FOREWORD

This investigation was initiated by the 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The study was carried out by the Boeing Company under Contract No. AF 44(616)-7945, Project No. 7164, "Space Biology Research" and Task No. 716403, "Environmental Biology." The research sponsored by this contract was begun in March 1961 and completed in April 1962. The principal investigators for the Boeing Company were Dr. A. J. Pilgrim and Dr. S. P. Johnson. The contract monitor was Dr. A. E. Prince, Chief, Biospecialties Section, Physiology Branch, Biomedical Laboratory, 6570th Aerospace Medical Research Laboratories.

ABSTRACT

The growth of a variety of Angiosperms was evaluated under controlled conditions in low intensity fluorescent light. Although a large number of species exhibited various abnormalities, three species, Brassica Chinesis, Chichorium endiva, Amaranthus gangeticus, were able to tolerate and grow normally under the conditions used. Studies of the photosynthetic activity of these plants showed that a great deal of variation is to be expected both under standard conditions of culture and in media containing increased salt concentrations. Studies of photosynthetic activity by the plants in an atmosphere with the nitrogen replaced with argon or helium indicated that these gases had no adverse effects. Analysis of the three plants included estimation of amino acids, carbon, water and alcohol soluble carbohydrates, protein, nitrogen, ash, lipid, and vitamins A, C, and E. The results indicate that the roots, stems, and leaves of these three species could provide a valuable nutritional supplement. Feeding of the plants to rabbits demonstrated that no acutely toxic compounds are present.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

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INTRODUCTION

During recent years, considerable attention has focused on the use of photosynthetic plants for atmosphere regeneration and food supplements during extended space flight. In most studies, emphasis has been placed on the utilization of microscopic algae. However, the use of higher plants for these purposes offers several potential advantages: they may be grown in light weight substrates; their requirements for environmental control are less critical; and many of the problems of gas exchange that may occur in the zero-gravity state with cultures of algae would not be anticipated with the leafed plants. Finally, the use of higher plants as a food is more appealing and probably will present fewer psychological problems than the use of algae pastes or powders.

The objectives of this study were to:

1. Conduct a survey of the literature with special attention devoted to photosynthetic and nutritional potential of Angiosperms grown under low intensity artificial light.
2. Select a group of plants for experimental studies based on the available information.
3. Evaluate selected plants by measuring photosynthetic activity under carefully controlled conditions of temperature, humidity, and light.
4. Select the three most promising species and carry out detailed studies of photosynthetic activity, water utilization, ease of reproduction, resistance to disease, and response to varying conditions of light, temperature, humidity, and atmospheric composition. Examine these plants for pharmacologically active substances.
5. Establish the food value of the selected plants by chemical analysis and preliminary animal feeding experiments.

There is little in the way of pertinent literature to support the selection of Angiosperms that have desirable characteristics for use in an extraterrestrial environment, since the need-to-know concerning specific response under modified conditions is of recent origin. Hence, the role of the literature review is relegated to one of establishing guide lines within which a group of plants may be selected that will, with a reasonable degree of proficiency, fulfill a role in respiratory support and as a food source for man in a space situation.

The principle points developed by a review of the literature led to the establishment of the following criteria for selection:

1. High photosynthetic efficiency under low artificial light conditions.
2. Production of edible parts or plants under low artificial light conditions.
3. Resistance to increasing osmotic pressures.
4. Absence of pharmacologically active substances.

In addition to the criteria above, certain other factors were taken into consideration in the final selection of plants. These were:

1. Compactness of plant. Since space and weight penalties are critical considerations in the selection of any part of system for a space vehicle or base, the volume occupied by the plant system must be kept at a minimum. Therefore, plants which have a maximum leaf area and a minimum of stem would be desirable.
2. Since respiratory support is of paramount interest, plants which tend to flower readily should be avoided since oxygen production declines rapidly after flowering.

Prior to selecting groups of plants that might serve in gas exchange systems, a survey of the plant kingdom as a whole was initiated and complete orders of plants were eliminated in the initial screening because of structure, function, edibility, or availability. The plant kingdom contains over 300,000 different kinds or species of plants that have been observed and described. The present investigation limits selection of plants to the Angiosperms. Taxonomic orders of the Angiosperms are listed with representative plants noted as familiar examples.

Piperales - Mostly tropical herbs and shrubs, peppers and peperomia are the more familiar examples.

Salicales, Juglundales, and Fagales - These orders are comprised of many of our woody plants. Willows, poplars, walnuts, hickories, birches, beeches, alders, and oaks are some of the best known examples.

Urticales - Woody and herbaceous plants are evident in this order. The woody plant representatives are elms, figs, and mulberries; the herbaceous, hemp, hop and nettles.

Santales - Parasitic herbs and woody plants. Mistletoe belongs to this order.

Aristolochiales - Very small order of herbs and woody plants, containing about 200 species.

Polygonales - Herbs and woody plants. Smartweed, dock, rhubarb, and buckwheat are familiar examples.

Centrospermales - An order of interest for this investigation. Examples of plants are goosefoot, beet, spinach, pigweed, coxcomb, salt-brush, and miners' lettuce.

Ranales - Herbs, shrubs, and trees. Common forms representing this order are buttercup, clematis, anemone, columbine, larkspur, and peony.

Papaverales - Another order of interest in the selection of plants. Mustards, radish, stocks, cabbage, and shepherds purse are examples.

Sarracenieles - A small order of insectivorous plants.

Rosales - Comprised of herbs, shrubs, and trees: gooseberries, currants, roses, strawberries, raspberries, cherries, apples, plums, hawthornes, beans, and peas are familiar plants.

Geraniales - Herbs and woody plants. Geranium, oxalis, linum, and citrus are example genera.

Sapindales - Mostly woody plants. Sumacs, hollies, maples, and buckeyes are best known in this order.

Rhamnales - Woody plants which include the familiar grapes, buckthorn, and Virginia creeper.

Malvales - Basswood, mallows, hollyhock, and cotton make up a portion of this order of herbs and woody plants.

Parietales - Comprised of herbs and woody plants of which the more familiar forms are violets and pansies.

Opuntiales - The cactus family is representative of this order.

Myrtales - A tropical order of herbs and woody plants. Myrtles, Eucalyptus, evening primroses, and Fuchsia are best known members.

Umbellales - Nearly all forms are herbaceous and include carrots, celery, parsnip, and dill.

Ericales - Mostly shrubs; azalea, rhododendrons, heather, and blueberries are representative plants.

Primulales - Chiefly herbs, in which primrose, saltwort, and shooting star represent common forms.

Ebenales - Ebony and persimmon are examples of tropical trees and shrubs in this order.

Gentianales - Olives, ashes, lilacs, privets, gentians, and milkweeds are representative of herbs and woody plants belonging to this order.

Tubiflorales - Comprised mostly of herbs of which morning glory, dodder, bluebells, verbena, sage, mint, tobacco, and snapdragon are familiar.

Plantaginales - Plantains are the principal plants of this order of herbs.

Rubiales - Coffee, elders, and honeysuckle make up a portion of this order.

Campanulales - Well-known plants belonging to this order are lettuce, dandelion, the cucurbits, aster, and sunflower. This order is of considerable interest in the selection of plants for the investigation.

It is felt at this time that representative monocotyledons such as cereals, grasses, sedges, palms, lilies, orchids, many aquatic and marsh plants, and cattails should await completion of the present investigation. Though of great interest, the intensive coverage of such a broad field is not considered feasible within the realm of this contract. It is suggested that investigations of the monocotyledons be considered as an extension of the present contract.

Photosynthesis and Respiration

References on the photosynthesis of plant species are voluminous, non-specific to the requirements of this investigation and in general, refer to various areas of interest such as agronomic crops (A7, A10), various sun and shade plants (A2, A3), or plankton algae, arctic, tropical, or alpine plants (A9, A11, A13).

No references on the effect of low intensity artificial light on the plants selected for this study were available, but the data consistently obtained in the laboratories of the authors indicate that photosynthetic rate is significantly depressed under these conditions. Also, even though no data were obtainable on the edibility of plants grown under low light conditions, according to Burton, it is likely that yields will be reduced considerably, that carbohydrate content will decrease, and that protein and mineral content will increase (A4).

On the basis of a literature review, the production of pharmacologically active substances in the selected species when grown under the environmental conditions of space travel cannot be predicted. The selected plants do not produce any known substances with undesirable pharmacodynamic activity in a terrestrial environment. Production of active substances in an artificial environment has not been studied and will require examination prior to elimination of the selected plants. For example, the presence of latex cells and laticiferous systems in Lactuca, Cichorium and Taraxacum is well known (J1); however, these specialized systems are confined primarily to the root and stem sections and not the leaves. Under adverse growing conditions a bitter principle occurs in the leaves (K1), but its pharmacological properties, if any, have not been defined. Although members of the genera Brassica and Raphanus contain mustard oils in the seeds, the young plant is considered edible (J2). Again, a type of bitter principle is produced by members of these two genera under high intensity light or elevated temperature but the identity of the principle is not known. The genus Ipomea contains a species, Ipomea purga, which contains a purgative (J1). Ipomea batatus is not known to contain this substance. The genus Amaranthus also contains species that cause poisoning in cattle due primarily to the accumulation of nitrates in the leaves and stem (J2). Eighteen other species of plants ranging from oats to algae and including Beta vulgaris also produce nitrate poisoning in cattle. No references to Tetragonia expansa, New Zealand spinach, were found.

In addition to the criteria for plant selection mentioned in the preceding section, it would also be desirable to screen plants which can utilize gas mixtures richer in carbon dioxide than normally occurs in the atmosphere; i.e., 10-100 x 0.03%. A carbon dioxide enriched atmosphere would

*The reference numbers in the Literature Survey text refer to those in the annotated bibliography in the Appendix.

not only enhance photosynthesis (A5), but would be compatible with the atmospheric composition proposed for man; 0.5% carbon dioxide at one atmosphere. The data found in the literature suggests strongly that plants tolerate higher than normal concentrations of carbon dioxide (A1, A12).

It would also seem desirable to study plant growth as a function of humidity. Few investigators have studied humidity relations in plants as they affect growth, although Nightingale and Mitchell (C3) do state that low relative humidity depresses growth. It is likely that a separate moisture control system will be required for the plants even though the literature only infers that low relative humidity may reduce growth rate, but this may well depend upon the kinds selected.

The optimum temperature for plant growth also requires a separate system from that recommended for man, i.e., $70^{\circ} \pm 5^{\circ}$F. The plants selected for the study generally have a requirement for a 70°F. day, 60°F. night regime (C4).

Other data on the adverse effects of continuous temperatures (C1), high temperatures (C2), and the requirement for thermoperiodicity (C5) tend to indicate that separate temperature control systems must be proposed for man and for plants.

The effects of the quality of the illumination and length of the illumination period (B1, B2, B3, B4) on plant growth will have to be determined for the species selected for the study. One article on beans and wheat (B6) suggests a combination of fluorescent and incandescent lamps as the best source of illumination for optimum growth of plants under low light intensities.

Freedom from toxic vapors is a prime requisite in a sealed cabin atmosphere. This means not only freedom from volatile components of the mechanical and electrical parts but of the closed ecological systems as well. Volatile materials such as the aromatic principles of apples, onions, garlic, and bananas are well known. A search of the literature on the selected plants revealed that ethylene (E2) may be produced by Brassica oleracea. It is possible, however, that a more critical evaluation of the selected plants would reveal a group of volatile compounds which may or may not fall into the classification of toxic substances.

Plant Pathology

When plant diseases are considered, it is the same as has been indicated in the other sections: little within the literature is directly applicable to the space mission environment. Therefore, this review was approached by studying the disease problems which might involve the potential plant candidates.

The storage diseases have been excluded from this review, since at the present time storage for long periods is not anticipated, especially with fresh fruits or vegetables. It is not generally believed that major or minor element deficiencies will exist because complete nutrient solutions and/or modified waste effluent will be utilized as a medium for growth (K3, I12); however, this should be considered before final selection is made.

A non-parasitic disease known as tipburn could occur in an environment where a broad differential between air and soil temperatures exists (I2), but a reasonably controlled space vehicle or extraterrestrial environment would resolve these potential causal factors. Although none of the known selected varieties of lettuce, cabbage or celtuce are completely free of susceptibility to tipburn, most of the commercial varieties are screened for tolerance.

The "damping-off" disease which includes both pre-emergence and post-emergence damping-off is a common disease complex that occurs frequently on a great number of seedling plants. The species of fungi that may be considered causal factors are Pythium, Sclerotinia, Botrytis and Pellicularia. Any one of these may induce the disease (I11, I16). Control of damping-off diseases in a terrestrial environment may be accomplished by application of Arasan, Spergon or Cuprocide to the seed, and at 10-day intervals to the plant growth medium.

The plant diseases which might commonly occur in the terrestrial environment may not manifest themselves in the controlled environment of a space vehicle. It is assumed that the diseases which might become evident in a space vehicle or an extraterrestrial site would in all probability be those which are seed borne, physiogenic, or inherent. The selection of plants is based upon the commercial screening programs on tolerance to diseases. Various diseases such as the mosaic of dandelions, chard, spinach and the yellows disease of lettuce could be seed borne and transmitted by insects. Other viral diseases similarly may be nondetectable as seed borne disease; however, a similar program of screening for resistance in the commercial market is beneficial in the selection of plants.

Diseases of plants caused by bacteria are generally present and active in the soil (I4, I5, I6), as well as the many diseases caused by the fungi (I3, I7, I9, I10). The source of the pathogen may be the preceding crop, plant debris, irrigation water, or rain. Winds are a major source of dissemination of plant disease. Many of these means of dissemination would be eliminated in a space vehicle.

The Pox of sweet potato, beet, and turnip, caused by a species of Actinomyces, is soil borne and causes a seedling infection (I1). A soil rot disease of sweet potatoes (I3) caused by an Actinomycete, is usually found in dry soil with a pH less than 5.2. Ring rot of sweet potato occurs in the field as a soil rot and wet soil favors growth of the fungus (I15).

Nutrition

The methods for determining the nutritional value of the plants were largely derived from a review of the literature presented in Section G, Nutritional Value, of the Appendix. The analytical methods to be used in this study of plant parts have been selected as the most accurate and precise methods which will provide the required data. Although there may exist alternatives for some substances, it is felt that many of them lack the desired sensitivity or precision. The Stein-Moore method for amino acids is, of course, the standard, and relatively absolute control is provided by Kjeldahl nitrogen determination. The Lowry modified Folin protein estimation is extremely reliable and of wide applicability with the obvious

advantage of being a colorimetric procedure. For the most part, the other methods selected are standard for application to this type of investigation.

Propagation

The propagation of plants under artificial illumination requires blue fluorescent lamps for best results. The blue lamps tend to offset the etiolation effects of low light intensity (F3). Preliminary slip culturing and seed-tuber culturing have been successfully accomplished in laboratories of the contractor.

Experimental guide lines in the propagation of selected plants are those noted in Section F of the Appendix, Propagation.

Methods of CO_2 and O_2 Analysis

The requirement for large numbers of routine carbon dioxide and oxygen estimations virtually necessitates a rapid instrumental method of measurement. Carbon dioxide is readily measurable by either infrared (H13, H23) or gas chromatographic techniques (H9, H12). Oxygen can also be determined by gas chromatography (H20, H21) or by paramagnetic instruments (H8) such as the Beckman oxygen analyzer, but not by infrared analysis. By the use of an appropriate double column system the gas chromatograph can give results for both carbon dioxide and oxygen from a single sample in a time period of less than 5 minutes. This allows frequent analysis of several different plant systems over the same growth period. The infrared and paramagnetic instruments are not so readily adaptable to multiple analyses. However, they may be utilized in the event that the gas chromatographic procedure should prove to be not sufficiently sensitive or accurate for the required measurements. Preliminary measurements at Boeing indicated that the gas chromatographic method gave satisfactory results for the plants tested.

Other methods of carbon dioxide and oxygen measurement are covered in the bibliography although it is not anticipated that any technique other than the ones indicated above will be required to obtain the desired results.

The attached bibliography is by no means an exhaustive review of the literature but is a representative sample of the literature pertinent to the problem. Every effort was made to obtain literature which applied directly to the plants under study.

On the basis of the above taxonomic survey, the designated criteria, and personal knowledge of the senior investigators concerning the horticultural programs of the USDA, the State Experiment Stations, and commercial firms, the following plants were selected for study in this program.

Lactuca sativa

1. Lettuce - variety Slobolt. Broadleaf, round head. Leaves edible.
2. Lettuce - variety Early Great Lakes. Broadleaf, round head. Leaves edible.
3. Lettuce - variety Great Lakes. Broadleaf, round head. Leaves edible.
4. Romaine - variety Paris Dark Green. Long, narrow leaf. Leaves edible.
5. Celtuce - variety Celtuce. Non-heading, large leaf. Edible, large, fleshy stem.

Members of the genus Lactuca were selected primarily because of their edibility, high ratio of leaf to stem area, and relative freedom from disease. Five species of the genus were selected in order to determine a range in response to treatment. Of the three lettuce varieties, Slobolt is a dwarf, compact plant, Great Lakes is a rather large plant, while the Early strain matures approximately one week earlier and is resistant to tipburn injury. The Romaine variety was included to see if the higher chlorophyll content, i.e., dark green leaves as opposed to the pale green leaves of lettuce, results in a significantly higher photosynthetic rate. Celtuce was added in view of the relatively low ratio of leaf to stem area.

Brassica chinensis

6. Chinese cabbage - variety Wong Bok Poatung. Round head, large, savoyed leaves. Leaves and stem edible.

Brassica chinensis was selected on the basis of edibility and very high leaf to stem ratio.

Brassica oleracea

7. Cabbage - variety Savoy Iron Head. Head flat, large leaf. Leaves and stem edible.
8. Cabbage, celery - variety Special Selections. Head elongate, large leaf. Leaves and stem edible.
9. Cauliflower - variety Snowball. Non-heading, very large leaf. Leaves edible.

10. Kale - variety Blue Green Curled. Non-heading, very large leaf. Leaves edible.

11. Kale - variety Georgia. Non-heading, very large leaf. Leaves edible.

12. Collards - variety Georgia. Non-heading, large leaf. Leaves edible.

These members of the cabbage family were selected also on the basis of edibility and high ratio of leaf to stem area. Savoy Iron Head is a small, early cabbage suitable for eating raw. Celery cabbage is a more succulent leafed variety with a loose open head. Snowball is a very large leafed variety of cauliflower which will be used to compare leaf size with the standard cabbage, Savoy Iron Head. The two Kales and Collards were included since these varieties are larger leafed cabbages and more heat resistant.

Brassica rapa

13. Turnip - variety Seven Top

Brassica rapa, Turnip was selected for the small elongate root, elongate leaf and petiole, and edibility of the entire plant.

Beta vulgaris cicla

14. Swiss chard - variety Fordhook Giant. Broad, succulent leaf. Leaves edible.

Swiss chard has been selected because of the large leaf area, deep green of leaves, edibility, and to serve as a comparison to the members of the cabbage family.

Cichorium endiva

15. Endive - variety Salad King. Finely curled, edible leaf.

The Salad King Endive is a dark green strain which can withstand hot weather or frost, and has vigorous growth.

Taraxacum officinale

16. Dandelion - variety Thick Leaf. Elongate, edible leaf.

The thick leaf dandelion is a member of the same order of plants as lettuce. It is far superior to the uncultivated plant, having large thick edible leaves and a large leaf area.

Raphanus sativas

17. Radish - variety Cherry Belle. Elongate leaf. Edible root.

The radish was chosen for its small compact growth with a small crisp edible root and short but broad leaves. Growth is moderately fast.

Tetragonia expansa

18. New Zealand spinach - no varietal name. Small, thick-leaf; prostrate habit of growth with young stem tips. Leaves edible.

New Zealand spinach was selected because of its resistance to heat and because it can be cut throughout its vegetative growth.

Amaranthus gangeticus

19. Tampala - variety Regular.

The tampala leaf is used like spinach, retains its tenderness for a long period, and is well suited for warm weather. The entire young plant is edible.

Ipomoea batatus

20. Sweet potato - variety Yellow Gem. Vine-like; large leaf. Edible fleshy roots.

The sweet potato has been selected for its vine-like growth, the edible tuberous roots, and large leaf area. Vegetative reproduction can be accomplished with comparative ease.

EXPERIMENTAL STUDIES*

A. Methods and Characteristics of Growth in Low Intensity Artificial Light.

Of the commonly available sources of low intensity artificial light, the fluorescent tube presents the most reasonable spectrum for plant growth and is the simplest to utilize under laboratory conditions (Figure 1). In addition, plants grown under "white" fluorescent light exhibit a minimum of stem elongation and appear to be somewhat more compact than plants grown under other sources. The lamps used in these experimental studies were General Electric F42T6 warm white, blue, green and pink. The lamps used in the Labline controlled environment chamber were General Electric F96T12 cool white.

In a previous study (Johnson, S. P. and Betty Benishek. Unpublished Reports. The Boeing Company. 1960), it was noted that plants exhibited a more normal appearance if the seeds were germinated and the seedlings grown under blue fluorescent tubes. Etiolation of stem, leaf, and petiole was considerably less in plants grown under the blue light than when either green, pink, or white fluorescent light was used. Preliminary studies evidenced a slightly higher dry weight of plants grown under green lights. Accordingly, for these studies the seeds were germinated under blue fluorescent lights in plywood cabinets painted a flat white and equipped with twelve fluorescent lamps each. Temperatures were held at $83^{\circ} \pm 2^{\circ}$F. during the day and $68^{\circ} \pm 2^{\circ}$F. at night. The length of the light period was 14 hours and the intensity, as measured by a Weston light meter, was 700 f.c. at plant height.

The growth medium was a mixture of equal parts of Perlite and peat moss moistened with a solution composed of 30 gm KNO_3, 30 gm $Ca(NO_3)_2$, and 30 gm KH_2SO_4 in 7 liters of water. The wet mix was limed so that random samples had a pH of 6.5 ± 0.2. Prior to planting, the seeds were soaked in warm water for one hour.

After two weeks under blue light, the seedlings were thinned to five per pot and placed under green fluorescent light, assuming an increase in dry weight was probable. Following two weeks of exposure to green light, the plants were transferred to cabinets containing white light for an additional two weeks. The six-week seedlings were then ready for experimental use. During the entire period, the plants were watered daily with tap water and weekly with dilute Shives Solution (Table I).

Growth Response to Low Intensity Artificial Light

Observations were made as to general growth characteristics under these conditions. Members of the genus Lactuca exhibited extreme etiolation (blanching) and elongation of the hypocotyl and epicotyl. Cotyledons and true leaves were strap-shaped and leaf margins were entire whereas the typical morphology is one of crinkled or curled broad leaves (9).

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*Reference numbers in this and following sections refer to those cited in the References section.

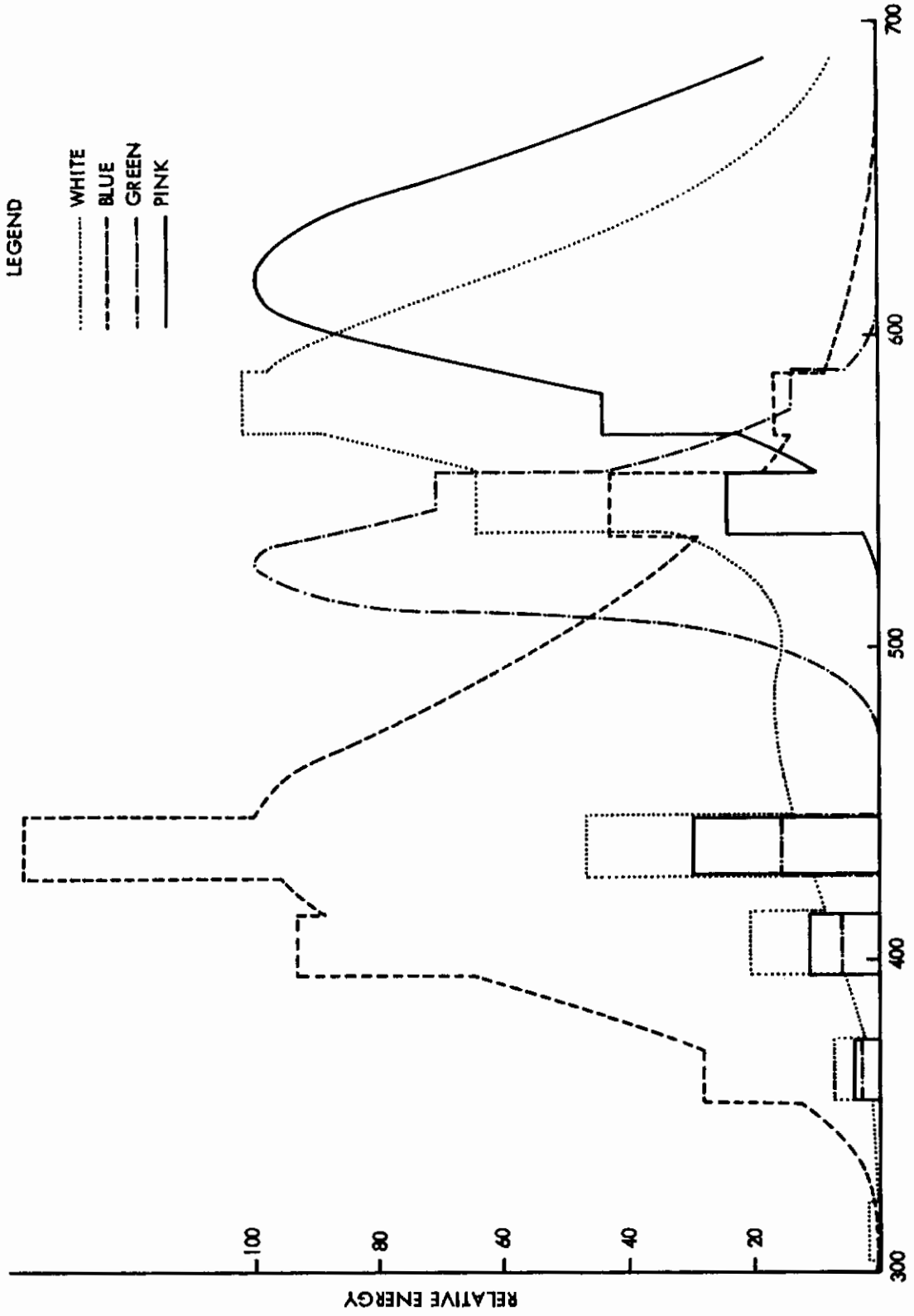


FIG. 1 SPECTRA OF WHITE, BLUE, GREEN AND PINK FLUORESCENT LAMPS

TABLE I

CHEMICAL COMPOSITION OF SHIVES SOLUTION*

Macronutrients	Grams per 20 liters
KH_2PO_4	5.9
$Ca(NO_3)_2 \cdot 4H_2O$	20.1
$MgSO_4 \cdot 7H_2O$	10.7
$(NH_4)_2SO_4$	1.8
Micronutrients	**In 500 ml H_2O**
H_3BO_3	0.8 gm
$MnSO_4$	0.8 gm
$ZnSO_4$	0.8 gm
$CuSO_4$	0.4 gm

*10 ml added to each liter of solution.

The leaves were a very pale green color instead of light green. No change was noticed when the seedlings were placed in the green light or later under the white light. Abnormalities of growth are shown in Figure 2. A similar situation existed when several varieties of lettuce were grown in pink light in an earlier study by S. P. Johnson and Betty Benishek at Boeing.

Similar observations were recorded for members of the genus Brassica with the exception that leaf margins were the normal crinkled or curled leaf. However, the stems and petioles of kale, varieties Blue Green Curled and Georgia, and the Georgia Collard remained etiolated. Swiss chard exhibited signs of etiolation of the hypocotyl but not the epicotyl (see Figures 3, 4, 5). However, the number of leaves and their size was considerably reduced.

Ipomoea batatus, sweet potato, grew normally for a short period of time, then the stems of succeeding flushes of growth produced abnormally small leaves (Figure 6).

Hypocotyl and epicotyl etiolation were also noted in the cabbage, variety Savoy Iron Head, although stem elongation was reduced as the plant aged. Epicotyl elongation was minor in the cabbage, variety celery. This variety, however, could not be distinguished from Chinese cabbage at maturity.

The plants eliminated from further testing for reasons cited above were:

Lactuca sativa

Lettuce - variety Slobolt

Lettuce - variety Early Great Lakes

Lettuce - variety Great Lakes

Romaine - variety Paris Dark Green

Celtuce - no varietal name

Brassica oleracia

Cabbage, celery - variety Special Selections

Kale - variety Blue Green Curled

Kale - variety Georgia

Collards - variety Georgia

Beta vulgaris cicla

Swiss chard - variety Fordhook Giant

Ipomoea batatus

Sweet potato - variety Yellow Gem

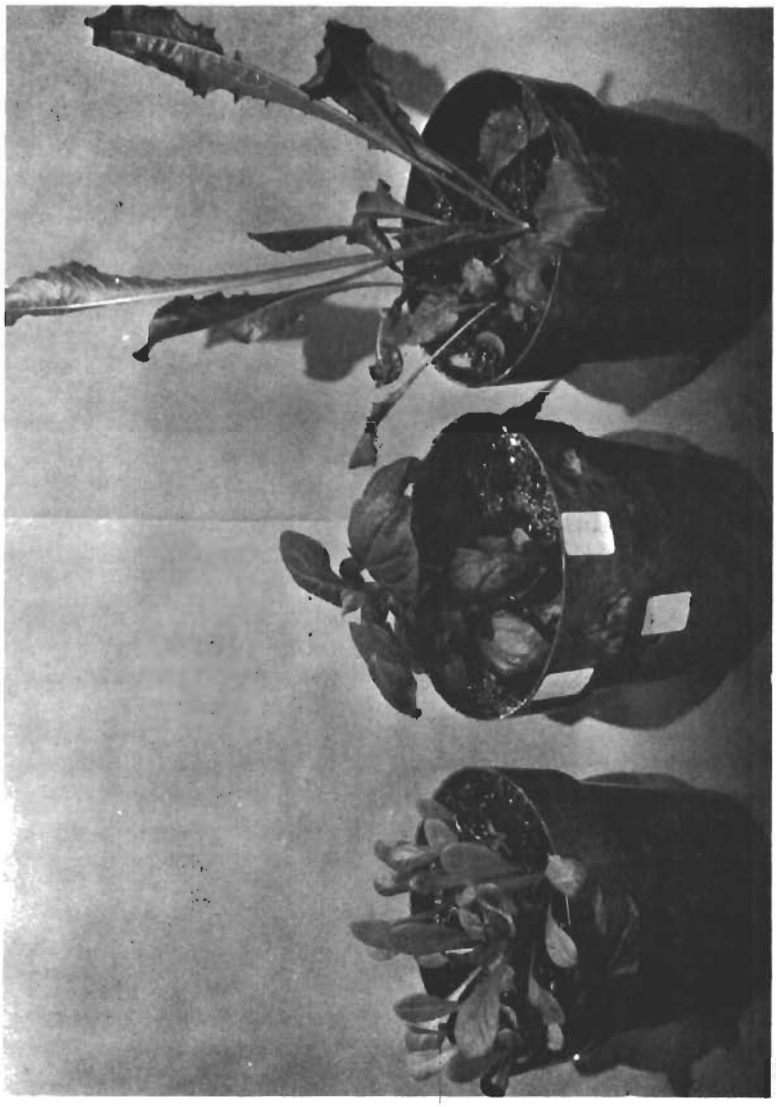


FIGURE 2 LEAF ETIOLATION IN LETTUCE (LEFT), NORMAL GROWTH IN NEW ZEALAND SPINACH (MIDDLE), AND EXTREME LEAF ETIOLATION IN DANDELION (RIGHT)

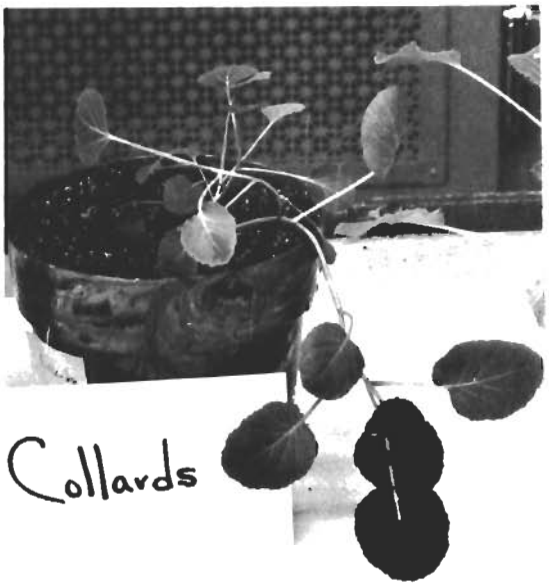


FIGURE 3 COLLARDS, ETIOLATION OF STEM AND PETIOLE

FIGURE 4 KALE, ETIOLATION OF STEM AND PETIOLE

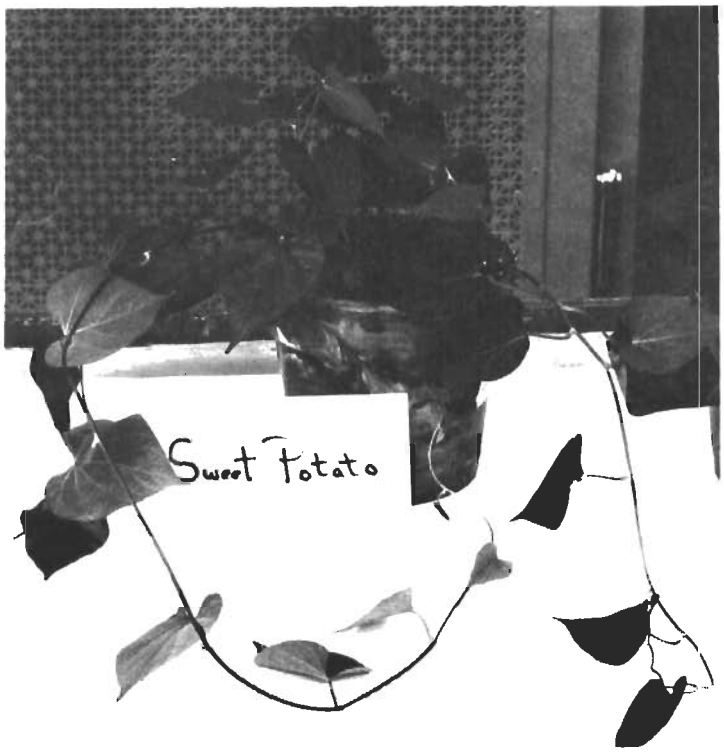


FIGURE 5 EXTREME VINE-LIKE GROWTH OF SWEET POTATO UNDER LOW LIGHT INTENSITY

FIGURE 6 PETIOLE ETIOLATION AND REDUCED INITIATION IN LEAVES OF SWISS CHARD

B. Preliminary Screening for Photosynthetic Activity

Two methods were employed for the measurement of photosynthetic activity. In the first, a well-watered plant was placed under a 5-liter glass battery jar which was sealed with vacuum grease to an aluminum base plate. The base plate contained inlet and outlet ports through which the atmosphere was recirculated by means of a small diaphragm pump (Dyna-pump). Two 10-lamp fluorescent light banks were placed on each side of the battery jar and a 500-watt photoflood lamp was placed directly above. The total incident light intensity, as measured by a Weston light meter, Model 756, was approximately 2000 f.c. from above and 1400 f.c. from the sides. The atmosphere was circulated in series through a Beckman E2 oxygen analyzer for measurements of oxygen concentration and an Aerograph gas chromatograph for carbon dioxide estimation. The system was cooled by an evaporative blower which maintained a temperature of 85 - 90°F. inside the battery jar.

A volume of carbon dioxide equivalent to two per cent of the volume of the system was injected into the battery jar and measurements of carbon dioxide utilization and oxygen production were started. After the initial period required for mixing of the atmospheric components, data were recorded at 15 minute intervals and are shown in Figure 8. During these measurements, humidity and temperature could not be adequately controlled and the condensate on the sides of the battery jar varied from plant to plant. Measurement of the effect of the condensate on light intensity showed a decrease of up to 11 per cent.

The alternate method was similar to that described above except that a lucite box (1 ft^3) was used in place of the battery jar. In addition, a dry ice-alcohol mixture was circulated through a coil within the plastic box to hold internal temperatures at or below 75°F. and to eliminate condensate. A humidistat placed within the plastic box revealed that as the coolant was shut off, the humidity rapidly increased up to 100 per cent and then decreased rapidly as the coolant was recirculated through the box during temperature control periods. The data obtained by this method are similar to the data obtained in Figure 8, and recorded in Table II.

Following the oxygen and carbon dioxide measurements described, further evaluation of the usable photosynthetic potential was made by measurement of the leaf area versus the area occupied by the whole plant. The plants were approximately 45 days old. The shadow leaf area of each species was photographed against a white background and printed on a sheet of photographic paper of known dimensions and weight. From the cut-out photograph, the leaf area could be calculated from its weight and expressed in square centimeters. In addition, the excised leaves were photographed, weighed, and the total leaf area calculated. These data are shown in Table IV. For comparative purposes the plant species are listed in order from highest to lowest parameters (Table III).

As may be seen, tampala, Chinese cabbage, turnip, the Savoy variety of cabbage, cauliflower, endive and New Zealand spinach appeared to be the most likely candidates for further study. Plants eliminated from this group were: cabbage, cauliflower, turnip and New Zealand spinach.

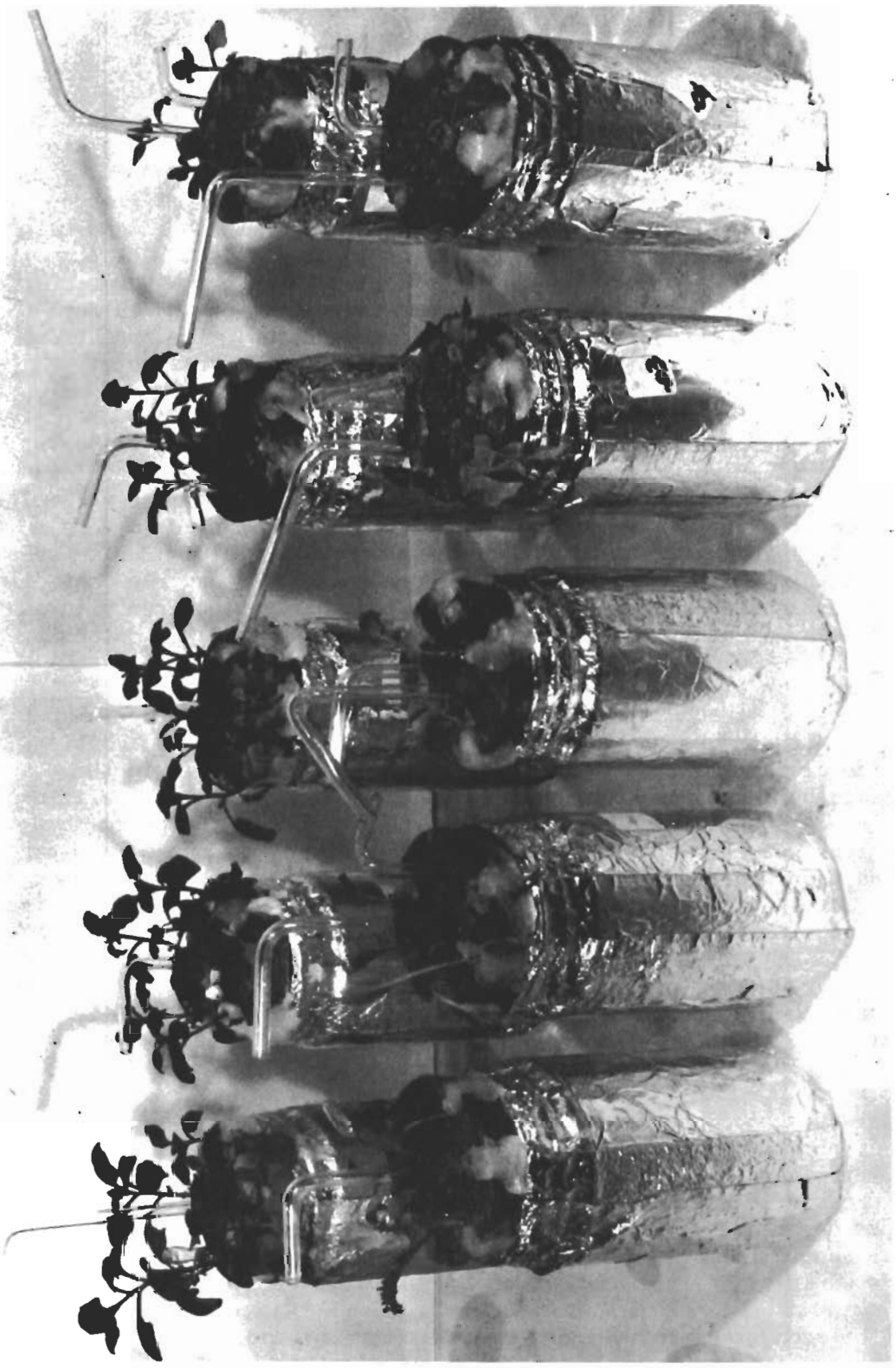
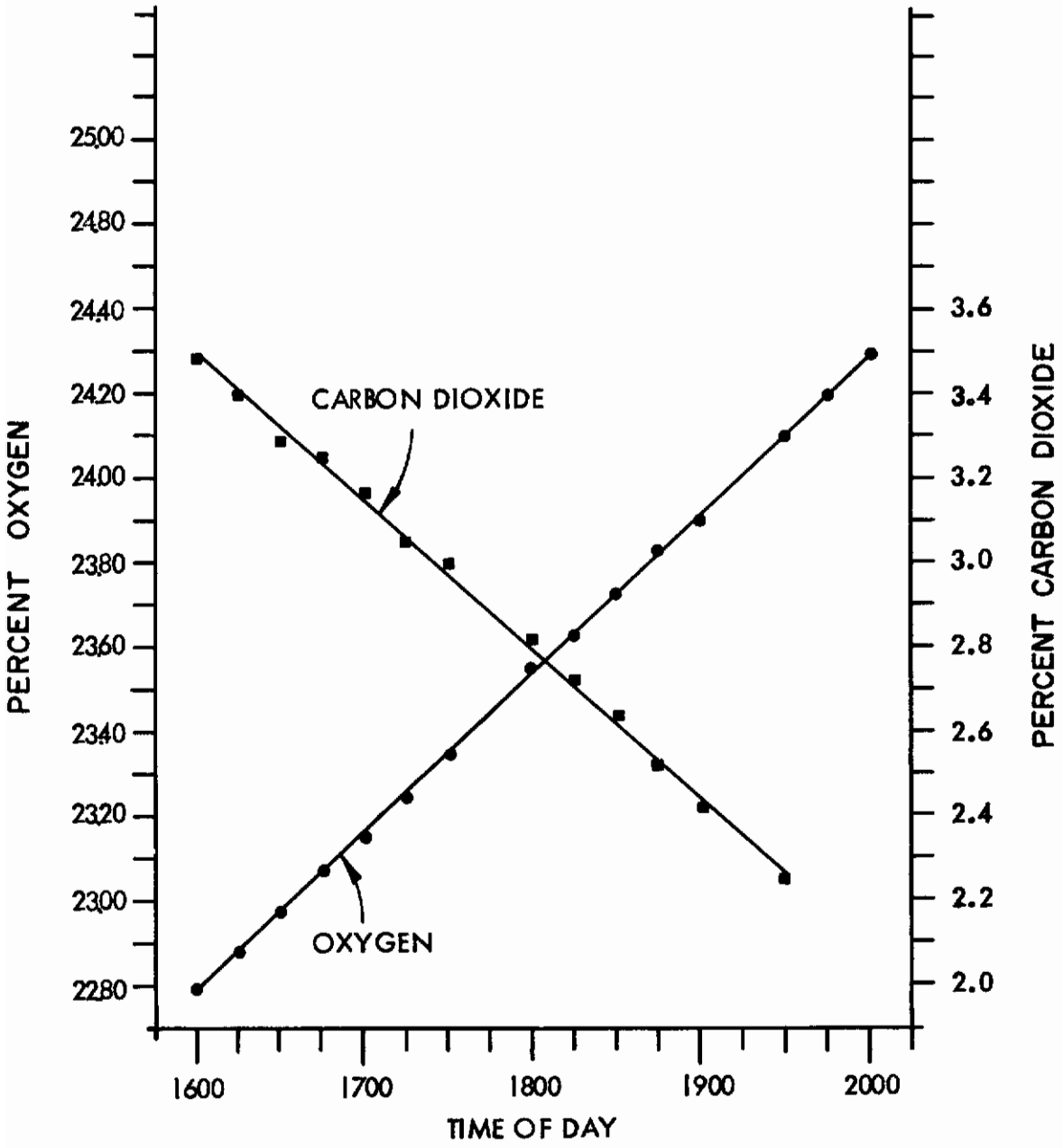


FIGURE 7 METHOD OF CONTAINING PLANTS FOR OSMOTIC PRESSURE TESTS



AN EXAMPLE OF LINEARITY OF DATA ON OXYGEN, CARBON DIOXIDE RUN.

FIG. 8 FEB 9, 1962 CHINESE CABBAGE

TABLE II

PRELIMINARY SCREENING FOR PHOTOSYNTHETIC ACTIVITY

Plant	Light Intensity (fc)	$ml/dm^2/hr$ $+O_2$	$-CO_2$
Chinese Cabbage	1200	3.8	6.8
	2000	3.6	3.0
	8000	6.2	5.1
	8000	7.0	5.6
Cabbage, Savoy	1500	4.4	11.6
	2000	15.1	9.8
	8000	11.2	13.5
Endive	1500	3.6	8.7
	2000	6.5	5.2
	2000	4.2	3.7
Tampala	1200	3.8	8.0
	1500	3.2	7.4
	2000	3.2	4.7
	8000	6.6	4.2
N. Z. Spinach	1500	6.6	11.3
	8000	13.2	13.4
Turnip	1500	6.8	17.5
	2000	16.4	13.7

TABLE III

Weight in Grams	Total Leaf Area	Area Covered By Plant	Total Leaf Area/ Area Covered By Plant
Chinese Cabbage	Chinese Cabbage	Cabbage, Savoy	Turnip
Turnip	Cabbage, Savoy	Chinese Cabbage	Chinese Cabbage
Cabbage, Savoy	Endive	Endive	Dandelion
Endive	Turnip	Cauliflower	N. Z. Spinach
Cauliflower	Tampala	Tampala	Endive
N. Z. Spinach	Cauliflower	N. Z. Spinach	Tampala
Tampala	N. Z. Spinach	Dandelion	Cauliflower
Dandelion	Dandelion	Turnip	Cabbage, Savoy

TABLE IV

WEIGHT AND AREA MEASUREMENTS OF LEAVES OF EIGHT OF THE SELECTED PLANT SPECIES

An Average of Three Plants

Variety	Leaf Weight/Plant in Grams	Area/1 gm of Leaf Tissue	Total Area in Sq. Cm.	Total Shadow Area Covered by Plant	Ratio: Total Area/ Area of Plant
Cabbage (Savoy Iron Head)	18.3	26.8	478.0	379.5	1.26
Cauliflower	10.3	23.0	237.1	188.10	1.26
Chinese Cabbage	22.8	23.9	533.0	261.4	2.04
Dandelion	3.8	40.8	155.2	72.0	2.16
Endive	15.4	28.9	444.6	254.9	1.74
New Zealand Spinach	7.3	23.6	172.0	85.1	2.02
Tampala	6.1	39.2	239.0	171.8	1.39
Turnip	19.4	22.2	431.3	8.0	53.9

Cabbage and cauliflower were eliminated because of the gas they produce upon ingestion which would result in extreme discomfort at high altitude. Turnip and radish exhibit an unfavorable mode of growth for maximum photosynthesis. In the 3 weeks following emergence, these species produced from 6 to 8 leaves and then the plants became dormant with only 3 to 4 leaves fully expanded. The remaining leaves were from 1 to 5 centimeters in length at maturity. During the dormant period of approximately 60 days the roots enlarged into the typical radish or turnip. The terminal of the turnip remained dormant for an additional 2 months, after which the turnips were discarded. However, the dormancy in the radish was broken and elongation of the stem occurred (Figure 14).

During the period of oxygen and carbon dioxide measurements, the New Zealand spinach plants flowered and were eliminated from further consideration.

The dandelion (Figure 10), although it maintained a rosette mode of growth, produced an insufficient number of leaves and was dropped from further consideration. The remaining 3 species, Chinese cabbage, endive, and tampala, were retained for additional study.

C. Tolerance to Salts

In closed ecological systems, the nutrient substrate for plant growth will be supplied by human waste products. One of the problems involved in using these waste products is the high ratio of sodium to other ions. Table V shows the ion content, i.e., calcium, magnesium, potassium and sodium, of human waste as opposed to a standard plant nutrient solution. Because of this high ratio the plants must have considerable tolerance to sodium chloride in the nutrient solution, if concentrations of calcium, magnesium and potassium are high enough to support adequate plant growth.

TABLE V

Comparison of a Plant Medium with the Average Composition of Human Excretion Products

	Plant Media (1) Mg/L	Excretion Products (2) mg per Kg Body Weight/24 Hrs.
Ca	168	10
Mg	50	4
K	241	34
Na	Trace	46

(1) Table 192, Synthetic Culture Media, Plant. Handbook of Biological Data. WADD Tech. Report 56-273. Oct. 1956

(2) Table 222, ibid.

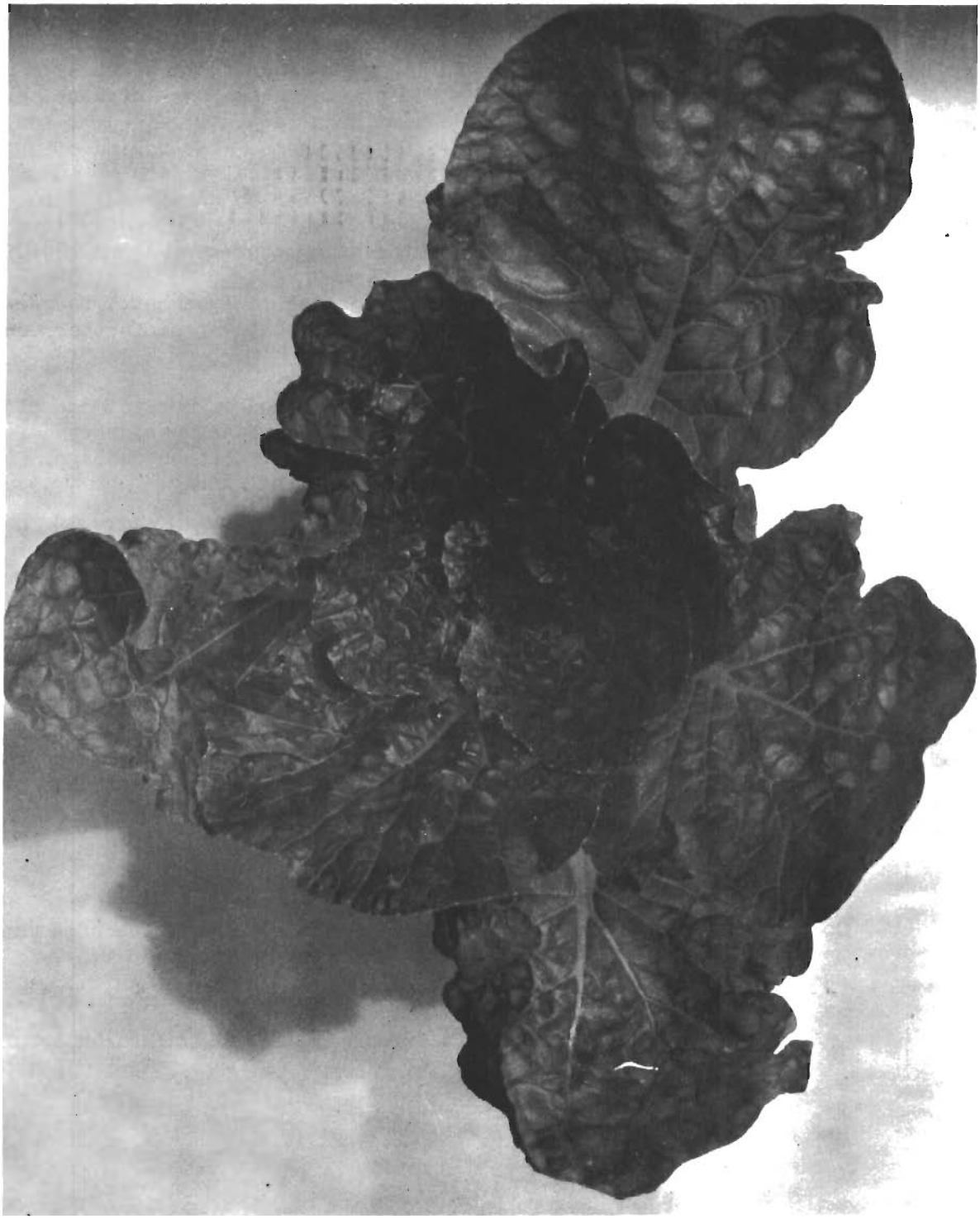


FIGURE 9 CHINESE CABBAGE (TOP VIEW)

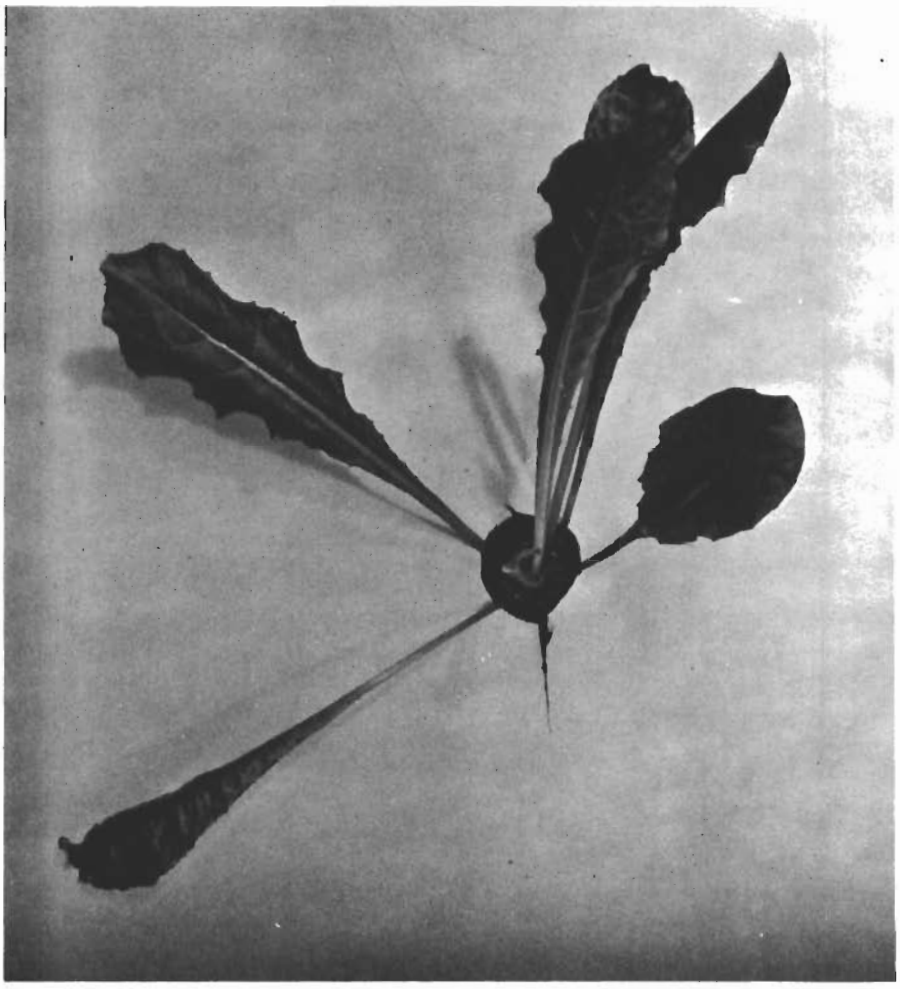


FIGURE 10 DANDELION (TOP VIEW)

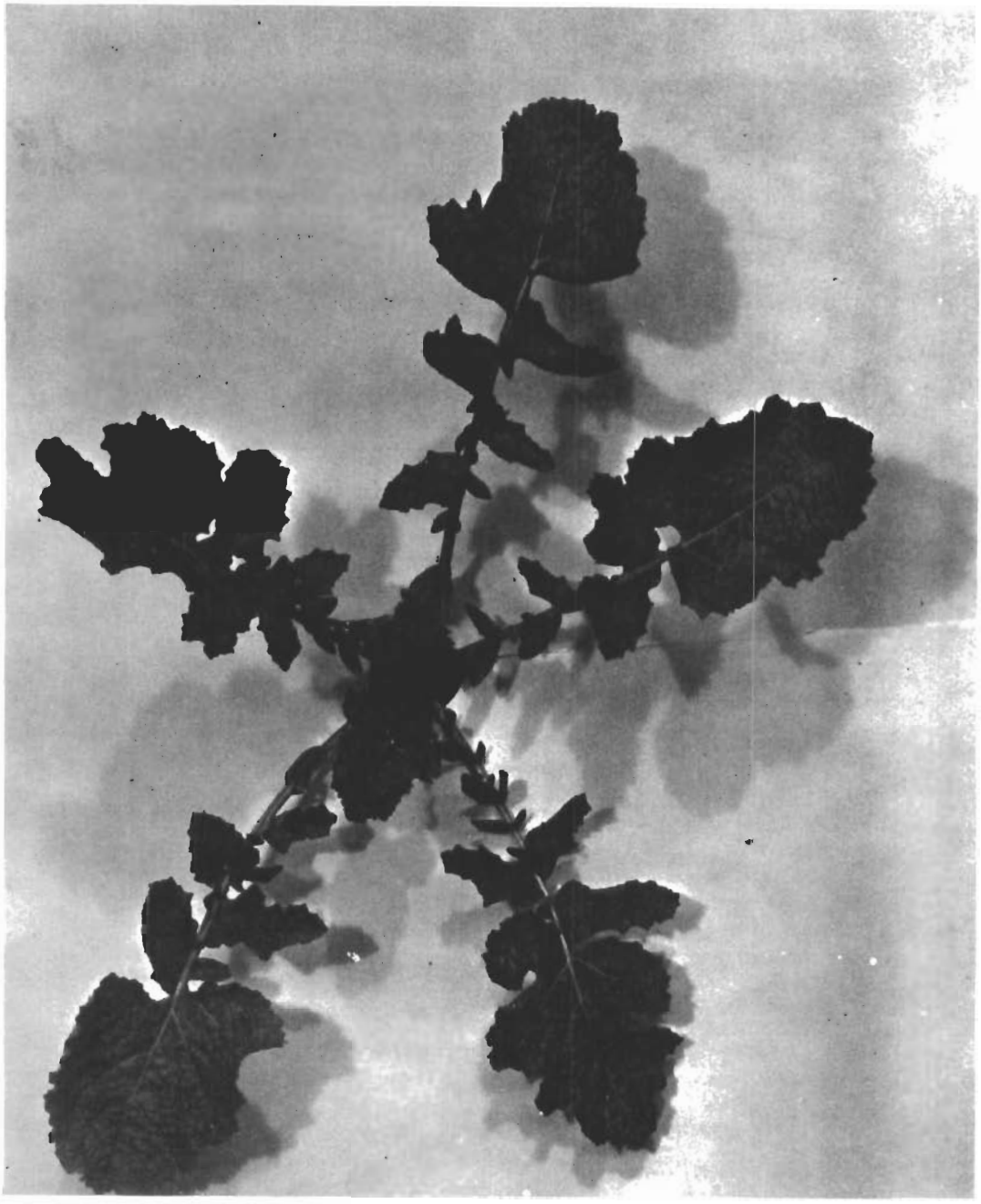


FIGURE 11 TURNIP (TOP VIEW)

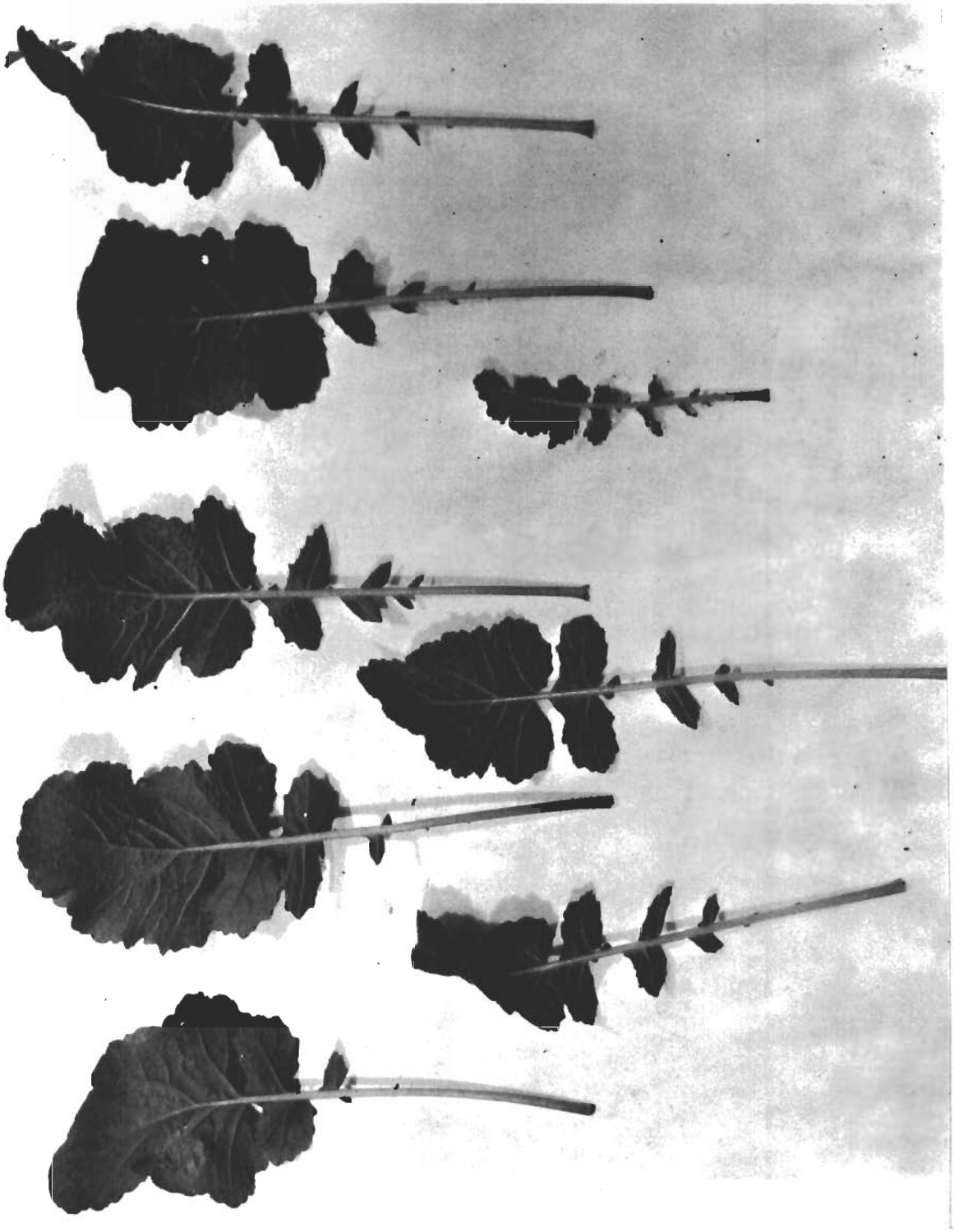


FIGURE 12 EXCISED TURNIP LEAVES

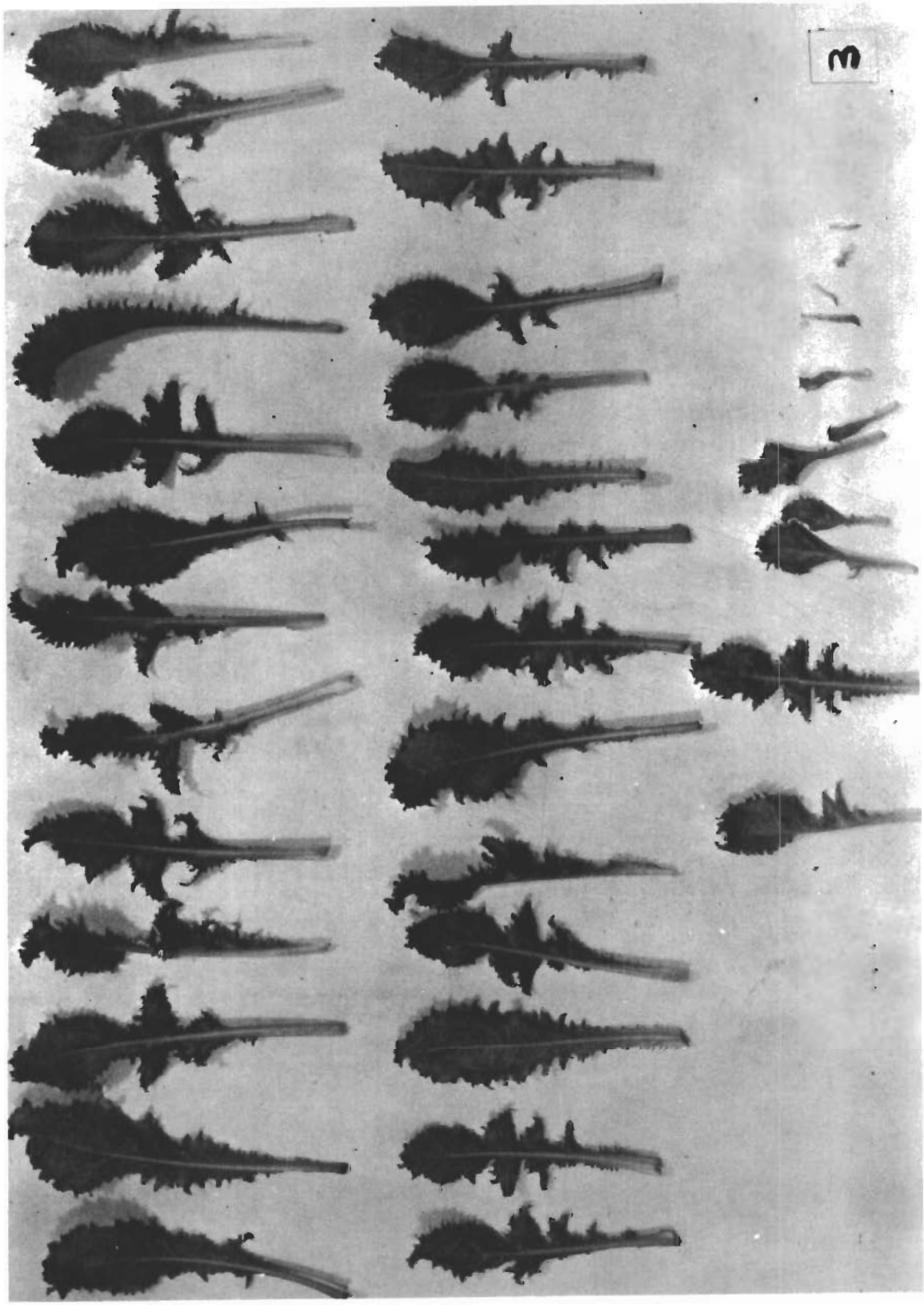


FIGURE 13 EXCISED ENDIVE LEAVES

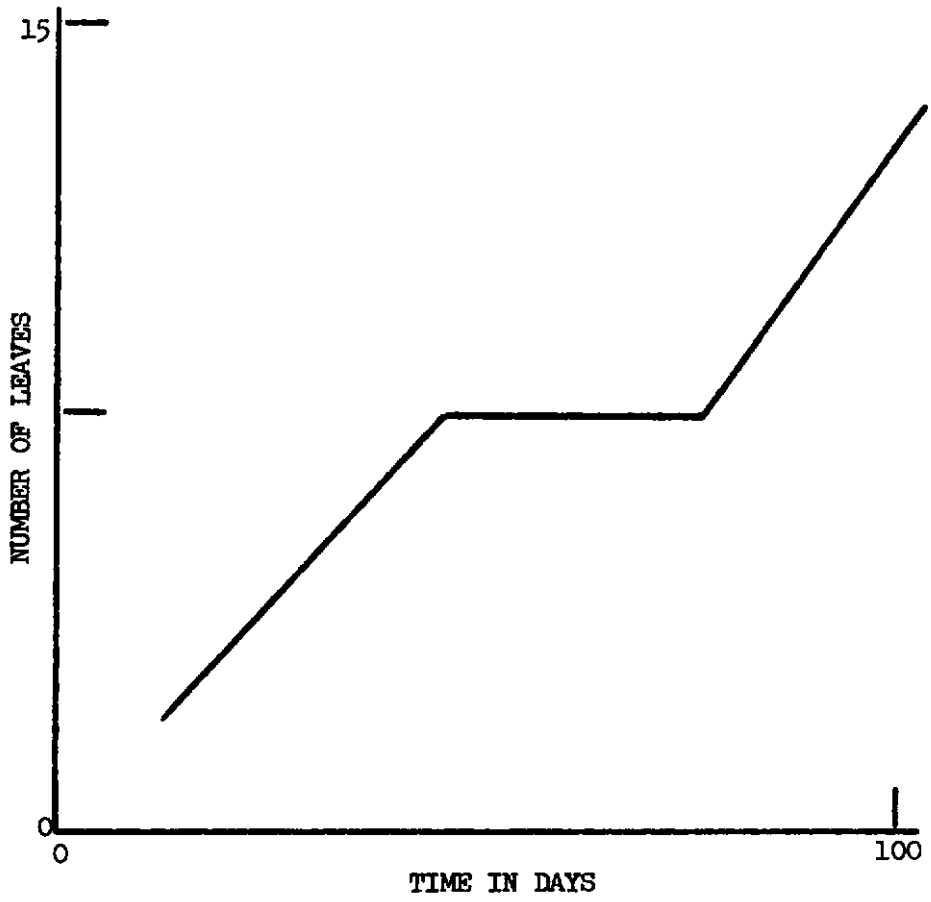


FIGURE 14 LEAF PRODUCTION, RADISH AND TURNIP

For preliminary screening purposes, a medium composed of 1/10 Shives solution (Table I) plus 0.01, 0.02, 0.03 or 0.04 molar sodium chloride were prepared. These solutions were equivalent to 585, 1170, 1755, and 2340 mg/l NaCl, respectively. Approximately 800 ml of each solution was placed in wide mouth one-quart mason jars leaving about one centimeter for airspace. Holes were drilled in a cork stopper so that it would hold 5 six week old seedling plants and an outlet and inlet glass tube for aeration. The seedlings were placed in holes in the stopper and sealed in with cotton and warm wax. Jars, in duplicate, containing the various solutions were connected to a humidified aeration line. The experimental technique is shown in Figure 7.

A preliminary evaluation of water loss indicated that approximately 1 ml of water was lost per day per mason jar. Water loss due to transpiration and evaporation was replaced on Mondays, Wednesdays, and Fridays during the 2 week run. Data were obtained on water loss and the death rate of the plants.

Tampala and cabbage showed a greater loss of water at high concentration of sodium chloride than was exhibited by the controls (standard Shives Solution). The reverse was true for Swiss chard and New Zealand spinach.

Of the 6 plants tested, only Chinese cabbage and endive were affected by the sodium chloride solutions. Endive would not tolerate 0.03 molar NaCl while Chinese cabbage showed a loss of 50 per cent of the plants after 6 days at a concentration of 0.04 molar. Browning of the root tips was the first evidence of injury followed by a general darkening of the entire root system with an accompanying wilt which remained permanent. Necrotic areas in the leaves also appeared at the onset of root system darkening.

A second series of tests were run with 0.01, 0.02, 0.03 and 0.04 molar calcium chloride and Shives Solution. The results of this test were similar to the results obtained with sodium chloride.

D. Photosynthetic Studies

Carbon dioxide utilization and oxygen production data were obtained on 2 species, endive and Chinese cabbage. Initially, tampala was selected for these studies but a large portion of this group developed a vascular disease of unknown origin. Sterile stem sections placed on agar developed a mycelial growth characteristic of the genus Fusarium. Other groups of plants grown from the same seed lot did not develop the disease.

The method used for photosynthetic measurement was quite similar to the methods described previously. Three plastic boxes, each with a capacity of approximately 130 liters, were placed on top of an aluminum base equipped with an inlet and outlet connection for atmospheric recycling, a cold water coil for cooling and control of condensation, and a tube for changing the nutrient substrate. In addition, the plastic box was equipped with a small fan to aid in rapid mixing of the atmospheric gases. A Labline controlled-environment chamber was programmed for a

14 hour light period and temperatures of 85°F. during the day and 65°F. during the night. The relative humidity in the boxes was not measured. The light intensity at plant level, as measured by an Eppley pyrheliometer, Model 50, is shown in Table VI.

TABLE VI

Light Intensity at Plant Height in the Labline as Measured by an Eppley Pyrheliometer, Model 50, Amplified by a Kintel Model III Amplifier and Head by a Fluke Differential Voltmeter, Model 801

	I	ΔI
	$(Cal/Cm^{-2}/min^{-1})$	
Plant alone	0.144	
Plant covered by box	0.124	-13.4%
Plant box partially covered with condensate	0.117	-18.6%

Twelve each of the endive and Chinese cabbage plants were placed in the chamber for a conditioning period of 10 days prior to start of the tests. Each group of 12 plants was divided into 3 lots. The endive plants were (a) the control, (b) 50 mg/l added sodium chloride, and (c) 100 mg/l added sodium chloride. The Chinese cabbage were (a) the control, (b) 250 mg/l added sodium chloride, and (c) 750 mg/l added sodium chloride.

One endive plant was placed in each of the 3 plastic boxes which were connected in series. A measured amount of carbon dioxide gas was introduced into the system. After allowing several hours for thorough mixing of the atmospheric components, data on carbon dioxide utilization and oxygen production were taken at 15 minute intervals. The leakage rate was negligible.

Data were obtained over a 12 hour period during which the box temperatures were 85° ± 1°F. Figure 18 illustrates the technique of utilizing the plastic box for measurement of O_2 evolution and CO_2 utilization.

In the Chinese cabbage (Table VII), a general increase in utilization of CO_2 and O_2 production were noted as NaCl concentration was increased.

Although endive was the most susceptible species to salt damage in the preliminary screening test, increases of salt concentration of up to 200 mg/l in the three plants used for the 100 mg/l experiment above were without visible effect. At 2200 mg/l some leaf curl appeared. The 750 mg/l Chinese cabbage plants were increased to 2500 ppm sodium chloride without visible signs of damage.

TABLE VII

AVERAGE OXYGEN PRODUCTION AND CARBON DIOXIDE UTILIZATION DURING LIGHT PERIOD BY ENDIVE AND CHINESE CABBAGE DURING LIGHT PERIOD

	$ml/dm^2/hr$*		Photosynthetic Quotient
	$+O_2$	$-CO_2$	O_2/CO_2
Endive, Control	3.38	4.63	0.73
Endive, +50 ppm NaCl	2.98	2.67	1.12
Endive, +100 ppm NaCl	2.85	2.80	1.02
Chinese Cabbage, Control	0.74	0.94	0.79
Chinese Cabbage, +250 ppm NaCl	1.10	1.32	0.83
Chinese Cabbage, +750 ppm NaCl	1.31	1.25	1.04

*Volume of gas utilized or produced per square decimeter of cross sectional area of plant leaf per unit time.

The data obtained on the endive plants was similar to that obtained in preliminary screening tests (Table II). Both carbon dioxide and oxygen measurements were essentially linear with time. No differences in oxygen production could be attributed to differences in carbon dioxide content of the atmosphere. Endive plants proved superior in oxygen production and carbon dioxide utilization. However, the differences may be due to experimental techniques rather than photosynthetic activity (Tables VII and VIII).

Differences occurred between the response of endive to sodium chloride treatments. As the salt content of the medium was increased oxygen production and carbon dioxide utilization decreased (Table VII).

E. Exotic Atmosphere Studies

Preliminary investigations concerning the replacement of nitrogen with an inert gas, helium or argon, were initiated. The plastic box described previously under photosynthetic studies was modified by placing an additional port on top of the box to facilitate gas mixing. The desired atmospheric composition was obtained by the simultaneous injection of each gas through a calibrated rotometer into a four-way mixing connector leading to the plant box. After allowing several hours for stabilization of the atmospheric compound, the oxygen and carbon dioxide measurements were recorded (Table IX).

While leakage problems were encountered with the helium, no leakage problems were encountered with argon. Data from these experiments (Table IX) indicate that these inert gases do not interfere with photosynthesis.

At the end of the run nitrogen was allowed to accumulate in the atmosphere of the box during the dark period. At 8:30 A.M. the following morning, the lights were turned on and the depletion rate of nitrogen was followed. These data are shown in Table X.

TABLE VIII

HOURLY OXYGEN PRODUCTION AND CARBON DIOXIDE UTILIZATION DURING LIGHT PERIOD BY ENDIVE AND CHINESE CABBAGE AS EXPRESSED IN MILLILITERS PER GRAM OF WET WEIGHT OF TISSUE PER HOUR

	ml/gm/hr	
	$+O_2$	$-CO_2$
Endive, Control	0.67	0.92
Endive, +50 ppm NaCl	0.62	0.55
Endive, +100 ppm NaCl	0.62	0.59
Chinese Cabbage, Control	0.12	0.15
Chinese Cabbage, +250 ppm NaCl	0.14	0.17
Chinese Cabbage, +750 ppm NaCl	0.19	0.19

TABLE IX

Photosynthetic Activity of Endive in Atmospheres Containing Helium and Argon

HELIUM

Time	ml $O_2/dm^2/hr$	ml $CO_2/dm^2/hr$	Photosynthetic Quotient
1st hour	4.60	4.68	
2nd hour	4.60	4.44	
3rd hour	4.21	3.98	
Average	4.47	4.37	1.02

ARGON

Time	ml $O_2/dm^2/hr$	ml $CO_2/dm^2/hr$	P.Q. Time	ml $O_2/dm^2/hr$	ml $CO_2/dm^2/$ hr P.Q.
1st hour	3.79	1.41	9-10 am	3.05	2.29
2nd hour	1.86	1.64	10-11	1.18	0.91
3rd hour	2.34	2.34	11-noon	3.86	2.29
4th hour	3.79	2.29	1-2	2.80	1.64
5th hour	2.57	2.34	2-3	4.68	5.61
6th hour	1.64	2.57	3-4	2.80	2.34
7th hour	2.34	2.34			
Average	2.62	2.13	1.23	3.06	2.51

TABLE X

DEPLETION RATE OF NITROGEN

	Time	Percent N_2 in box
AM	8:30	13.0
	9:30	12.4
	10:30	11.7
PM	1:30	11.2

The utilization of nitrogen by endive at the rate shown was not expected. However, in view of the reports of nitrogen utilization by non-leguminous plants (1), this phenomenon should be investigated further.

F. Vegetative Propagation of Plants

There are two methods of propagation which may be considered for space systems: seed propagation and vegetative organ propagation. Seed propagation is the easiest to accomplish yet increases the possibility that seed borne disease may be introduced into the plant system. Vegetative propagation, while somewhat more difficult, offers a higher degree of protection from disease. In view of the lack of reports on the propagation of organs of plants grown under low intensity artificial light, a preliminary investigation of this problem was initiated.

Two types of propagating medium were used. These were: Perlite with lime mixed into the top inch, and a mixture of one-half peat moss, one-half fine sand with lime mixed into the top inch. Ten mature endive and Chinese cabbage plants were fractionated into leaf and stem sections. The leaf sections consisted of the lower third planted upright and the top two-thirds of the leaf placed horizontal on the propagating medium. The midribs of the latter were scarified with a sterile razor blade. The stem cuttings consisted of 4 vertical sections. Mature tampala cuttings consisted of stem sections, lateral branch sections from laterals 3 to 4 centimeters long, and leaf cuttings. Stem sections were of two types, basal sections and terminal sections. In addition, the root system of each species was planted in order to determine if bud initials were present in the root system.

The results of these experiments were disappointing. All the endive and Chinese cabbage cuttings died within 2 weeks due to a disease similar to the various soft rots of vegetables as described by Walker (16). The tissues below ground darkened and became soft and slimy. Water soaked areas also appeared above the soil line. Roots were found, however, at the basal ends of the leaf cuttings.

FIGURE 15 TAMPALA, AERIAL ROOTS ON THE BASAL PORTION OF A STEM

FIGURE 16 LATERAL BRANCHES FROM THE STEM PORTION OF A TOPPED CHINESE CABBAGE PLANT

FIGURE 17 THE THREE SPECIES SELECTED FOR PHASE II STUDIES. ENDIVE (LEFT), TAMPALA (MIDDLE), AND CHINESE CABBAGE (RIGHT)

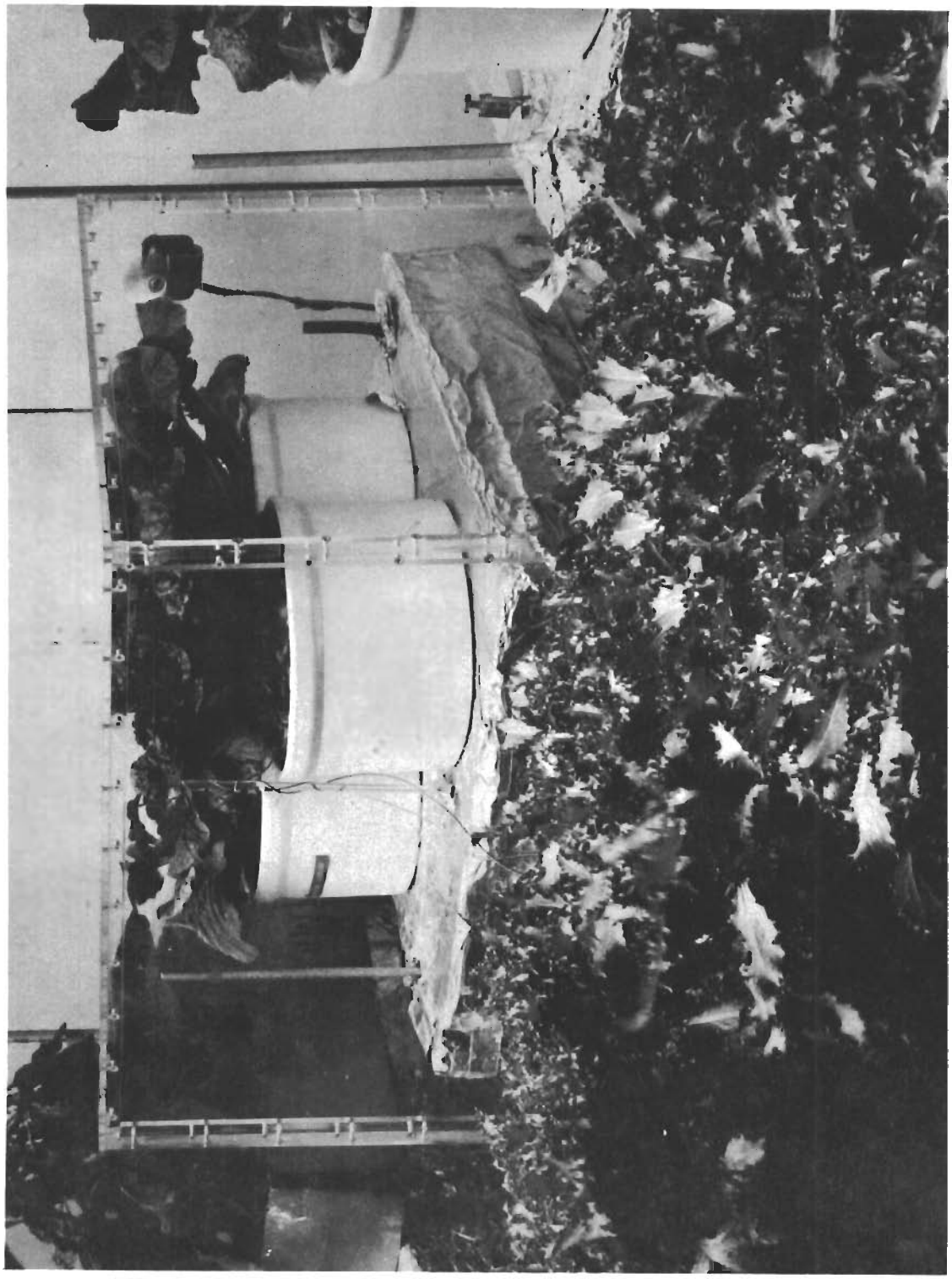
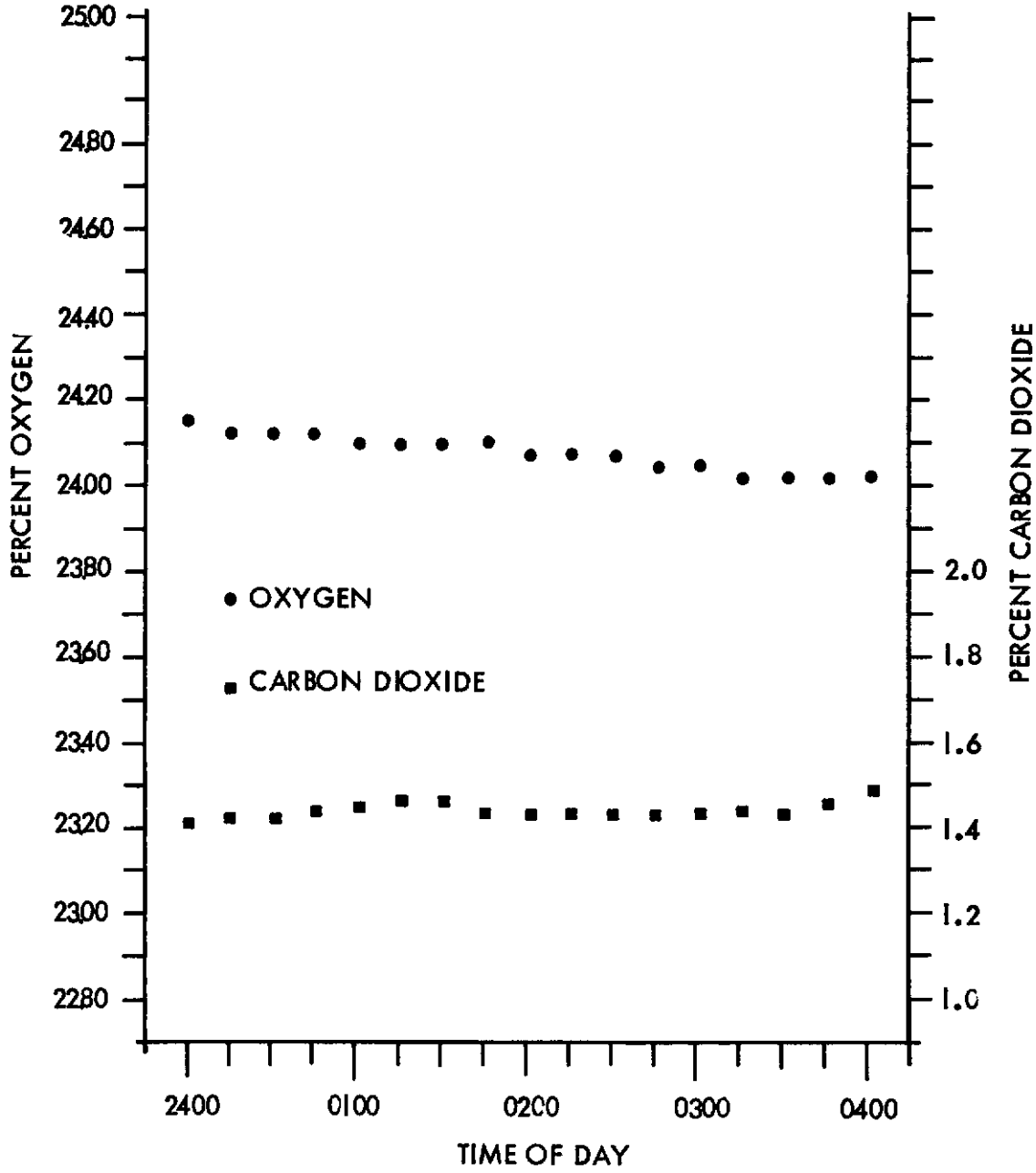


FIGURE 18 PLASTIC BOX USED IN PHASE II PHOTOSYNTHETIC STUDIES



EXAMPLE OF OXYGEN AND CARBON DIOXIDE DATA OBTAINED DURING DARK PERIODS.

FIG. 19 JAN. 24 2400 - 0400 NIGHT RUN

G. Nutritional Studies

This study was conducted in two parts. The first consisted of chemical analyses designed to reveal information pertaining to the nutritional value of the selected species and the second part to determine if the plants were toxic during short-term animal feeding tests.

The plants selected for study during Phase II were grown for a 6 week period in the manner described previously. The more sturdy of these plants were then transplanted into one gallon crocks and placed under white light at 700 f.c. (plant height) for further growth. In order to minimize the problem of separating the root systems from the soil substrate prior to chemical analysis, the plants were grown in washed river gravel ranging from 1/4 inch to 1/2 inch in size. The plants were fed daily with 750-800 ml of the nutrient solution described in Table XI.

TABLE XI

COMPOSITION OF NUTRIENT SOLUTION USED TO SUPPORT PLANT GROWTH IN ONE GALLON CROCKS

Macronutrients	Moles per liter
KH_2PO_4	0.01
KCl	0.25
KNO_3	0.50
$MgSO_4$	0.40
$CaSO_4$	0.20
$(NH_4)_2SO_4$	0.10

Micronutrients as in Shives Solution (Table I)

Fe^{+++} added as FeKEDTA at the rate of 0.5 ml per liter of solution

Chemical Analyses

The analyses which were performed on the plants were chosen because of the relatively complete picture of dietary essentials which could be assembled from the data. All major groups of essential nutrients were studied and, although a finer definition within some groups may be desirable, it is felt that the results reported leave little to be desired, at least for a preliminary screening.

The environmental conditions and the analytical procedures used were controlled as closely as possible. All plants used for analysis were brought to maturity under identical conditions of temperature, light, and humidity, and all plants of a given species were 12 weeks old when taken for analysis. The tissues of 10 plants of each species were pooled, and samples for analysis were drawn from this pool. All procedures were carried out as rapidly as possible to minimize the effect of autolytic changes in labile components.

A group of plants was harvested, weighed, and rinsed in cold running tap water. The roots, stems, and leaves were separated, and the tissues frozen between blocks of solid carbon dioxide. After freezing, the tissues were pooled and ground to a fine powder in a mortar and pestle with powdered dry ice. Following thorough mixing, the ground tissues were stored at -20°C. in polyethylene bags until used. The wet weights of the plants are shown in Table XII.

TABLE XII

Wet Weight, of Leaf, Stem and Root Tissues
of Endive, Chinese Cabbage and Tampala (One Plant)*

	Leaf	Stem	Root	Total Weight
Endive	212.4	5.6	54.4	272.4
Chinese Cabbage	151.9	4.2	11.4	167.5
Tampala	37.3	11.1	17.1	65.5

*Each value represents the average of data from 10 plants.
Weights in grams.

The analytical procedures employed are, for the most part, slightly modified from standard methods reported in the literature. In most cases, the modifications were necessitated by the limited amount of material available for analysis. All the procedures and any necessary modifications were thoroughly checked in this laboratory prior to use in order to assure quantitative recovery and adequate precision.

All reagents and chemicals used were of the highest purity available. In some cases, inadequacies in commercially available reagent chemicals necessitated the preparation or purification of chemicals in this laboratory.

Methods and Results

Moisture and Ash: The water and ash content of the plant materials were estimated by standard methods and reported in Table XIII. Moisture was determined as weight loss after drying to constant weight at 100°C. and ash is reported as residue remaining after ignition for 4 hours at 600°C.

TABLE XIII

Water and Ash Content

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Moisture*	92.8	85.7	95.8	82.3	86.6	92.6	91.7	87.7	93.2
Ash**	16.39	6.71	20.48	10.6	8.06	15.0	13.25	9.76	20.0

*As percent of wet weight.
**As percent of dry weight.

Total Nitrogen: The nitrogen content of the 3 tissues of each plant was estimated by the addition of Nessler's reagent to a Kjeldahl digest of the frozen tissue. The results are recorded on Table XIV.

Protein: A weighed amount of frozen tissue was homogenized in a Waring Blendor with 25 ml of Tris* buffer, 0.2 M, pH 7.6. After centrifugation the residue was extracted twice more with 25 ml portions of the buffer (preliminary experiments showed that this procedure resulted in essentially quantitative recovery of the plant protein). Sufficient cold 50% trichloroacetic acid was added to the combined extracts to result in a final concentration of 5% and the extracts were allowed to stand at 3° for one hour. The precipitate was collected by centrifugation, extracted twice with cold 5% TCA and washed twice with a cold acetone-ether mixture (1:1) to remove adsorbed pigments and residual TCA. The precipitate was dissolved in dilute NaOH and an aliquot taken for the estimation of protein by the method of Lowrey, et.al. (I2). The results are expressed as gm of protein per 100 gm of dry tissue in Table XIV.

*Tris (hydroxymethyl) amino methane.

TABLE XIV

Total Nitrogen and Protein*

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Nitrogen %	4.65	2.52	6.20	4.08	2.46	3.85	3.70	3.68	6.93
Protein %	8.83	3.78	2.70	6.90	1.79	2.97	6.56	0.90	1.74

*Data are expressed as percent of dry weight.

Amino Acids: The amino acid content was estimated by the procedures of Spackman, Stein, and Moore (15). Proteinaceous material was extracted as described for the determination of total protein except that the precipitated material was dried following acetone extraction. Samples of the dry material were weighed into Pyrex tubes with 10 times their weight of 6 N HCl. The tube was sealed and the material hydrolyzed for 18 hours in the autoclave. The hydrolysate was then treated with Norite, filtered, and repeatedly evaporated to dryness under a stream of nitrogen. The residue was dissolved in distilled water and an aliquot subjected to the Stein-Moore procedure. The results are presented in Table XV, expressing amino acid concentration as gm per 16 gm N.

Total Carbon: The method used is based upon the Schöniger technique as modified by Cheng and Smullin (7). The results are reported as percent carbon in Table XVI.

Alcohol Soluble Carbohydrate: The procedure used for determination of alcohol soluble carbohydrate is the same as that reported below for water soluble compounds except that 80% ethanol was used as the solvent. The results are expressed as gm. glucose per 100 gm. dry plant tissue in Table XVI.

Water Soluble Carbohydrates: Water soluble carbohydrates, including the soluble polysaccharides, in the plant tissues were estimated by application of the anthrone reagent to water extracts. Samples of plant tissues were dropped into boiling water and allowed to boil for 30 minutes. The entire contents of the flask were then transferred to a Waring Blendor, ground for 2 minutes, and transferred to an alundum or pyrex extraction thimble. Extraction in a Sohxlet apparatus was continued for no less than 8 hours. The results are reported as gm. glucose per 100 gm. dry plant tissue in Table XVI.

TABLE XV

AMINO ACID RECOVERY - GRAMS PER 16 GRAMS OF NITROGEN

Amino Acid	Chinese Cabbage Leaves	Chinese Cabbage Roots	Tampala Leaves	Tampala Roots	Tampala Stems	Endive Leaves	Endive Roots	Endive Stems
Cystic Acid	1.874	0.518	0.151	0.858	1.318	0.973		5.924
Aspartic Acid	7.784	14.927	12.393	12.517	10.007	10.078	10.409	5.441
Threonine	3.995	6.031	5.477	3.077	5.660	5.595	5.342	2.522
Serine	2.099		5.154	2.192	4.248	5.062	4.918	3.759
Glutamic Acid	10.598	11.091	12.796	7.873	12.417	9.919	10.563	7.732
Proline	3.095	5.004	5.193	5.452	8.084	3.089	3.394	1.009
Glycine	4.785	6.275	5.033	7.761	5.909	5.204	6.208	4.493
Alanine	4.840	5.849	6.315	7.257	6.256	6.205	6.149	3.512
Valine	0.260	0.319			2.742			1.881
Methionine	3.308	9.978	6.860	9.140	9.523	8.676	11.860	6.861
Cystine		0.199	0.743					0.885
Isoleucine	3.636	3.389	5.528	3.779	4.744	4.903	4.625	2.872
Leucine	6.617	5.382	9.599	7.080	7.721	7.753	11.135	5.074
Tyrosine	0.552	0.440	1.804	0.421	4.755			
Phenyl-alanine	0.549	0.402	3.164		6.443			
Lysine	8.509	10.575	8.962	10.358	7.050	9.284	10.215	6.616
Histidine	3.182	3.490	3.805	3.288	3.851	5.511	3.680	3.737
Arginine	7.592	8.710	9.849	9.907	4.184	16.329	5.583	4.613
Ammonia	2.075	3.064	1.959	3.111	1.928	2.693	3.323	2.404

Fiber: Crude fiber in each of the plant tissues was estimated by a slightly modified AOAC method (18) which involved serial extraction with ether, sulfuric acid, and sodium hydroxide. The results are expressed as gm. per 100 gm. dry plant tissue in Table XVI.

Lipid: Lipid content of the plant tissues was estimated as weight loss after extraction for 8 hours with ethanol-ether 2:1. The results are expressed in gm. per 100 gm. dry tissue.

TABLE XVI

MAJOR CARBON FRACTIONS

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
CHO %* (H_2O Sol)	24.6	63.7	33.1	17.63	42.99	29.54	40.12	43.85	16.71
CHO %* (Alc. Sol)	23.51	45.94	34.05	7.10	28.7	21.67	30.2	40.65	14.79
Total C	42.2	41.1	41.7	42.4	41.9	41.5	40.2	41.9	38.0
Fiber %	10.83	11.61	12.38	8.25	18.66	15.14	7.89	21.87	18.5
Lipid %	6.30	2.03	4.52	2.49	5.45	4.59	5.66	2.76	2.65

*as mg. glucose per 100 gm. dry weight

Ascorbic Acid: Ascorbic acid in the plant tissues was measured by the method of Roe and Oesterling (14). Because of the labile nature of this substance, the determinations were performed with minimum delay. Tissues taken for ascorbic acid analysis were extracted within 24 hours after harvest. The results are expressed as mg. per 100 gm. dry tissue in Table XVII.

Carotenes: The method used for the estimation of carotenes in the plant tissues is a modification of the methods reported by Wall and Kelly (17). A 10-gm. sample of ground plant tissue was homogenized for 5 to 10 minutes in the Waring Blender with a mixture of 40 ml. of acetone, 60 ml. of hexane, and 0.2 gm $MgCO_3$. The homogenate was filtered and the filtrate washed twice with 25 ml. portions of acetone-hexane (3:4) and 5 times

TABLE XVII (Vitamins)*

	Endive			Tampala			Chinese Cabbage		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
A-Ascorbic Acid (mg/100 gm)	168.5	52.0	105.5	242.4	53.7	5.9	135.5	28.5	86.0
C-Carotene I.U./100 gm	96,931	3.727	41,785	69,265	12,269	15,310	69,880	9,211	7,838
E-Tocopherol (mg/100 gm)	22.5	2.59	12.62	2.88	3.21	5.54	24.8	3.01	10.00

*Data given are per 100 gm dry weight.

with 100 ml. portions of water. The extract was diluted to a volume of 100 ml. with hexane and used for chromatographic purification of the carotenes. A 150 mm X 8 mm column of activated magnesia and diatomaceous earth was used to remove interfering substances. Following elution of the carotenes with 1:9 acetone-hexane, an aliquot of the eluate was evaporated to dryness under a stream of nitrogen. The residue was dissolved in reagent grade chloroform and the carotenes were measured with antimony trichloride according to the method of Carr and Price (5). Replicate checks on standards showed that this procedure resulted in quantitative recovery of the carotenes and the values of known solutions were reproducible to within 10%. The results of the carotene estimation are reported as International Units per 100-gm. plant tissue in Table XVII.

Tocopherols: The procedure used for the estimation of tocopherol content of the plant tissues is a modification of the method of Wall and Kelly (17). This procedure consists of removal of interfering substances with concentrated H_2SO_4 and measuring the tocopherols colorimetrically after reaction with α-α- dipyridyl-$FeCl_3$. The standard used in this procedure was a solution of α-d-tocopherol in cottonseed oil. Replicate determinations agreed within 5%. The results are expressed as mg. per 100 gm. dry tissue in Table XVII.

A survey of the literature has failed to reveal any compositional studies of these particular plants grown under similar carefully controlled conditions which might be used for comparative purposes. Some of the values reported here for certain constituents are outside the range which has been reported for many related plants, but it is not possible to say whether this is an inherent characteristic of these species or a function of the techniques and conditions used in their cultivation.

Animal Feeding Tests: Short-term animal feeding tests rarely reveal any useful information concerning the nutritional value or acceptability to humans of various plants. However, short-term feeding tests may reveal any acute toxicity, such as the "sudden death factor" present in blue green algae. Since the formation of toxic compounds in plants grown under the high level of ultraviolet radiation produced by fluorescent lamps has not been studied, it was felt that only short-term toxicity studies were required. Of the plants under investigation, only tampala has a history of containing toxic substances. Muenscher (12) reported that the high nitrate concentration in Amaranthus produces cattle poisoning when grown on soils fertilized with nitrate fertilizers. Both lactiferous ducts and oil ducts are found in the genus Cichorium (9). The toxicity of latex or oil produced under our conditions of growth was not known. No inference that the genus Brassica might be toxic was found.

Toxicity data are usually obtained through rat feeding tests. However, such tests are lengthy and require special handling facilities For the purpose of these tests, it was felt that short-term feeding of rabbits and guinea pigs would suffice for purposes of obtaining preliminary acute toxicity data.

The first test consisted of feeding rabbits the 3 plants, tampala, endive, and Chinese cabbage, both as a sole diet and as one-half the daily ration of food. Each rabbit received all 3 plants. Three New Zealand white male rabbits, 8 weeks old, and weighing approximately 4 pounds each, were housed in a cage $2\frac{1}{2}$ feet x 4 feet x 4 feet for the feeding tests. The rabbits were fed only "chow" prior to the tests. The plants, approximately 4 months old, were fractionated into leaf,

stem, and root tissue and placed, uncooked, in each cage at 9:00 a.m. each morning for a total feeding time of 3 days.

Chinese cabbage and endive appeared to be highly acceptable. No preference was shown for leaf, stem or root fractions. The plant material was preferred to "chow". Tampala was rejected over the 3 day feeding period. A minor amount of nibbling of roots occurred and a small amount of sampling of stem sections was attempted. Tampala leaves were neither sampled nor nibbled. Leaf particles of tampala were mixed with Chinese cabbage and endive leaf particles and fed to one lot of rabbits. After initial sampling of the leaf mixture, no further attempt at eating was observed. Since tampala is classed as a potherb, i.e., eaten after boiling or steaming in water, one lot of tampala leaf tissue was briefly cooked in boiling water and then fed to one lot of rabbits. This tissue was eaten although not as rapidly as other material. Evidently the offensive fraction was removed by boiling.

Three guinea pigs 8 weeks old and weighing $2\frac{1}{2}$ pounds were fed fresh tissues of the 3 plants while 3 additional guinea pigs were fed the standard chow ration as controls. Each 3 foot x 3 foot x 3 foot cage contained 3 animals. These animals previously had been fed on "Oat Groats" as a sole source of chow. The guinea pigs preferred the leaves first, then the stems. No root fractions were consumed. All plant material was preferred to the standard chow ration.

The animals were observed for a period of 2 weeks after the feeding tests. The animals reverted to the original chow without an interruption of feeding. No diarrhea was observed during the test nor during the 2 week observation period. Taste tests of the 3 plants were made periodically by members of the research team. Other than a slight bitterness in all 3 of the plant species, no adverse problems were encountered.

In addition, the leaves of the 3 species were analyzed for nitrate and oxalic acid (Tables XVIII and XIX). No evidence exists that tampala accumulated an excessive amount of nitrate. Oxalic acid content in tampala is higher than in endive or Chinese cabbage. However, spinach leaves usually have 3 times as much oxalic acid and are not known to be toxic.

TABLE XVIII

Nitrate Concentration of Leaves
(Brucine Method)

	NO_3 (mg/gm dry)
Endive	32.2
Chinese Cabbage	44.3
Tampala	40.5

TABLE XIX

Oxalic Acid Content of Leaves[1]

	Oxalate (mg/gm dry)
Endive	11.2
Chinese Cabbage	21.3
Tampala	35.0

[1]Gravimetric dry precipitate with calcium.

DISCUSSION

The screening of a wide variety of plants for possible use in closed ecological systems is a desirable area of study. Although little or no significant information to assist in the selection of plants was developed from the literature survey, a consideration of ecology, structural characteristics, and environmental requirements of a large number of plants was helpful in limiting the initial study to a few familiar species.

Although the fluorescent lamp is the simplest to use in laboratory studies a large number of plants were unable to develop normally under this type of light, and it would appear that a major effort is needed in the field of lighting technology if wider use is to be made of broadleaf plants. Detailed photosynthetic and nutritional studies were limited to those plants which grew in weak fluorescent light without marked deviation from normal growth and developmental characteristics. In the experimental studies concerned with photosynthetic activity and tolerance to salt a great deal of variability was observed between species and between individual plants of the same species. From the data which are included in this report, it is not possible to say what portion of this variation may be inherent in plants and what portion must be ascribed to lack of sufficient control and precision in experimental techniques.

Consideration of the analytical data does reveal that the 3 plants selected may provide a valuable nutritional supplement for man in a closed system. One of the most important considerations in assessing nutritive value in material of this type is accurate values for the content of amino acids, especially those which are indispensible to man. Inspection of the tables reveal that these plants contain adequate quantities of all these compounds. The content of vitamins A and C would also provide valuable supplies of these substances.

The only additional work necessary to completely characterize these plants and define their nutritive value would be estimation of the members of the B group of vitamins.

The preliminary feeding tests served only to demonstrate that the plants did not contain any highly toxic materials. Before they could be used as a food source for man, the fraction of these or any other uncommon plant would have to be thoroughly tested in long term feeding experiments.

The refusal of tampala by rabbits is not explained by any of the studies included here. The bitter taste of tampala would necessitate additional studies to identify the substance or substances which are involved. In view of the massive numbers of plants which may be useful in a closed system, however, it would be of doubtful value to attempt such a study.

The studies which are reported represent a fairly thorough screening of the 3 species of plants and represent the type of data which will be required to evaluate the potential of any plant species in a closed system. One additional study which is necessary is evaluation of the response of the plant when human waste materials or their degradation products are used as the growth substrate. This evaluation would be of primary interest in any

further study, which should also include:

1. The screening of more species of plants, in particular those non-commercial horticultural lines which have a rosette mode of growth or a minimum of stem area such as is found in the double dwarf types.

2. Determine the optimal means of plant reproduction (vegetative vs. seed production) under conditions which will exist in closed ecological systems.

3. Determine photosynthetic activity as a function of the quality and quantity of artificial light.

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APPENDIX

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INVESTIGATIONS OF SELECTED HIGHER PLANTS AS GAS EXCHANGE MECHANISMS FOR CLOSED ECOLOGICAL SYSTEMS

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Amer. Journ. Bot. 30:214. 1955.

Yields of crop plants were considerable depressed at exchangeable levels of Ca of 20% or less. Native plants, however, were only moderately reduced at 3% exchangeable Ca.

18. Wilson, C. C., W. R. Boggess, and P. J. Kramer.

Diurnal fluctuations in the moisture content of some herbaceous plants.
Amer. Jour. Bot. 40:97. 1953.

The moisture content of the leaves reached a minimum during the afternoon and attained a maximum between 12 and 4 a.m. The moisture content of the roots and stems reached a maximum between 6 and 10 a.m.

19. Woolley, J.

Sodium and silicon as nutrients for the tomato plant.
PP 32:317. 1957.

Evidence was presented that Na was possibly a micronutrient. Silicon did not give a response.

E. VOLATILE PRODUCTS OF PLANTS

1. Biale, J., R. Young and A. Olmstead.

Fruit respiration and ethylene production.
PP 29:168. 1954.

Fourteen species of fruits were investigated for ethylene production. The ratio production was highest for apple, followed by sapote, pear, cherimoya, peach, papaya, feijoa, avacado, persimmon and banana. Oranges and lemons produced no ethylene.

2. Leiberman, M.

Oxygen tension in relation to volatile production in broccoli.
ASHS 65:381. 1955.

Ethylene was identified as an emanation from broccoli under aerobic conditions but not anaerobic.

F. PROPAGATION

1. Dunham, C. W.

Use of methylene blue to evaluate rootings of cuttings.
ASHS 72:450. 1958.

The colorimeter reading obtained from a solution of methylene blue dye displaced from plant roots was found to be a reliable measure of the amount of roots present. In addition some measure of the absorbing surface of the roots was obtained.

2. Hartmann and Kester.

Plant Propagation
Prentice-Hall, Englewood Cliffs, N. J. 1960.

3. Stoutemeyer, V. T., and A. Close.

Propagation by seedage and grafting under fluorescent lamps.
ASHS 62:459. 1953.

Blue fluorescent tubes were needed for best propagation. Blue tended to offset the etiolation effects of low light intensity.

G. NUTRITIONAL VALUE

1. Anderson, D.

Amino acid content of foods. Private communication quoted in Block, R. J. and Weiss, K. W.
Amino Acid Handbook. C. C. Thomas, Pub.,
Springfield, Mass., 1956.

Pea meal was shown to contain the following amino acids: arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine.

2. Block, R. J. and Bolling, D.

The amino acid composition of proteins and foods.
C. C. Thomas, Pub., Springfield, Mass., 1951.

Compilation of the amino acids of a wider variety of food and miscellaneous materials.

3. Block, R. J. and K. W. Weiss.

Amino Acid Handbook.
C. C. Thomas, Pub., Springfield, Mass., 1956.

An exhaustive compilation of the amino acid content of a variety of plant and animal materials.

4. Bonnetti, D.

Boll. Soc. Ital. Biol. Oper. 25:337 (quoted in Borne, G. H. and G. W. Kidder, Biochemistry and Physiology of Nutrition.)
Academic Press, New York, 1953.

5. Edwards, C. N., L. P. Carter, and C. E. Ostland.

Cystine, Tyrosine, and essential amino acid contents of selected foods.
J. Agric. and Food Chem. 3:952. 1955.

Microbiological estimation of the amino acids of cabbage revealed arginine, histidine, lysine, tyrosine, tryptophane, phenylalanine, cystine, methionine, threonine, leucine, isoleucine, and valine.

By microbiological assay, collards were shown to contain arginine, histidine, lysine, tyrosine, tryptophane, phenylalanine, cystine, methionine, threonine, leucine, isoleucine, and valine.

6. Gustafson, F. G.

Influence of photoperiod on thiamine, riboflavin and niacin content of green plants.
Amer. Jour. Bot. 40:256. 1953.

At temperatures of 14°, 20°, and 26°C. there was a decrease in thiamine content as the plants were exposed to longer photoperiods. On the contrary, riboflavin and niacin increased with an increase in photoperiod.

7. Holmes, P.

The amino acid composition of certain seed proteins.
Austral. Jour. Exper. Biol. & Med. Sci. 31:595. 1953.

Paper chromatographic estimation of the amino acids in pea seeds showed arginine, histidine, lysine, tyrosine, phenylalanine, methionine, threonine, leucine, isoleucine, valine, glutamic acid, aspartic acid, glycine, and alanine.

8. Horn, M. J., A. E. Blum, C. E. F. Aersdorff and H. W. Warren.

I. Sources of error in microbiological determinations of amino acids on acid hydrolystates.
II. Apparent losses of amino acids on storage.
Cereal Chem. 32:64. 1955.

9. Hurich, J. S., A. D. Niles and A. R. Kemmerer

Essential amino acid content of several vegetables.
Food Res. 17:442. 1952.

Microbiological assay of amino acids of spinach showed that arginine, histidine, lysine, tryptophane, phenylalanine, threonine, leucine, isoleucine, and valine are present. The values in this case differ somewhat from those reported by Kelley and Baum. 1953.

10. Kelley, E. G. and R. R. Baum.

Protein amino acid contents of vegetable leaf proteins. J. Agric. and Food Chem. 1:680. 1953.

The amino acids of spinach, pea leaf meal, and the pea vine were estimated by microbiological methods. In spinach arginine, histidine, lysine, tryptophane, phenylalanine, cystine, threonine, leucine, isoleucine, and valine were found.

Pea leaf meal was shown to contain arginine, histidine, lysine, tryptophane, phenylalanine, methionine, threonine, leucine, isoleucine, and valine. Formic acid extracts of pea vine contained arginine, histidine, lysine, phenylalanine, methionine, threonine, leucine, isoleucine, and valine.

11. Lowrey, O. H., N. J. Rosebrough, A. L. Fare and R. S. Randall.

J. Biol. Chem. 193:265. 1951.

This paper describes a method for the estimation of protein employing a modified Folin reagent and copper tartrate.

12. Moore, S., D. H. Spackman and W. H. Stein.

Anal. Chem. 30:1185. 1958.

A description of improved methods of separating and estimating amino acids on columns of Amberlite 120.

13. Roe, J. H. and C. A. Keuther.

J. Biol. Chem. 147:399. 1943.

Describes the original method for the estimation of ascorbic acid, ascorbone, and diketogulonic acid in plant cells with a reagent containing 2.4 dinitrophenylhydrazine.

14. Robinson, W. B.

J. Agric. Res. 78:257. 1959.

It was shown that shading of whole strawberry plants results in a decrease of the ascorbic acid content. Shading of fruit alone resulted in no such effect.

15. Sommers, B. A., W. C. Kelley and K. C. Hamner.

Arch. Biochem. 18:59. 1958.

A study of the relation between the ascorbic acid content of leaf discs and CO_2 and illumination. Adequate CO_2 and illumination for free ascorbic acid in the disc.

16. Spector, Harry and D. W. Calloway.

Reduction of X-radiation mortality by cabbage and broccoli. Proc. Soc. Exp. Biol. and Med. 100:405. 1959.

Exposure to 400 r of whole-body X-radiation resulted in 100% mortality in 10-15 days of young male guinea pigs fed a basal diet of bran and oats plus ascorbic acid. Supplementation with cabbage or broccoli for two weeks before radiation and during 30 days after radiation significantly reduced mortality.

17. Williams, H. H.

"Essential" amino acid content of animal feeds. Cornell University. Memoir 337. 1955.

A survey of the essential amino acid content of a variety of plant materials important in animal nutrition.

H. METHODS OF CARBON DIOXIDE AND OXYGEN ANALYSIS

1. Balazs, O.

Respiration of Plants - I - Electrometric method for the determination of oxygen consumed during respiration. Acta. Biol. Acad. Sci. Hung. 7:301. 1957.

A new electrometric respirometer is described in which the amount of O_2 in a given air space can be directly measured quickly and continuously. The apparatus has a sensitivity of 0.05 - 0.1%.

2. Barley, T., J. H. Elliott, and R. P. Kissey.

Photoelectric absorptiometer for determination of small amounts of gases in air. U.S. Pat. 2,829,032, April 1, 1958.

The apparatus described determines the amount of one gas in another with the accuracy of 0.5 ppm. It records continuously the color changes in a specific solution, using alkaline pyrogallol or ammoniacal $CuCl_2$ for O_2.

3. Behmann, F. W.

Methods for analysis of biological gas mixtures. Arch. Tech. Missen, Lfg. 262, 245-8. 1957.

A review of the Haldane, Scholander and interferometric apparatus and their use for determination of CO_2 and O_2 in gas mixtures.

4. Catsky, J. and B. Slavik.

A field apparatus for the determination of intensity of photosynthesis.
Biol. Plantarum 2(2): 107-112. 1960.

An apparatus for the determination of photosynthetic intensity under field conditions is described. The apparatus operates on the basis of a continuous flow gasometric method with a visual colorimetric determination of CO_2 concentration in air.

5. Decker, J. P.

Some effects of temperature and carbon dioxide concentration on photosynthesis of Mimulus.
Plant Physiol. 34(2):103. 1959.

CO_2 exchange by intact attached leaves was studied at 20, 30, and 40°C. and from 20 to 300 ppm CO_2 using an infrared gas analyzer.

6. Farhi, Leon E.

A method for microanalysis of carbon dioxide and oxygen in minute samples of gas.
J. Appl. Physiol. 11:139. 1957.

Use of the Scholander - Roughton syringe is described for analysis of respiratory gases in 50 cu. mm of gas. The standard deviation of the error of the method as checked against the Scholander gas analyzer is 0.1%.

7. Goodwin, R. D.

Automatic recording of carbon dioxide by conductometry.
Anal. Chem. 25:263. 1953.

Barium hydroxide is used in a conductometric cell to measure total CO_2.

8. Greene, W. J.

Magnetic apparatus for determining the oxygen content of mixed gases.
U.S. Patent 2,689,332. September 14, 1954.

9. Keulemans, A. I. M.

Gas chromatography, 2nd Ed.
Reinhold Publishing Corp., New York. 1959.

10. Linhart, K. and J. Zagman'

Polarographic determination of oxygen.
Chem. Anal. 2:183. 1957.

A polarographic method for determining O_2 in gases (containing small and large amounts of O_2) utilizes the reaction of O_2 with Cr^{++}. The method can be extended to acid gases and the results are reproducible.

11. Loveland, J. W., et.al.

Spectrophotometric titration of ppm of carbon dioxide in gases.
Anal. Chem 31:1008. 1959.

CO_2 is absorbed in dilute sodium hydroxide and the excess caustic is back-titrated with dilute HCl. The titration is followed at 555 mu on a spectrophotometer using phenolphthalein as an indicator. Sensitivity is about 1 ppm of CO_2 in 20 l. of gas.

12. Pecsok, R. L.

Principles and practices of gas chromatography.
John Wiley and Sons, Inc., New York. 1959.

Extensive bibliography - 710 references.

13. Rosenbaum, E. J., R. W. Adam and H. H. King.

Monitoring trace hydrocarbon in air by catalytic oxidation and nondispersive infrared analysis.
Anal. Chem. 31:1006. 1959.

Liston-Becker Model 21 Infrared Gas Analyzer. Linear range 1-300 ppm CO_2. Calibrated with known gas mixtures which were analyzed by spectrophotometric method of Loveland, et.al. Mixtures of low concentration had CO_2 absorbing on cylinder walls to give low results.

14. Scott, F. D.

Sonic gas analyzer for measurement of carbon dioxide in expired air.
Review of Sci. Inst. 28:914. N. 1957.

A sonic gas analyzer intended primarily for estimation of CO_2 in the physiological range of 0-10% is described. The instrument is intended for continuous sampling at a rate of about 2 l/min. The time of response is about 0.5 sec. for full deflection.

15. Stickland, R. G.

Polarographic measurement of oxygen uptake by pea-root mitochondria.
Biochem. Jour. 77(3) 636-40. 1960.

A simple polarograph and a cell containing platinum and Ag/AgCl electrodes suitable for measurement of O_2 uptake are described.

16. Todt, F., K. Damaschke and L. Rothbuhr.

The electrochemical measurement of oxygen turnover in photosynthesis.
Biochem. Zeitschr. 325(3):210. 1954.

In the range of low O_2 concentration an almost unlimited degree of accuracy in time analysis to fractions of a second is attainable by methods described. This makes possible very exact measurements of O_2 in studies of photosynthesis even where low O_2 concentrations are used or after respiration has ceased.

17. Toreri, P. E. and B. J. Heinrich.

Determination of carbon dioxide in gas streams.
Anal. Chem. 29:1854. 1957.

The sample to be analyzed is passed through a saturated solution of an alkaline earth carbonate containing excess solid carbonate. At equilibrium, the pH of the solution is measured and the carbon dioxide concentration from 1 ppm to 100% is determined from a previously prepared calibration chart. The method is precise to within $\pm$ 5% of the CO_2 content measured.

18. Vejlby, K.

Induction phenomena in photosynthesis. Simultaneous measurements of carbon dioxide and oxygen exchange.
Physiol. Plantarum. 12(1):162. 1953.

The initial uptake of CO_2 and the formation of O_2 during photosynthesis in the moss species Polytrichun attemuatum and in Crowberry leaves (Empetrum migrum) were studied by the gas thermal conductivity method, using gas mixtures of He, O_2 and CO_2.

19. Vizard, G. S. and A. Wynne.

Determination of argon and oxygen by gas chromatography.
Chem. and Ind., p. 196-7. Febr. 1959.

20. Wenke, K.

Chromatographic method for quantitative gas analysis.
Chem. Tech. (Berlin) 9:404. 1957.

Mixtures of N_2, O_2, H_2, CO, CH_4, and CO_2 were analyzed at a constant temperature of 29°. A column 210 cm long with a diameter of 5 mm packed with activated carbon is used. CO_2 cannot be detected since its discharge from the column packing is slow and irregular.

21. White, C. S., L. C. Watkins and E. G. Fletcher.

Emissive spectroscopy in analysis of respiratory gases. IV. Calibration characteristics of oxygen emission in the near infrared.
J. Aviation Med. 28:406. 1957.

A circular quartz discharge tube fitted with external electrodes and adapted to the Beckman DU spectrophotometer is described. Calibration data show that the emission intensity vs. O_2 concentration is a linear function for 3-30% O_2 in N_2 and for 0-21% O_2 in CO_2.

I. PLANT DISEASES

1. Adams, J. F.

An Actinomycete, the cause of soil rot or pox in sweet potatoes.
Phytopathology 19:179-190. 1929.

Studies of the pox or soil rot in sweet potatoes indicated that the causal agent was a species of Actinomyces and found pathogenic on fleshy roots and cut slices of sweet potato, white potato, beet and turnip; however, negative results were obtained with carrots and dalhia. Seedling infections were decreased by seed treatments with common fungicidal dust.

2. Andersen, E. M.

Tipburn of lettuce. Effect of maturity, air and soil temperature and soil moisture tension.
N. Y. (Cornell) Agr. Expt. Sta. Bul. 829, 1946.

Discusses a non-parasitic disease known as tipburn which under conditions of a broad differential between maximum air and soil temperatures causes a burning of the leaves.

3. Atkinson, R. G.

Studies on the parasitism and variation of Alternaria raphani. Canadian Jour. of Research. Sect. C. 28:288-317. 1950.

The most rapid progress of this disease occurred at temperatures of 22° to 25°C. Increased soil moisture is associated with increased seedling disease. The fungus appears capable of surviving at least 18 months in dry soil cultures with no loss of culture habit virulence or sporulation.

4. Brown, N. A.

Some bacterial diseases of lettuce.
J. of Agri. Res. 13:367-388. 1918.

Two bacterial diseases of lettuce are described. It appears that both of these organisms are present and active in soil in which there is abundant green manure or stable manure which has not been thoroughly decomposed. If conditions are such that the plant keeps up a steady growth and is not checked, these bacteria do not enter. When conditions are such that the plant is weakened or growth checked, an entrance is gained and disease follows.

5. Brown, W. and N. Montgomery.

Problems in the cultivation of winter lettuce.
Ann. Appl. Bio. 35:161-180. 1948.

In a 4-year study of winter lettuce cultivation, attention has been given to the effects of variety, dates of sowing and transplanting, method of preparing seed-beds, nature of field soil and ungicidal treatments upon % survival and date of maturity of the crop.

6. Burkholder, W. H. and W. L. Smith, Jr.

Erwinia atroseptica (Van Hall) Jennison and Erwinia carotovora (Jones) Holland.
Phytopathology 39:887-897. 1949.

Sixty-one isolates of Erwinia were studied in this investigation. They could be divided into two species, E. atroseptica and E. carotovora. The former appears to be a more stable species than latter.

7. Harter, L. L. and J. L. Weimer.

A monographic study of sweet potato diseases and their control.
U. S. Dept. of Agriculture Tech. Bul. 99, 1929.

An extended account of the storage diseases, field diseases, and physiological diseases are described with regard to their occurrence, extent of damage and cause.

8. Hooker, W. J.

Comparative studies of two carrot leaf diseases.
Phytopathology 34:606-612. 1944.

The leaf blights of carrots have been studied under controlled conditions to determine what might be the underlying causes of the striking contrast in season cycles of the 2 diseases.

9. Jagger, I. C.

Brown blight of lettuce.
Phytopathology 30:53-64. 1940.

Brown Blight is a disease of lettuce that has been shown to be soil-borne. Certain varieties are highly resistant or entirely immune, but are commercially useless in the infested regions.

10. Jagger, I. C.

Sclerotinia minor, N. Sp., The cause of a decay of lettuce, celery, and other crops.
J. Agri. Res. 20:331-334. 1920.

Sclerotinia minor, N. Sp., produces a decay of lettuce and other plants similar to that produced by S. libertiana. It is known to occur in Massachusetts, New York, Pennsylvania, and Florida.

11. Leach, L. D.

Growth rates of host and pathogen as factors determining the severity of preemergence damping-off.
J. Agri. Res. 75:161-179. 1947.

Studies on growth rates of pathogens under varied environmental conditions on spinach, watermelons, and beets inoculated with the Pythium, Rhizoctonia, and Phoma.

12. Nusbaum, C. J.

Internal Brown Spot, a boron deficiency disease of sweet potato.
Phytopathology 36:164-167. 1946.

The description on the effects of boron deficiency on sweet potato.

13. Person, L. H. and W. J. Martin.

Soil rot of sweet potatoes in Louisiana.
Phytopathology 30:913-926. 1940.

The soil rot organism has been isolated and is described as a new species of Actinomyces. Soil rot is more serious in dry soils and in dry seasons and is not usually found in soils with the pH above 5.2.

14. Pinckard, J. A.

Physiological studies of several pathogenic bacteria that induce cell stimulation in plants.
J. Agri. Res. 50:933-952. 1935.

Several pathogenic bacteria were cross-inoculated with small pieces of gall tissue and single cell tissues on several horticultural crops. It appeared that the organisms were pathogenic only on the host from which it was isolated.

15. Poole, R. F.

Sweet-potato ring rot caused by Pythium ultimum.
Phytopathology 24:807-814. 1934.

The causal fungus occurs in the field as a soil rot. Extended wet soils favor growth of the fungus.

16. Rangel, J. F.

Two Alternaria diseases of cruciferous plants.
Phytopathology 35:1002-1007. 1945.

Discusses the morphological differences and cultural characteristics of two alternaria species which cause leaf spot, pod spot, burning of heads of cauliflower and broccoli, damping-off, wire stem and spotting of seedlings. The spores may retain their viability for more than 6 months, and the sources of inoculum are the dead lesions and the decaying plant parts on the soil. Water is the main agent of dissemination.

17. Walker, J. C.

Diseases of cabbage and related plants.
U. S. Department Agriculture Farmer Bulletin 1439, 1927; Revised, 1948.

Diseases of the cabbage family are discussed and the means by which these diseases are preventable.

J. PHARMACEUTICAL BOTANY

1. Fernald, M. L., S. C. Kinsey and R. C. Rollins.

Edible Wild Plants of Eastern North America.
Harper and Bros., New York. 1958.

2. Muenscher, W. C.

Poisonous plants of the United States.
Macmillan Co., New York. 1958

3. Youngken, H. W.

Pharmaceutical Botany
Blakiston Co., Philadelphia. 1951.

K. GENERAL SECTION

1. Bonner, J.

Plant Biochemistry.
Academic Press. New York. 1950.

2. Cruess, W. V.

Commercial Fruit and Vegetable Products.
McGraw-Hill, New York. 1948.

3. Ellis, Carlton and N. W. Swaney.

Soilless Growth of Plants. 2nd Edition, Revised and Enlarged by Tom Eastwood.
Reinhold Publishing Corp., New York. 1953.

4. Haupt, A. W.

Plant Morphology.
McGraw-Hill Book Co., Inc. New York. 1953.

5. Jacobs, M. B.

The Chemistry and Technology of Foods and Food Products II
Foods Interscience. New York. 1951.

6. Machlis, L. and J. G. Torrey.

A Laboratory Manual of Plant Physiology.
Freeman and Co., San Francisco. 1959.

7. Meyer, B. S. and D. B. Anderson.

Plant Physiology.
D. Van Nostrand Co., New York. 1952.

8. Rabinowitch, E. I.

Photosynthesis.
Interscience, New York. Vol. I, 1945; II, 1951; II_2, 1956.

9. Shoemaker, J. S.

Vegetable Growing.
Wiley and Sons, New York. 1947.

10. Sinnot, E. W. and K. S. Wilson.

Botany: Principles and Problems.
McGraw-Hill Book Co., Inc. 1955.

11. Walker, J. C.

Diseases of Vegetable Crops.
McGraw-Hill Book Co., Inc., New York. 1952.