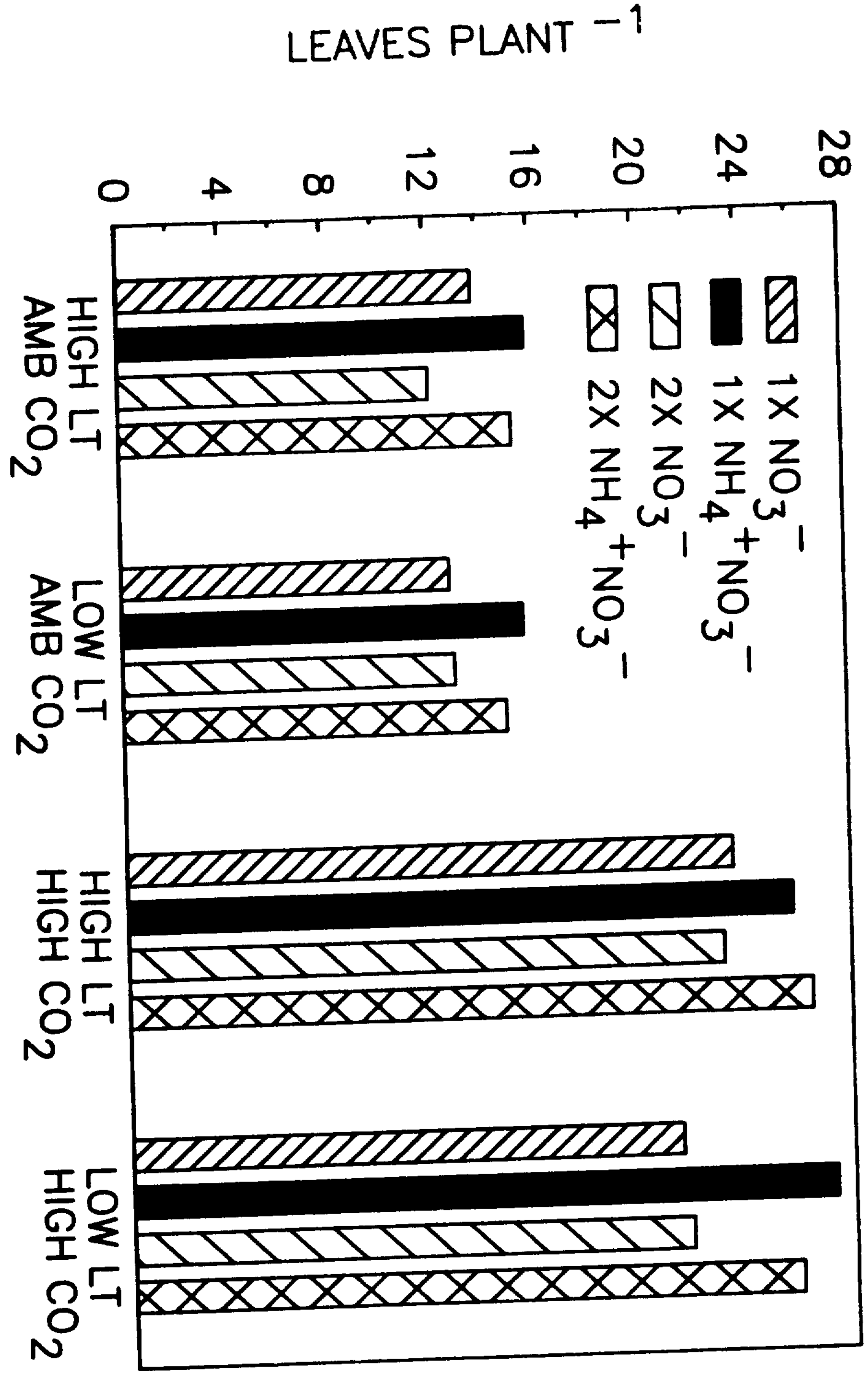
JUSTIFICATION FOR OPTIMIZING PRODUCTIVITY OF LEAF LETTUCE (*Lactuca sativa* L.) IN THE CELSS PROGRAM:

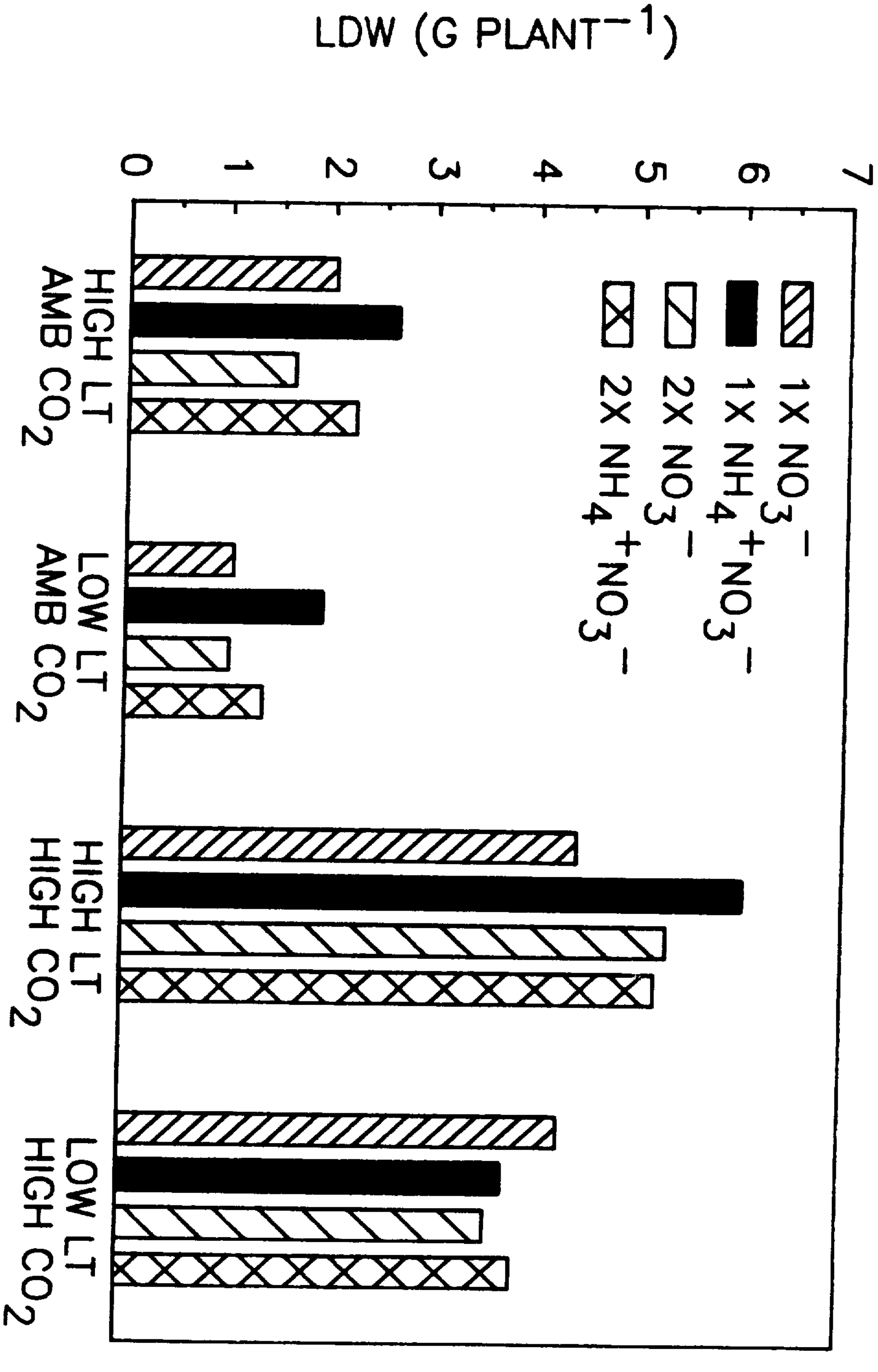
- Short production cycle ($\leq$ 22 days by CEA vs. 55-70 days by OFA).
- Promising yield rate (2.6 g DW edible biomass m^{-2} day^{-1} from OFA vs. 16.4 g m^{-2} day^{-1} from unoptimized CEA vs. $\geq$ 60 g m^{-2} day^{-1} from "optimizing" CEA vs. __?__ g m^{-2} day^{-1} from optimum CEA).
- Favorable harvest index ($\geq$ 80% of total DW).

  Excellent dietary enhancement food for the psychologically satisfying diet (is the traditional salad "base" in our culture).
- Provides some vitamins, minerals, and fiber for a nutritionally balanced diet.
- Sustains high level of Pn (O_2 ↑ and CO_2 ↓) throughout production as a leafy salad crop.
- Suitable for all forms of soilless culture (hydroponics, aeroponics, NFT, tubular membrane system, etc.).
- Excellent tolerance of NH_4^+ beyond the seed-germination stage, especially in the presence of NO_3^- and radiation enhancement.
- Ideal model system for maximization of vegetative growth, photosynthesis, and productivity without complications arising from source/sink movement and monocarpic senescence.
- Diminutive stem in vegetative stage to pose few gravitropism problems in hypogravity.
- Extensive data base on culture to build upon.

Cultural conditions to give $\geq$ 60 gDW m^{-2} day^{-1} of edible biomass for 19-day-old 'Waldmann's Green' leaf lettuce:

- 13.5-cm spacing of plants
- continuous 25°C air temperature
- 85% relative humidity
- 20-h photoperiod
- 900 μmol m^{-2} s^{-1} of PAR from 84% incandescent + 16% fluorescent radiation from days 11-19
- single-strength Hoagland's nutrient solutions. pH 6.0 ± 0.2, containing 5 mM NH_4^+ + 25 mM NO_3^-
- 1500 μl l^{-1} CO_2 from days 11-19


LEAVES PLANT $^{-1}$
0
4
8
12
16
20
24
28
1X NO_3^-
1X $NH_4^+NO_3^-$
2X NO_3^-
2X $NH_4^+NO_3^-$
HIGH LT AMB CO_2
LOW LT AMB CO_2
HIGH LT HIGH CO_2
LOW LT HIGH CO_2

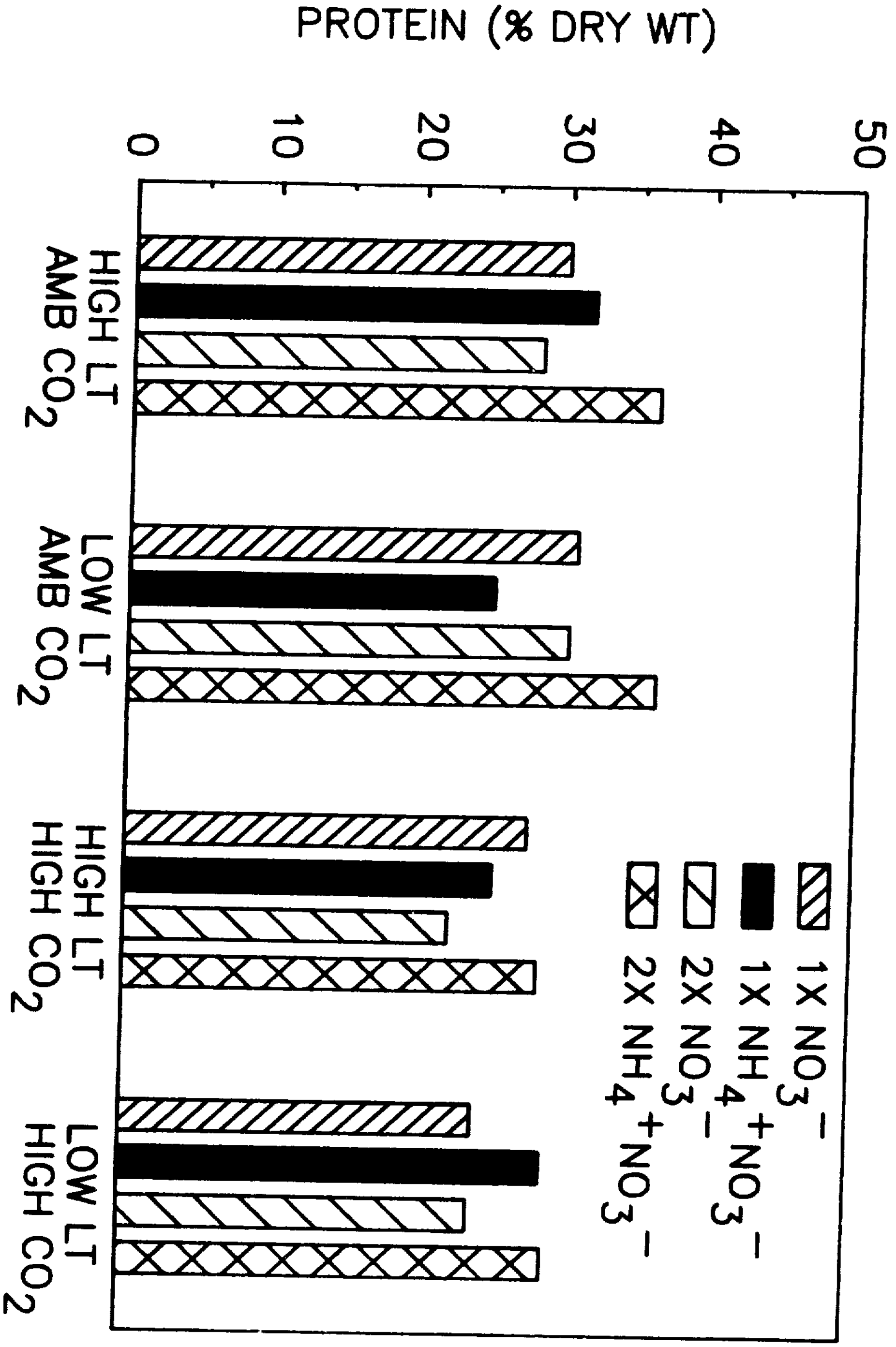

LDW (G PLANT^{-1})
0
1
2
3
4
5
6
7
1X NO_3^-
1X $NH_4^+NO_3^-$
2X NO_3^-
2X $NH_4^+NO_3^-$
HIGH LT AMB CO_2
LOW LT AMB CO_2
HIGH LT HIGH CO_2
LOW LT HIGH CO_2

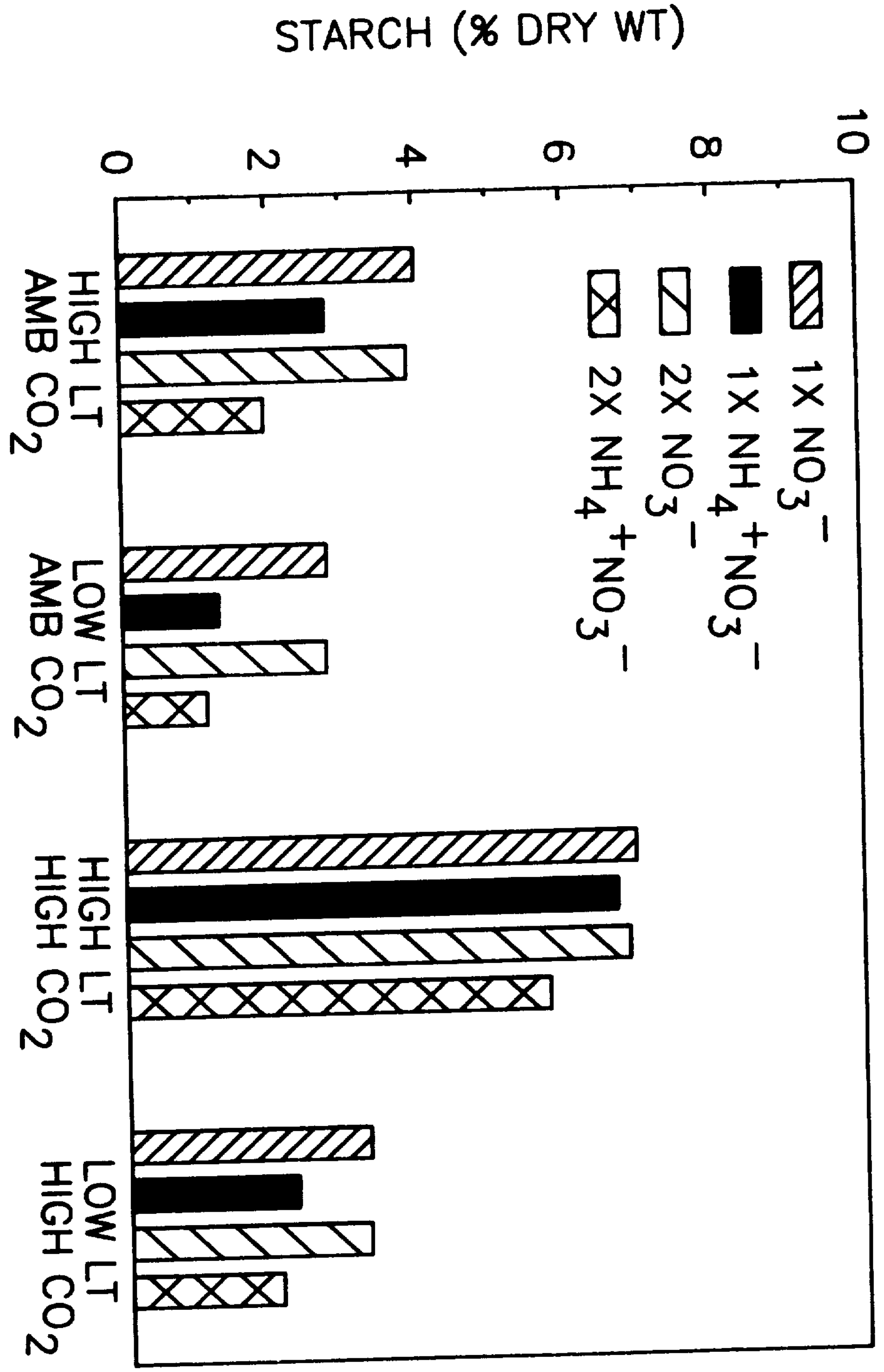
Proximate composition[1] of field-grown loose-leaf lettuce.

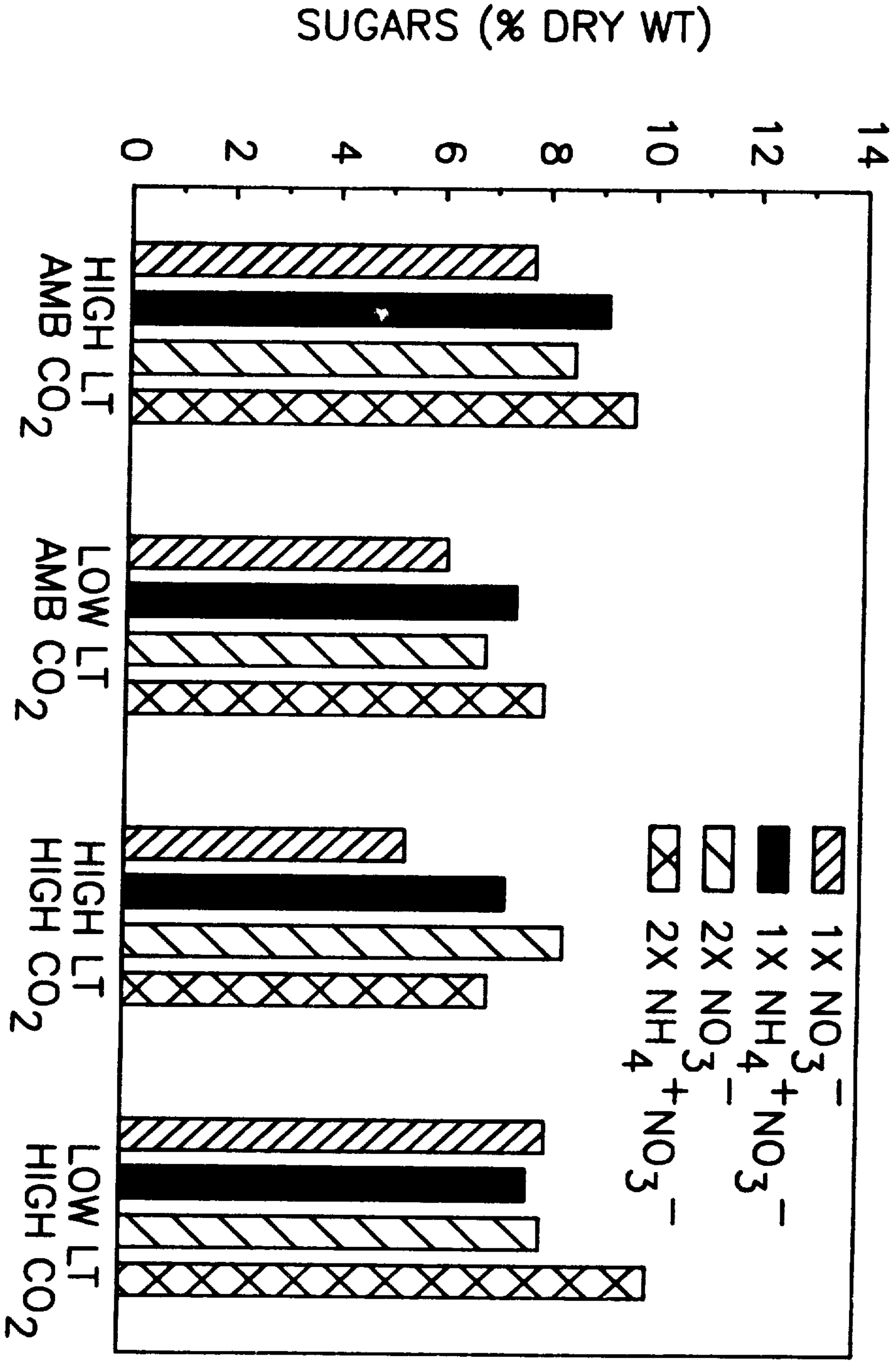
Component	Composition (% of DW)
Protein	22
Carbohydrate[2]	58
Fat	5
Ash	15

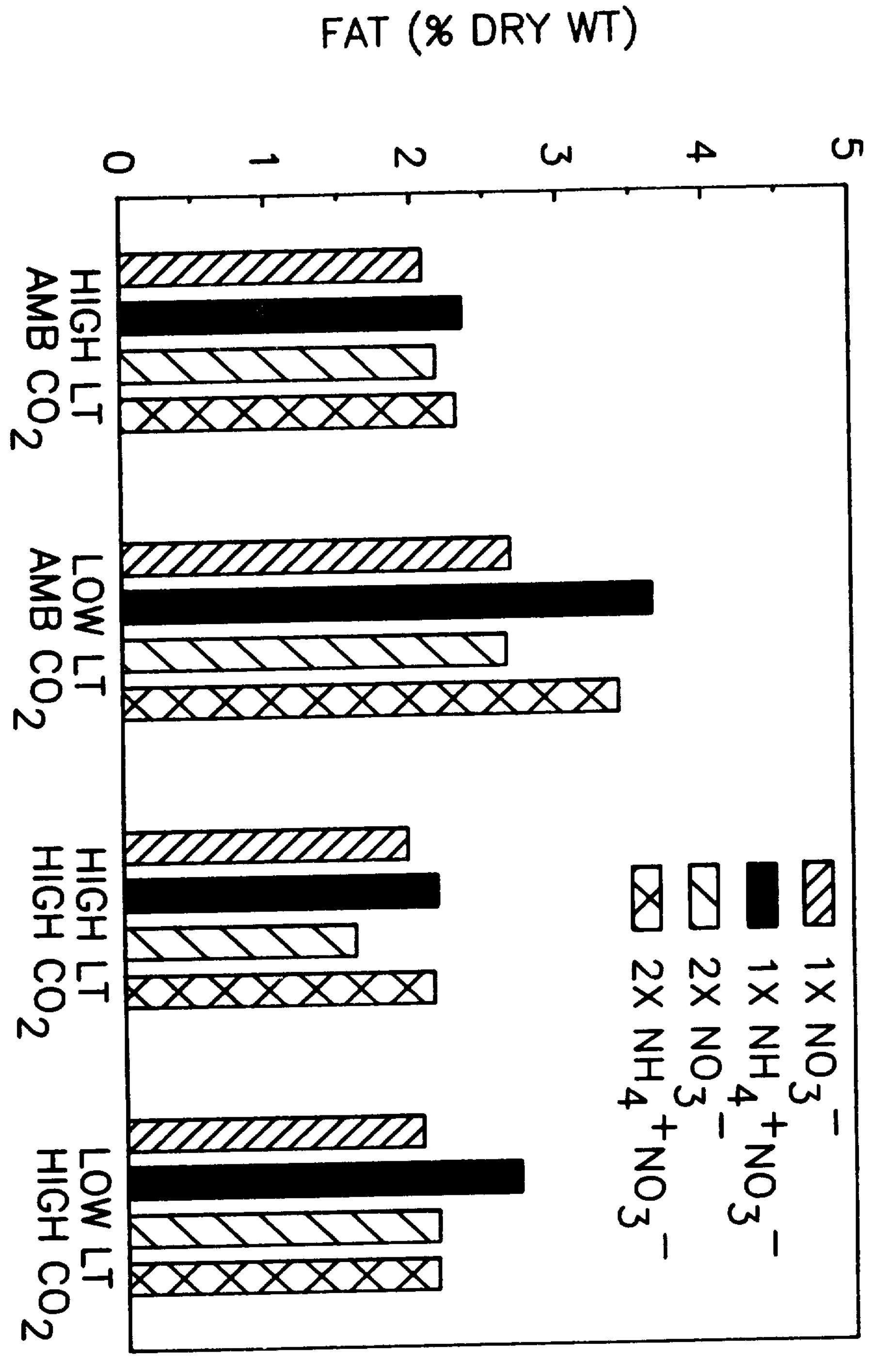
[1] Composition of Foods. Agriculture Handbook No. 8. USDA, ARS.

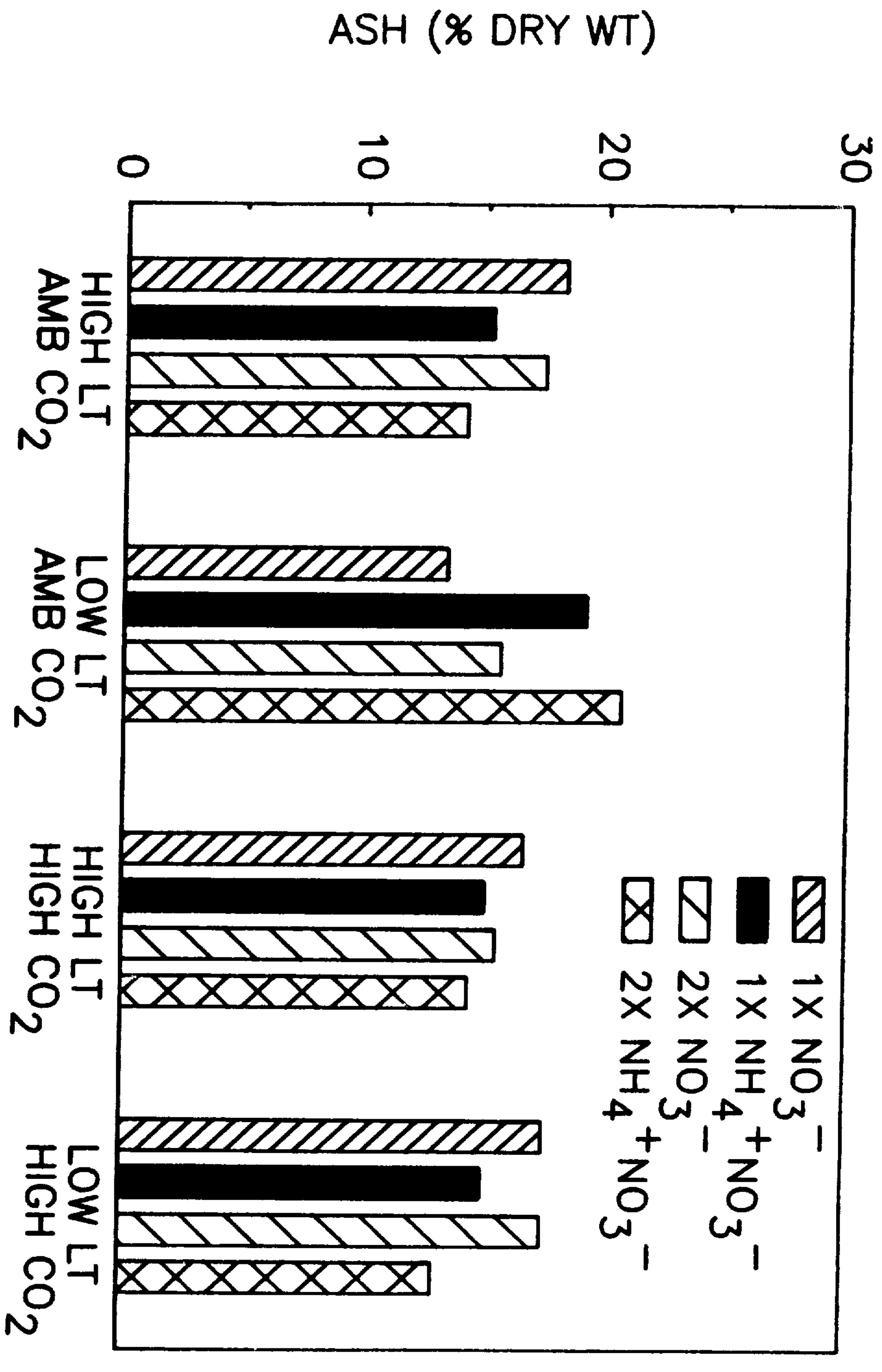
[2] Includes total structural and non-structural carbohydrate.


PROTEIN (% DRY WT)
0
10
20
30
40
50
1X NO_3^-
1X $NH_4^+NO_3^-$
2X NO_3^-
2X $NH_4^+NO_3^-$
HIGH LT AMB CO_2
LOW LT AMB CO_2
HIGH LT HIGH CO_2
LOW LT HIGH CO_2


STARCH (% DRY WT)
0
2
4
6
8
10
1X NO_3^-
1X $NH_4^+NO_3^-$
2X NO_3^-
2X $NH_4^+NO_3^-$
HIGH LT AMB CO_2
LOW LT AMB CO_2
HIGH LT HIGH CO_2
LOW LT HIGH CO_2


SUGARS (% DRY WT)
0
2
4
6
8
10
12
14
HIGH LT AMB CO$_2$
LOW LT AMB CO$_2$
HIGH LT HIGH CO$_2$
LOW LT HIGH CO$_2$
1X NO$_3^-$
1X NH$_4^+$NO$_3^-$
2X NO$_3^-$
2X NH$_4^+$NO$_3^-$


FAT (% DRY WT)
0
1
2
3
4
5
HIGH LT AMB CO_2
LOW LT AMB CO_2
HIGH LT HIGH CO_2
LOW LT HIGH CO_2
1X NO_3^-
1X $NH_4^+NO_3^-$
2X NO_3^-
2X $NH_4^+NO_3^-$


ASH (% DRY WT)
0
10
20
30
HIGH LT AMB CO$_2$
LOW LT AMB CO$_2$
HIGH LT HIGH CO$_2$
LOW LT HIGH CO$_2$
1X NO$_3^-$
1X NH$_4^+$NO$_3^-$
2X NO$_3^-$
2X NH$_4^+$NO$_3^-$

Biomass distribution in Vigna unguiculata cv. IT84E-124 as a function of traditional seed harvest (75 days), vegetative harvest (40 days), or mixed seed/vegetative harvest.

Plant part	Seed harvest (g)	Vegetative harvest (g)	Mixed harvest (g)
Leaves	24	34a	7c
Stem	28	20b	12c
Pod	46	-	13
Seed	35	-	10
Total	117	60b	44

Yield characteristics of *Vigna unguiculata* cv. IT84E-124 as influenced by harvest strategy.

Harvest Parameter	Seed harvest		Mixed harvest		Vegetative harvest
Seed yield (g $plant^{-1}$)	35	**	10		-
Edible leaves (g $plant^{-1}$)	-		15	**	34
Total edible yield (g $plant^{-1}$)	35a		25b		34a
Harvest index (%)	30c		72a		58b
Time to harvest (days)	75a		75a		40b
Daily yield (g $plant^{-1}$ day^{-1})	0.46b		0.33c		0.85a
Yield/area (g m^{-2} canopy)	69b		46c		105a
Yield efficiency (g m^{-2} day^{-1})	0.92b		0.60c		2.64a

Proximate composition of expanding (7-10 day old) and fully expanded leaves (22-25 days old) as well as seeds of Vigna unguiculata cv. IT84E-124.

Component	Expanding leaves (%)	Fully expanded leaves (%)	Seed (%)
Carbohydrate	32c	43b	56a
Protein	43a	30b	31b
Fat	5a	5a	1b
Ash	14a	15a	4b