PLANT NUTRITION CHEMICAL PACKAGE

CODE 5940

QUANTITY	CONTENTS	CODE
250 mL	*#1 Calcium Nitrate, 1M	*5341-K
120 mL	#2 Magnesium Sulfate, 1M	5342-J
250 mL	*#3 Potassium Nitrate, 1M	*5343-K
60 mL	#4 Potassium Phosphate, 1M	5344-H
120 mL	#5 Calcium Phosphate, 0.05M	5345-J
60 mL	#6 Potassium Sulfate, 0.5 M	5346-H
60 mL	*#7 Magnesium Nitrate, 1M	*5347-H
4 x 250 mL	#8 Calcium Sulfate, 0.01M	5348-K
60 mL	*#9 Iron-EDTA	*5349-H
60 mL	#10 Trace Elements	5350-H
1	Plant Nutrition Studies	1596

*WARNING: Reagents marked with an * are considered to be potential health hazards. To view or print a Safety Data Sheet (SDS) for these reagents go to www.lamotte.com. Search for the four digit reagent code number listed on the reagent label, in the contents list or in the test procedures. Omit any letter that follows or precedes the four digit code number. For example, if the code is 4450WT-H, search 4450. To obtain a printed copy, contact LaMotte by email, phone or fax.

Emergency information for all LaMotte reagents is available from Chem-Tel: (US, 1-800-255-3924) (International, call collect, 813-248-0585).

To order individual reagents or test kit components, use the specified code number.

Warning! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision.

PROCEDURE

See *Plant Nutrition Studies* (Code 1596) for suggested experiments using the Plant Nutrition Chemical Package. Use the amounts of nutrient solutions listed in the chart below to prepare water culture solutions.

Composition of Various Water Culture Solutions

Type of Nutrient Solution	mL of Stock Solutions/Liter of Distilled Water									
	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**
Complete nutrient	5	2	5	1	–	–	–	–	1	1
Minus calcium	–	2	5	1	–	–	–	–	1	1
Minus magnesium	4	–	6	1	–	3	–	–	1	1
Minus potassium	5	2	–	–	10	–	–	–	1	1
Minus nitrogen	–	2	–	–	10	5	–	200	1	1
Minus phosphorus	4	2	6	–	–	–	–	–	1	1
Minus sulfur	4	–	6	1	–	–	2	–	1	1
Minus trace element	5	2	5	1	–	–	–	–	1	–
Minus iron	5	5	2	1	–	–	–	–	–	1

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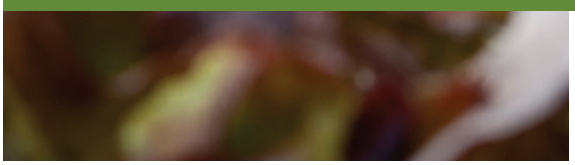
Plant Nutrition Studies



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Introduction

Among the multitude of human problems, malnutrition is conspicuous. The rapid growth of the population has equaled, or exceeded, any gains of agricultural science and probably will continue to outstrip agricultural advances at an accelerating pace for some decades to come. At least one-half of the Earth's human population is existing at a deficient nutritional level.

Although agriculture has made great gains in food production in the last century, one of the most critical factors is, and will continue to be, food production.

This booklet is intended to open the study of food production to the general student through readings and activities in plant nutrition.

The Significance of Green Plants for All Life

There was a time on this planet when there were no green plants—when all organisms subsisted on organic molecules synthesized in air and in water. The inefficiency of this process was a limiting factor for the growth and evolution of the biosphere, and it was not until the advent of *chlorophyll a*, with its light-absorbing power, that the door was opened for the expansion of life forms, leading to our present flora and fauna. Other pigments are involved in the capture of light energy, but *chlorophyll a* seems to be the key to the reaction, for it occurs in all photosynthetic organisms, except some of the sulfur bacteria, where bacteriochlorophyll does the work. *Chlorophyll a* is always accompanied by beta-carotene, whose function is not clearly understood, although it probably is important.

The universal fitness of chlorophyll a to carry on photosynthesis is due to its ability to capture packets of light energy, especially in the blue and red bands of the spectrum. A molecule of *chlorophyll a* (Figure 1) is a series of atoms connected by regularly alternating single and double chemical bonds (a conjugated system). This type of structure is common to all the other chloroplast pigments. In such a system, some of the electrons forming the bonds between atoms are free to move about in the molecule without changing the basic molecular structure. When light energy enters such a molecule, some electrons are elevated to higher energy levels, and, if they can be captured, their energy can be converted to use in photosynthesis.

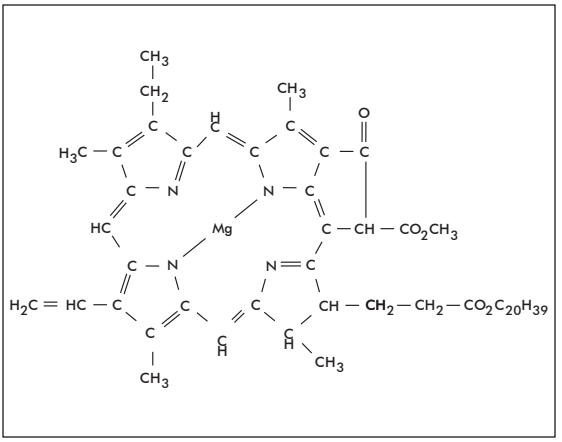

Figure 1: Molecule of chlorophyll a.

The efficiency of *chlorophyll a* is increased by the arrangement of the molecules in an orderly

array in small structures called grana, which are themselves marshaled in effective order in small organelles called chloroplasts. It is in these microscopic structures that the energy-rich foods that nourish the entire biosphere are manufactured.*

Synthesis of Carbohydrates

Units of light energy especially in the blue and red bands excite electrons in chlorophyll molecules. When these electrons are captured by electron acceptors, their energy is utilized to form the energy-packed molecules, i.e., adenosine triphosphate (or ATP), and reduced triphosphopyridine nucleotide, $TPNH_2$ (also known as $NADPH_2$). The hydrogen which reduces TPN, yielding $TPNH_2$, comes from water, which also is the source of the oxygen given off from green plants. This part of photosynthesis is called the light phase.

The energetic partners, ATP and $TPNH_2$, now bring about the combination of carbon dioxide with certain carbohydrate molecules to form glucose, $C_6H_{12}O_6$. This part of photosynthesis is known as the dark phase, since light is not required.

Photosynthesis requires organized chlorophyll, light, water and carbon dioxide, all made available together in the structures of green plants. From the basic substance glucose, plants are able to synthesize all of the many complex substances they use. It is on these substances that animals depend for their energy. Even the release of energy in animal bodies requires the oxygen which green plants have added to the earth's atmosphere. When we study plant nutrition we are studying the very hub of the biosphere.

The Mineral Needs of Plants

Plant growth requires more than sunlight, carbon dioxide, chlorophyll and water. Many plants require fertile soil containing mineral substances derived from the decomposition of the parent rock. These substances are absorbed into plants through their roots and move to the cells where foods are synthesized. These minerals as "plant foods" but they are really raw materials, along with water and carbon dioxide. The substances that provide structure, energy and life processes for plants are manufactured in the cells, with the participation of mineral substances from the soil.

There are more than one hundred elements in soils of various kinds, and all of them are found to some extent in some plants. What are their roles, if any? Are they all essential to plant life? Answers to such questions are complicated by the complexities of soil structure and soil chemistry which make it difficult to measure the mineral ions available in the soil.

Prominent among early investigators of mineral nutrition in plants were two plant physiologists, Sachs and Knop, who showed that certain common salts in solution could support the lives of many different plants (Table 1).

* blue-green algae and sulfur bacteria do not have discrete chloroplasts

Table 1. Sachs' Nutrient Solution

Salts	Anions	Cations
Calcium nitrate, $Ca(NO_3)_2$	NO_3^-	Ca^{+2}
Magnesium sulfate, $MgSO_4$	SO_4^{-2}	Mg^{+2}
Ferrous sulfate, $FeSO_4$	SO_4^{-2}	Fe^{+2}
Potassium acid phosphate, KH_2PO_4	PO_4^{-3}	K^+, H^+

Ten elements are essential to plants: calcium, hydrogen, magnesium, oxygen, potassium, carbon, iron, nitrogen, phosphorus, and sulfur. When any of these elements are absent from the culture solution, characteristic deficiency symptoms are observed and the plants finally die.

To establish an element as essential it must be shown that the plant cannot complete its life cycle without it, and that the element is directly involved in the plant life processes (rather than in an indirect effect, such as an influence on the availability of some other element in the soil mixture).

With the invention of water culture techniques, it became possible to establish the roles of the principal essential elements, but new requirements have been discovered in recent years. These substances were overlooked in earlier studies because they are required in minutes amounts, and the chemicals used in early nutrition experiments contained traces of these elements as impurities. The importance of these trace elements was not discovered until highly purified compounds became available*. The trace elements now established as essential are: boron, copper, manganese, zinc and molybdenum.

To this list should be added chlorine and sodium, which probably are required in very minute amounts by some plants. It is likely that new requirements will be discovered as culture media are refined.

Mineral Nutrients from the Soil

With the exception of nitrogen, nutrients required for plant growth originated from minerals by the process of weathering. They may occur as cations (Ca^{+2}, Mg^{+2}, K^+, Na^+, Fe^{+2}, NH_4^+); anions (SO_4^{-2}, PO_4^{-2}, NO_3^-); or in complex mineral or organic molecules not readily usable by the plant. The colloidal particles of the soil are negatively charged and therefore attract cations. This ability of soil particles to attract, hold and exchange cations is referred to as the exchange capacity of the soil and is related to the particle size. Since smaller particles have a larger surface area-to-volume ratio than larger particles, they usually have a greater exchange capacity.

The power of ions to displace each other on the colloidal soil particles occurs in a specific order, i.e., H^+, Ca^{+2}, Mg^{+2}, K^+, NH_4^+, and is important in determining the availability of the ions to the plant. The supply of hydrogen ions determines the pH of the soil: a large number in acid soils, fewer in alkaline soils (Table 2). Since hydrogen ions will displace any of the other cations, they play a major role in the presence and availability of the other cations. In acid soils the large

* Although required in minute amounts, the role of iron was among the early discoveries.

number of hydrogen ions may displace the other cations which can then be leached from the soil, resulting in low fertility. However, the release of hydrogen ions in the soil aids in the exchange of cations, and makes them available to the plant through the soil solution. The production of hydrogen ions is accomplished by the reaction of carbon dioxide, produced by plant roots and microorganisms in the soil, with soil water as illustrated by the reaction:

$$CO_2 + H_2O \leftrightarrow \underset{\text{carbonic acid}}{H_2CO_3} \leftrightarrow H^+ + HCO_3^-$$

These hydrogen ions are then available to release cations from the soil particles (Table 2).

Table 2. Effects of soil reaction

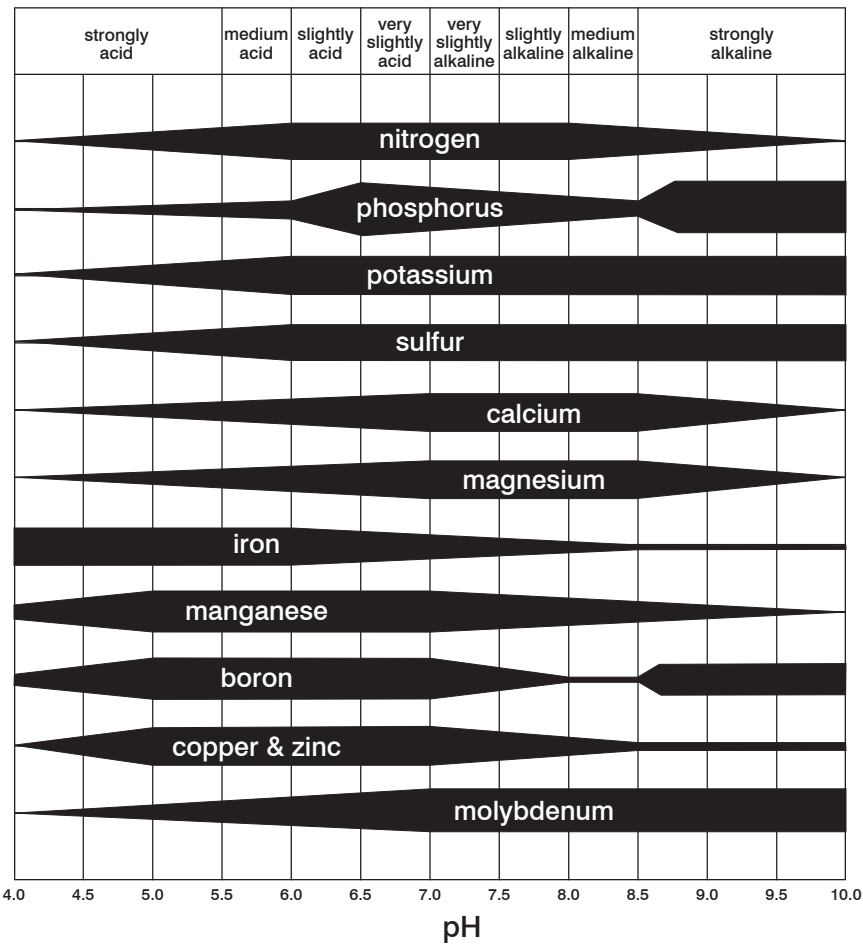

Table 2: Effects of soil reaction on availability to plants of soil nutrients (after Truog). The width of the bar determines the relative availability of each element with a change in soil reaction.

There are other effects that pH may have on the availability of nutrient ions. Soil organisms that break down organic materials and those that "fix" atmospheric nitrogen do not grow well in highly acid soils. A large part of the nitrogen used by plants is contained in the organic materials, but the material must undergo mineralization before the nitrogen is in a form available for plant use. This is the job of soil microorganisms. Chemical reactions may also make nutrient ions unavailable. For example, phosphorus combines with iron and aluminum compounds in highly acid soils, and in alkaline soils iron and manganese form insoluble compounds.

We also find that the ions absorbed by soil particles and the ions in the soil solution are normally in equilibrium. As the ions in the soil solution are absorbed by the plant roots they are replaced by the ions held on the soil particles, or the ions released by the breakdown of mineral and organic matter in the soil. Fertilizers are used to shift the equilibrium or to supply cations of one kind in such large numbers that they will displace others from soil particles. The fertility of a soil is determined by its ability to supply mineral ions to the plants. Soils may contain large quantities of minerals and organic material but be infertile because the ions are not available to the plant.

Anions are also held by soil particles, but not as strongly as cations, and they may therefore be more readily lost by leaching. Anions, being negatively charged, are held by positive charges in the soil complex and are normally made available to plants by the mineralization of humus. The exchange capacity of the soil is usually inversely related to soil pH, i.e., increasing as pH decreases.

Movement of Ions into Plants

Plant cells absorb ions in two major ways: (1) simple exchange through the cell membrane (diffusion) and (2) active transport. Ion exchange by diffusion is normally reversible, independent of metabolism and primarily concerned with cation exchange. It is also independent of aeration and moderate temperature changes, and equilibrium is reached very quickly. Active transport, for the most part, is irreversible, dependent on metabolism, and involves both cation and anion exchange. Active transport is also sensitive to changes in aeration and temperature, and equilibrium is established very slowly, if at all.

There have been many attempts to explain the transport of ions into the plant cell. The carrier hypothesis, supported by many investigators, envisions a carrier molecule similar in function to enzyme molecules. The carrier molecule combines with the nutrient ions at the surface of the cell membrane, transports them across the membrane, releases them, and is then available to combine with additional nutrient ions. Cells may accumulate certain ions since the carrier molecules are selective in the ions they will combine with and transport.

The mobility of ions in the soil also affects their availability to the plant. Phosphate ions are highly immobile and must be in almost immediate contact with the root surface before they can be absorbed, whereas mobile nitrate and potassium ions in solution may move greater distances to the root surface.

Suggested experimental procedures using tomato plants

The early mineral nutrition experiments, along with other interesting studies, can be carried out using the general procedures described below. Many possibilities for varying these techniques will occur to the investigator.

Procedure:

Clean glassware by rinsing with dilute hydrochloric acid and distilled water. Prepare nutrient stock solution* according to Table 3.

Table 3. Stock Solution for Water Culture

Solution #	Salts†	g/L of glass-distilled water
1	lM $Ca(NO_3)_2 \cdot 4H_2O$	236.2
2	lM $MgSO_4 \cdot 7H_20$	246.5
3	lM KNO_3	101.1
4	lM KH_2PO_4	136.2
5	0.05M $Ca(H_2PO_4)_2$	12.6
6	0.5M K_2SO_4	87.1
7	lM $Mg (NO_3)_2 \cdot 6H_2O$	256.5
8	0.01M $CaSO_4$	1.36
9	Iron tartrate‡	0.5
9a	Iron EDTA	(see appendix, page 45)
10	Trace elements	
	H_3BO_3	2.86
	$MnCl_2 \cdot 4H_2O$	1.81
	$ZnSO_4 \cdot 7H_2O$	0.22
	$CuSO_4 \cdot 5H_2O$	0.0
	$H_2MoO_4 \cdot H_2O$	0.028

Distilled water will have a pH of about 5.0-6.0 because of the absorption of carbon dioxide from the air, forming carbonic acid.

The pH of the nutrient solutions should be adjusted to about 6.0 or 6.5 by adding small amounts of potassium hydroxide. For nutrient solutions deficient in potassium, use sodium or ammonium hydroxide. The pH can be measured reasonably well with pH paper or more accurately with a pH meter. To lower the

* See Appendix (page 46) for suggested stock solution bottle.

† C.P. or Reagent grade chemicals should be used to minimize impurities. Stock solutions for water culture of plants can be obtained from LaMotte Company.

‡ Add 1mL/litre twice a week.

pH (make the solution more acid) use hydrochloric or nitric acid. In all cases use "Reagent" grade chemicals and clean equipment.

Table 4. Composition of Various Water Culture Solutions*

Type of nutrient solution	mL of stock solutions/Liter of distilled water									
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Complete nutrient	5	2	5	1	—	—	—	—	1	1
Minus calcium	—	2	5	1	—	—	—	—	1	1
Minus magnesium	4	—	6	1	—	3	—	—	1	1
Minus potassium	5	2	—	—	10	—	—	—	1	1
Minus nitrogen	—	2	—	—	10	5	—	200	1	1
Minus phosphorus	4	2	6	—	—	—	—	—	1	1
Minus sulfur	4	—	6	1	—	—	2	—	1	1
Minus trace element	5	2	5	1	—	—	—	—	1	—
Minus iron	5	5	2	1	—	—	—	—	—	1

Any of the nutrient solutions in Table 4 can be made by adding the quantity of stock solution indicated to about 500 mL of distilled water. Bring the total amount of solution up to 1 L by adding water. This will tend to avoid precipitation.

Chelates

Assuring a sufficient supply of iron in the nutrient solution is difficult since many iron compounds precipitate. Notice that stock solutions 9 and 9a are iron chelates. Chelating agents are compounds that will bind metal ions. The metal ion combines with electron donors to form a ring configuration with the chelate molecule. The metal, then, no longer exhibits ionic properties. Iron held this way is less likely to precipitate in nutrient solutions. The chelate-iron complex, being soluble, keeps the iron in a form available to the plants growing in solution. If iron salts are used they must be added to the solution frequently to prevent iron deficiency, whereas a single addition of an iron chelate will suffice for an extended period of time. Several natural chelates have been used for iron, such as citric and tartaric acid, but the most widely used chelate is ethylenediaminetetraacetic acid (EDTA). Iron combined with EDTA is designated as FeEDTA†.

Chelates are affected by pH, but within the range normally found in nutrient solutions EDTA is an effective chelate for iron. FeEDTA or iron tartrate stock solutions are added in the same quantity (1.0 mL), but FeEDTA need not be added as frequently.

* Reagent-grade chemicals should be used to minimize impurities. Stock solutions for water culture of plants can be obtained from LaMotte Company.

† See Appendix (page 45) for preparation of FeEDTA.

Germination of Seeds

Seeds should be germinated in sand. Other substrates for germination (such as vermiculite) may be hard to separate from the roots and may serve as a surface for adsorption of nutrient ions. Water the seedlings with complete nutrient applied evenly to the sand through funnels. To produce vigorous seedlings, illuminate strongly in a greenhouse or with growth lights.

Always start three or four times as many seedlings as you will need so that a group of plants of equal size and vigor may be selected for the investigation. When the seedlings are about three inches tall they can be gently flooded out of the sand and placed on wet towels for classifying. Be careful to avoid exposure of the roots to dry air.

Some means of holding the plants is needed. Clean sand, vermiculite, or perlite can be used, but control and observation of the plants may be more effective in water culture.

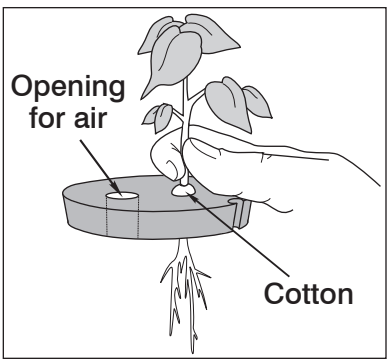

Figure 2a: Preparation of seedling for water culture.

For water culture, use wide-mouth jars of 500 mL or larger. If it is planned to grow large plants to maturity use jars of one-half gallon or more. To hold the plants with their roots in the nutrient solution, use large, flat corks, about one-half inch thick. It is important to obtain tapered corks that fit the jars precisely so that the plants will be held firmly upright and so that the corks can be readily removed from the jars. To hold the seedlings, make two or three half-inch holes in the cork with a sharp corkborer. Make a smaller hole or notch on the edge of the cork for the air line. Now impale the corks on dissecting needles, dip them in hot paraffin, and place on paper towels to cool. Take care to avoid igniting the paraffin, but be sure it is hot enough so that only a thin layer remains on the corks. The paraffin will seal the corks to prevent absorption of nutrients and will help to eliminate microorganisms.

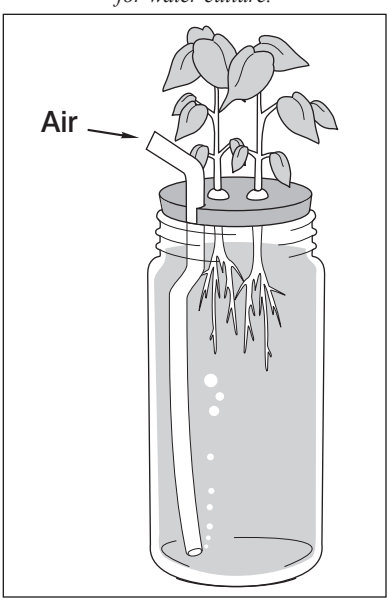

Figure 2b: Completed setup for seedlings.

Wrap the stems of the seedlings with nonabsorbent cotton sufficient to hold them in the cork. Do not allow the cotton to project below the cork because it will accumulate nutrients which may encourage the growth of microorganisms around the plant stems (Figure 2a).

If the seedlings are no more than three inches tall, their root systems probably can be inserted through the holes in the corks without damage. If the root systems are too large to go through the corks, wedges can be cut from the margin of the cork and re-fastened with pins when the plant is in place. The jars should be darkened with paint or foil, or placed in an apparatus, as diagramed in Figure 3.

The apparatus in Figure 3 provides a place to fasten air valves for control of aeration and a place to fasten sticks or wires to support the plants, if necessary. It also provides a convenient means of carrying an entire experiment into the classroom for discussion and demonstration. In this system, the roots can be observed at any time, and the rate of bubbling and the water level can be readily checked.

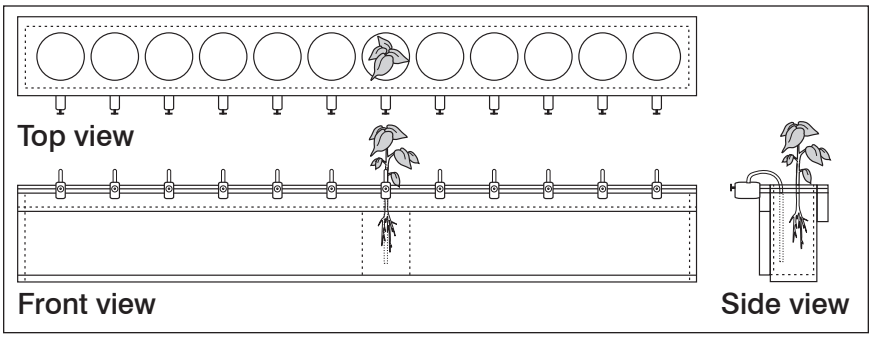

Figure 3: Water culture rack.

Aeration of the nutrient solution is important for many species and can readily be provided with an aquarium pump*. If there is any possibility that oil or other foreign matter could be introduced into the system by the air pump, a filter should be placed in the air line (Figure 4).

For each experiment, use new plastic tubing for air lines or rinse the old tubing thoroughly in dilute hydrochloric acid and then distilled water.

To be certain that light is not a limiting factor, set up a fluorescent fixture with four-40 watt plant growth tubes and provide for control of photoperiod with a clock. A plastic sheet can be placed over the light fixture to help in controlling humidity and temperature.

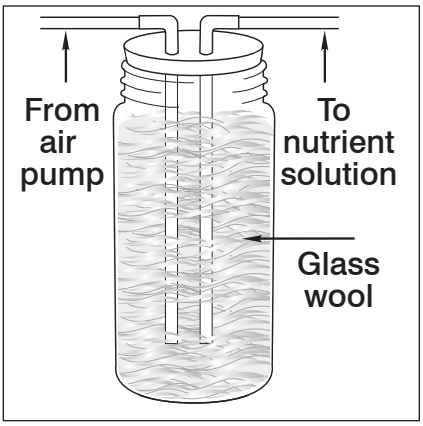

Figure 4: Airline filter.

* See Appendix (page 45) for construction of airbreaker.

Plant growth is promoted by fluorescent tubes designed to emit strongly in the red and blue bands of the spectrum. Fixtures holding four-40 watt tubes are convenient, and are adequate for these suggested investigations, if the plants have some sunlight or incandescent light to provide the infrared radiation needed for normal development. The fluorescent tubes should be placed about 12 inches above the plant tops, where the light intensity should be about 700 footcandles. Daylight, of course, is favorable for plant growth but even in a greenhouse the intensity may vary from a high of 250 ft-c on a dull day up to 10,000 ft-c in full sunlight. For many investigations fluorescent lights offer a desirable control.

This system is a convenient means of controlling various factors in the investigation of plant growth and will be the basis of many of the experiments suggested. With this nutrient culture system, the basic requirements can be provided and a variety of plants can be raised to maturity.

The tomato plant, *Lycopersicon esculentum*, is favorable for plant growth studies (Went 1957). Its growth is more or less continuous, and it is light saturated at about 1,000 ft-c. The tomato is not sensitive to photoperiod for flowering but needs at least eight hours of illumination per day for vegetative growth. However, growth decreases with continuous light. It grows well winter or summer without much trouble from pests or disease. The seeds germinate well in sand at about 26°C with seedlings emerging better in the dark.

Five or six days after planting at 26°C, the seedlings should be ready for illumination. About two weeks after planting, the seedlings should be ready to transplant to nutrient solutions. Always start about three or four times more plants than needed so that the tallest and shortest can be discarded and a uniform growth rate can be attained. For general purposes, most varieties are acceptable but there are varieties adapted to greenhouse conditions, such as Michigan State or Essex Wonder.

Most garden and field plants will grow satisfactorily in nutrient solution. The LaMotte Plant Nutrition Kit (Model AM-41) is used to test the nutrient solutions to see if they contain the mineral elements prescribed. Included in the kit are the directions for the use of the tests for:

Ammonia nitrogen	**Calcium**
Iron	**Magnesium**
Nitrate nitrogen	**pH**
Phosphorus	**Potassium**
Sulfate	

Hydroponics, or soil-less culture of plants, is used commercially where the roots are held by sand or gravel. On a large scale, where the primary aim is production of a crop rather than experimentation, sand and gravel provide a rooting medium and minimize the problem of plant support. Gravel culture, which involves a coarse substrate and sub-irrigation, is probably the most widely used commercial method.

What are the effects of mineral deficiencies on plant growth?

If you have a system such as the one shown in Figure 3, make up one liter of complete nutrient and one liter of each of five deficient nutrients. This will provide two jars of each type of solution with two or three plants in each jar. If two "bubble machines" (Figure 3) with 12 jars each are available other variations of nutrient solutions can be included, or the number of plants in each type of solution can be increased. The latter may be advisable because in the first investigation plants may suffer physical damage due to the inexperience of the investigator.

For quick and impressive results, use 10-14 day-old tomato seedlings and replace the nutrient solution about once a week.* Be sure the water level is maintained in the jars. As the plants grow larger they transpire a surprising amount. Remember that an empty jar may look like it is full of water. Between changes of nutrient solution, maintain water level with distilled water. Within two weeks or less the tomato seedlings will show conspicuous symptoms of nutrient deficiency as indicated in Table 5. If the nutrient solutions are unknown, try to identify them by the symptoms exhibited by the plants.

Symptoms of Mineral Deficiency

Nitrogen Deficiency

Signs of nitrogen deficiency normally appear in the older leaves first and as the deficiency becomes more pronounced, proceed up the plant to the newer growth. The leaves lose their normal green coloration and become a pale yellow-green. When grown in a nitrogen-deficient nutrient solution the entire plant may become a pale yellow and premature leaf-fall may occur. The young leaves remain small and the growth of the entire plant, including the root system, is very poor. The lack of lateral branching gives the plant a spindly appearance. In tomatoes the leaves become yellow with purple veins and the roots eventually turn brown. The stems may be stiff.

* In studies concerned with nutrient uptake by the plant from the solution, test nutrient solutions before replacing.

Phosphorus Deficiency

Phosphorus deficiency produces many of the same effects as nitrogen deficiency: retarded growth, spindly appearance, small leaves and the premature loss of leaves with a dark bluish-green to purple color. This is especially true of tomato plants, where the undersurface of the leaves develops a reddish-purple to purple color. This coloring develops along the veins and then spreads to the rest of the leaf. On the upper surface the veins usually show a purplish tinge, contrasting with the deep bluish-green of the leaf blade. In later stages, thin, dry, brown spots may occur on the leaves.

Potassium Deficiency

Plants suffering from potassium deficiency grow very slowly and are usually stunted. The symptoms first occur on older leaves as a speckling along the margins. The edges of the leaf turn yellow and then brown as the edges curl downward. Tomato plants in potassium-deficient nutrient solutions grow slowly and have a bluish-green color at first. New leaves have a distorted, crinkled appearance and brown spots may develop rapidly, which are dry and brittle. The leaf color around the margins and between the veins fades to a pale grey. The stems of the plants are woody and the poorly developed roots often turn brown.

Magnesium Deficiency

Symptoms of magnesium deficiency normally appear on the older leaves first and progress toward the younger growth as the deficiency becomes more severe. Interveinal chlorosis is quite common, although some plants may show yellow or brown spotting of the leaves. The entire leaf may turn brown and fall from the plant. Tomatoes grown in magnesium-deficient nutrient solutions show a chlorosis of the older leaves with the veins remaining green. The leaves become brittle and tend to curve upward along the margins.

Calcium Deficiency

Calcium deficiency appears first in the young leaves of the plant. The leaves turn yellow or brown and the edges may begin to curl. The upper leaves and stems may develop brown spots. The plants are weak and the stems often lack turgor and are unable to support the plant. The roots are poorly developed: short, with many branches, and often brown. The terminal buds often die.

Sulfur Deficiency

The symptoms of sulfur deficiency resemble those of nitrogen deficiency, but in tomato plants grown in nutrient solutions the symptoms do not appear as rapidly as with nitrogen deficiency. The first sign is usually chlorosis of the older foliage. Even though there is considerable increase in the length of the roots and stems, neither exhibits much increase in diameter. Stems are woody and hard, and growth of the plant is upright.

Iron Deficiency

Iron deficiency symptoms occur first in the youngest leaves of the plant. They show a mottling or interveinal yellowing which progresses until they become completely pale yellow to almost white. The stems near the growing tip of the plant become a yellowish-green and growth of shoots is very poor if the deficiency persists.

Manganese Deficiency

Manganese deficiency in tomato plants produces chlorosis in the leaves, which first have a mottled appearance and then turn a pale yellow, although the veins remain green. In later stages, dead spots occur on the yellowed leaves. The chlorosis progresses from the younger leaves to the older ones, and in severe deficiencies the entire plant becomes a light green.

Boron Deficiency

In tomatoes, the terminal shoot tends to curl upward, yellow or blacken, and die. Plants take on a bushy, flat-topped appearance as the lateral branches show growth, and then die back. Younger plants and leaves show purplish tints and older ones yellowish tints. Stems and petioles adjacent to the terminal growth become very stiff and brittle. Root growth is very poor, with the roots turning yellow or brown.

Copper & Zinc Deficiencies

Both copper and zinc are essential micronutrients for plant growth. Producing the deficiencies in nutrient solution growth can be difficult since impurities in the chemicals used for the solution may include enough copper and zinc for normal plant growth. Of the two, copper deficiency is usually more difficult to produce. Many of the symptoms are similar to those produced by macronutrient deficiencies and therefore procedures must be very exacting to prevent misinterpretation.

Nutritional deficiencies are indicated in the growth of leaves, stems and roots, and, if plants are grown to maturity, also in the production of flowers, fruits and seeds.

Unknown Nutritional Deficiencies

Unknown nutritional deficiencies of plants can often be identified by the use of the key in Table 5.

Sand Culture

In some cases it may be more convenient to grow plants in sand, watering them with various nutrient solutions. With sand culture it is not necessary to aerate the roots but it is necessary to devise some system for a steady supply of the nutrients to the plants. This can be achieved by placing the pots in dishes of the nutrient solution, or by supplying the nutrient from the top by means of a steadily dripping funnel. With this method the nutrient solutions are more subject to contamination, but this will probably not be a significant factor except when studying the micronutrients. To reduce contamination and evaporation the pot could be covered with foil. In sand culture the roots, of course, are not visible, which may or may not be a consideration.

If the sand culture method is chosen, clean, fine sand from a stream bed can be used for many experiments. It should be washed with distilled water if possible, or with tap water. For more carefully controlled experiments with micronutrients, it will be necessary to clean the sand by washing with hydrochloric acid and autoclaving. The removal of all impurities from sand is a difficult and tedious process.

Table 5. General key to foliar symptoms of mineral deficiencies in plants*

I. Initial injury on the mature foliage

- **A.** Site of general injury
  - **1.** Necrosis of tissue†
    - **a.** Stunted, light green plants; older leaves yellow-green to yellow in color, followed by drying and browning in advanced stages. Mineral deficiency caused by Nitrogen.
  - **2.** No necrosis of tissue
    - **a.** Stunted, abnormally dark green plants usually with narrow petiole angles; abundant reddish or purplish pigmentation; sometimes chlorosis of older leaves. Mineral deficiency caused by Phosphorus.
- **B.** Site of localized injury
  - **1.** Chlorosis starts at tips and margins of older leaves, progressing between veins, followed by brown necrotic spots which usually fall out, giving ragged appearance; leaves crinkled and curled, most noticeable in early stages. Mineral deficiency caused by Potassium.
  - **2.** Irregular chlorotic spots between veins of older leaves, followed by rapid necrosis and defoliation; die-back of twigs and small-leaved rosettes common in fruit trees. Mineral deficiency caused by Zinc.
  - **3.** Chlorosis starts between veins in older leaves; leaves become yellow or almost white with veins usually remaining green; necrosis not usual. Mineral deficiency caused by Magnesium.

* This table was originally prepared by T.M. Eastwood and C.H. Hobbs for a Plant Physiology Seminar Course at Purdue University, Lafayette, Indiana, 1942.

† Localized death of living tissue.

II. Initial injury on immature foliage

A. Site of general injury

1. Entire plant light green to yellowish-green in color; chlorosis most pronounced in young leaves which become yellow. Mineral deficiency caused by Sulfur.

B. Site of localized injury

1. Necrosis of tissue

a. Intervenal chlorosis of young leaves; leaves become yellow or white in color, all veins remaining green; small, brown necrotic spots follow chlorosis. Mineral deficiency caused by Manganese.

b. Chlorosis generally begins at bases and margins of young leaves, followed by necrosis; leaves become distorted or in more severe deficiencies terminal buds die and turn brown or black in color; gummy or corky deposits occur in fleshy organs. Mineral deficiency caused by Boron.

c. Chlorosis generally begins at tips and margins of young leaves, progressing between veins, followed by necrosis; leaves become distorted or in more severe deficiencies terminal buds die and turn brown or black in color; roots characteristically short, bulbous, with necrotic apical meristems. Mineral deficiency caused by Calcium.

2. No necrosis of tissue

a. Intervenal chlorosis of young leaves, veins remaining green; entire leaf including veins becomes yellow or white in color. Mineral deficiency caused by Iron.

b. Plants exhibit lack of turgor; wilting most pronounced in tops; sometimes chlorosis of young leaves. Mineral deficiency caused by Copper.

Tissue Analysis

In studies of plant nutrition it is important to know the amounts of certain nutrients that have been absorbed by various tissues.

Tissue Analysis with Chemicals

The LaMotte Plant Nutrition Kit (Model AM-41) contains materials and instructions for preparing tissues for analysis. Tests should be performed on equal portions of the control plants and the experimental plants at the same time. The tests suggested can be easily and quickly performed by students. Quantitative chemical tests are not generally proposed.

A new razor blade should be used to finely dice the plant material to be analyzed. Samples can be equated by weight or increments can be punched out with a corkborer. Measured amounts of the plant material and distilled water

(according to instructions with the kit) are placed in a vial, capped and shaken vigorously. The material is then filtered and, if excessively turbid or colored, activated charcoal is added. The solution is then shaken again and refiltered. Various tests can then be performed on the filtrate. If colorimetric tests are run on control and experimental plant filtrates at the same time, the tubes can be held side by side to compare the intensities of color.

In this way the mineral content of different plants and different parts of the same plant can be compared. For example, petioles can be compared to leaves from the bottom of the plant, leaves from iron and nitrogen deficient plants compared with completely nourished plants, etc.

The technique of tissue analysis can also be used with algae. The algae are separated from the nutrient solution by filtering or centrifuging. The cells can be broken up by the use of a glass homogenizer. If large masses of algae are available, the colonies can be broken up in a blender. In some cases the density of the algal population can be measured by colorimeter, or drops of the homogeneous suspension can be placed on a filter paper and the density of the color spots compared. When using radioactive materials, drops of algal suspension can be placed on filter paper, dried and autographed, giving a fairly reliable measurement of uptake.

Tissue Analysis with Radioactive Tracers

In recent years, tissue analysis has been greatly facilitated by the use of radioactive substances which can be detected by the use of a Geiger tube or by making a radio-autograph on X-ray or photographic film.

Radioactive substances correspond with their non-radioactive forms. They are not foreign or chemically different; they are not added material; in fact, they are the material being studied. Radioactive isotopes are natural traces and, as far as biological processes are concerned, are not significantly different from other atoms of their kind except for their ability to send out signals to indicate their presence.

Radioactive isotopes of the plant nutrient elements can be obtained from various sources. No license is required for small amounts of isotopes at low levels of energy.

Since phosphorus is involved in vital life processes of several types and is relatively easy to handle, having a half-life of 14.3 days, and a readily detectable radiation, it is ideal for studies of plant nutrition. Conventional methods of analysis of phosphorus and other nutrients are often difficult because of their involvement in the soil content, the life processes, the pH, the other ions present and other complicating factors. Radioactive substances can be detected in place without destroying the organism or disrupting its life processes.

Since phosphorus 32 has a half-life of 14.3 days, investigations using phosphorus should be planned carefully. The supplier will ship the isotope to arrive with a certain level of microcuries (μc). In the case of phosphorus 32 each vial will probably contain not more than 10 μc in the form of $NaH_2P^{32}O_4$, sodium dihydrogen phosphate. You will be permitted to purchase as many as ten units of this size per shipment.

Carbon 14 is less energetic than phosphorus 32, and can be purchased in units of 50 microcuries. It has a half-life of 5,568± years, so the dates of your investigation are not so important with carbon 14. Iodine 131, however, has a half-life of only 8 days, that is, its activity will diminish by half in eight days.

By using color tests for tissue analysis a great deal can be learned about the location, translocation and rate of uptake of nutrient substances. By the use of radioactive substances, further studies are possible. If a scaler-ratemeter is available, the passage of mineral nutrients can be traced through the plant with a Geiger tube. By placing a lead shield with a small hole in it over the end of the tube the investigator can locate radioactive materials accurately without harming the plant. Many questions can now be investigated. How fast is phosphorus 32 absorbed into the top of the plant? Is P^{32} absorbed through leaves? Is P^{32} translocated from one part of the plant to another? Does P^{32} accumulate in certain parts of the plant? Is the uptake of P^{32} affected by environmental conditions such as pH, temperature and aeration? Does radioactive P^{32} harm the plant? Is P^{32} as effective in plant growth as P^{31}? Activities of this type can be carried out with small amounts of radioactive material. About 0.5 microcuries of P^{32} will give impressive results in 500 mL of nutrient solution with small tomato plants, or you could use a much smaller amount of nutrient solution for short term experiments. Thus, one order of 10 microcuries of P^{32} could activate 20 jars of nutrient solution or more.

The investigator's imagination may move on to more complex questions, e.g., what is the fate of P^{32} in an ecosystem such as an aquarium? (Mayer, 1970).

It is advantageous not only to trace the path of mineral nutrients with the Geiger tube but also to make radioautographs of the material. To make radioautographs, the investigator will need the previously described plant nutrition system, plus radioisotopes of known energy, no-screen X-ray film (5" x 7" or 8" x 10") obtained from a local medical supply company or scientific supply company, developer, stop bath, fixer, film holders, developing trays and a dark room.

In the basic procedure, simply introduce 0.5 μc of P^{32} into 500 mL of nutrient solution and monitor its progress through the plant with a Geiger tube. After 24 hours, harvest the plant, wash the roots in nutrient solution (without P^{32}) and blot dry. To obtain the best radioautographs, the plant should be pressed flat until dry, remembering the size and shape of the X-ray film. Care in laying out and pressing the plant at this time will be rewarded when the films are developed.

When the plant is pressed flat and dry, take it into a darkroom and place it directly on the emulsion side of the X-ray film (dull side). Fold the plant and film in a film holder and press flat with a weight. This will help to insure a sharp focus of the plant tissues. Although the length of exposure will vary with the size of the plant and the concentration of P^{32}, try 24 hours. If this proves faulty, recalculate the exposure, allowing for decay (half-life 14.3 days), and try again. When the top of the plant is properly exposed, the roots will probably be overexposed. If this is a problem, autograph them separately. Note the variable uptake of P^{32} in the tissues. The roots, fruits, stems and young leaves are overexposed, and the uptake beyond the point of overexposure is not known.

A more accurate assay of the radioactivity of separate plant parts can be obtained through the use of a Geiger tube and scaler.

Generally, the procedure used to assay the activity of tissues is to collect a measured increment of the tissue and dry it under a lamp or in an oven. Place it squarely under the Geiger tube at a measured distance and count nuclear disintegrations for one minute or more. The longer the count, the more reliable the data. Calculate the counts per minute per gram. Use a dosage of isotopes sufficient to give a count at least double the background count.

The basic procedures described briefly above can be the basis of an extended series of investigations.

Solutions Arithmetic

Several common methods of expressing the amount of a particular element present in a solution are described below to facilitate comparison of solution formulas expressed in different terms.

A one molar (1M) solution results when one gram-molecular weight of a substance is dissolved in a liter of distilled water.

Example:

- How many grams of potassium nitrate are needed to make a 1M solution? The formula for potassium nitrate is KNO_3. The atomic weights are K-39.1, –14.0, 0-16.0. Thus, one gram-molecular weight is:

$$\frac{39.1}{K} + \frac{14.0}{N} + \frac{[(16) \times 3]}{0_3} = 101.1$$

  Therefore, 101.1 g of KNO_3 dissolved in a liter of water gives a 1M solution.

- If 50.55 g of KNO_3 are dissolved in one liter of water, what is the molarity

$$\text{Molarity} = \frac{\text{No. of grams of substance - Liter}}{\text{one gram - molecular weight of substance}}$$

  of the solution? We can use the formula:

$$M = \frac{50.55}{101.1} = 0.5$$

  and for the above problem we then have:
  The solution is 0.5 Molar.

- Another common measurement used is parts per million (ppm). One part per million is one gram dissolved in 1,000,000 milliliters (1,000 Liters) of water.

  How many ppm of K are there in a 1M solution of KNO_3? A lM solution of KNO_3 contains one gram-atomic weight of K (39.1) in one liter of water. Therefore,

$$\frac{39.1\text{ g}}{1\text{ Liter of water}} = \frac{39.1\text{ g x }1{,}000}{1\text{ Liter x }1{,}000} = \frac{39{,}100\text{ g}}{1{,}000{,}000\text{ mL}}$$

  This is a concentration of 39,100 parts per million. Note that a concentration of 1 g in 1,000 Liters of water is equivalent to 0.001 grams in 1 Liter (all parts divided by 1,000).

- Suppose we have a stock solution that will be further diluted, then what happens to the concentrations? Checking Table 4 on page 10 we find that the minus phosphorus solution contains 6 mL of stock solution #3 (KNO_3) per liter as the only source of K.

  How many ppm of K is this? The stock solution KNO_3 is 1M. Therefore, it contains 39.1 g of K per 1,000 mL. If we use 6 mL of this solution in 1,000 mL of distilled water, we have

$$6\text{ mL x }\frac{39.1\text{ g}}{1{,}000\text{ mL}}\text{ x }\frac{1}{1{,}000\text{ mL}} = \frac{234.6\text{ g}}{1{,}000{,}000\text{ mL}}\text{ or 234.6 ppm}$$

  In the complete nutrient we use 5 mL of stock solution #3 (1M KNO_3),

$$5\text{ mL x }\frac{39.1\text{ g}}{1{,}000\text{ mL}}\text{ x }\frac{1}{1{,}000\text{ mL}} = 195.5\text{ ppm}$$

  plus 1 mL of stock solution #4 (1M KH_2PO_4),

$$1\text{ mL x }\frac{39.1\text{ g}}{1{,}000\text{ mL}}\text{ x }\frac{1}{1{,}000\text{ mL}} = 39.1\text{ ppm}$$

  for a total of 234.6 ppm (195.5 + 39.1) of K in the complete nutrient. Thus both the complete nutrient and the minus phosphorus have the same concentration of K in ppm.

- The third measure of concentration often used is the milliequivalent (me). In using this measure the absolute value* of the total positive or negative valence of the ion must be considered. Thus:

$$\text{One milliequivalent/L} = \frac{\text{gram—atomic weight of ion}}{|\text{ valence of ion }|\text{ x }1{,}000\text{ L}} = \frac{\text{me}}{\text{L}}$$

* The absolute value of an ion is symbolized by [L],

Since potassium has a valance of +1, one milliequivalent of K in KNO_3 is:

$$\frac{39.1 \text{ g}}{|\ 1\ | \text{ x } 1{,}000 \text{ L}} = 0.0391 \text{ g/L}$$

In our nutrient solutions above we used 234.6 g of potassium per 1,000 liters or 0.2346 g/L.
If one milliequivalent of K is 0.0391 g/L, then 0.2346 g/L divided by 0.0391 g/L gives us the number of milliequivalents of K in the solution:

$$\frac{0.2346 \text{ g/L}}{0.0391 \text{ g/L}} = 6 \text{ me of K}$$

Note that the number of milliequivalents = $\dfrac{\text{ppm}}{\text{gram—atomic wt/ } |\ \text{valence}\ |}$

Thus:

$$6 = \frac{\frac{234.6}{39.1}}{\text{L}}$$

Consider another example. In stock solution #1 we find 40.0g of calcium in one liter of the 1M solution. 5 mL of this is added to 1 liter of the complete nutrient. This is equivalent to:

$$5 \text{ mL x } 40 \text{ g/L x } \frac{1}{1{,}000 \text{ g/L}} = 0.20 \text{ g/L of Ca}$$

Calcium has a +2 valence and gram-atomic weight of 40.0. So by substituting into the formula for milliequivalents, we have:

$$1 \text{ me} = \frac{40.0 \text{ g}}{[2] \text{ x } 1{,}000 \text{ g/L}} = 0.20 \text{ g/L of Ca}$$

Dividing this into the 0.20 g/L of Ca in the complete nutrient, we find that it contains $\dfrac{0.20 \text{ g/L}}{0.02 \text{ g/L}}$ or 10 milliequivalents of Ca.

Investigations

Now that the basic experimental system has been established and the importance of complete nutrition has been observed, what doors are open for further investigation? Can you now design and carry out investigations of these and other questions?

1. **What effect would various concentrations of nutrient solutions have on the plants?**

   The standard nutrient solutions described previously and provided with the kit are satisfactory for many plants. The supply of ingredients is probably greater than needed by most plants, but perhaps plants would grow even better if two or three times the prescribed concentration of a solution were used. It is possible that serious toxicity may occur at higher concentrations, which could be determined for each nutrient element by variations of the nutrient solution. It is also possible that increased nutrient concentrations could have harmful osmotic effects on plants. On the other end of the scale, minimal requirements for certain minerals and certain plants could be studied. The same line of investigation would also be possible with algae.

2. **Is growth and the uptake of nutrients affected by light intensity?**

   The uptake would be quite different in the dark than in the light. Tissue analysis by color tests or radioactive tracers could be used to investigate this question, while controlling other factors such as humidity and temperature as much as possible (an electric fan may help). Light intensity varies inversely with the square of the distance from the source, so small changes in distance from the lamps will make substantial changes in light intensity. A light meter reading in foot-candles or lux would be helpful (1 ft-c = 10.7 lux). Using fluorescent lamps, illumination will be close to minimal at best. The light intensity can be raised with flood lamps but heat is a problem. Many algae reach light saturation at lower intensities than higher plants. This saturation point may be reached with the fluorescent lamps.

3. **Is growth and the uptake of nutrients affected by light quality?**

   Unless ways can be devised to stabilize light intensity while varying quality, this question will be hard to investigate conclusively. However, there may be significant differences in growth between ordinary fluorescent tubes and plant growth tubes which are comparatively high in red and blue radiations, thereby providing more energy for photosynthesis.

4. **Is growth and the uptake of nutrients affected by humidity- transpiration?**

   Humidity is related to transpiration, which may or may not be directly related to mineral uptake. To investigate this question, set up two jars of tomato plants with complete nutrient, with or without P^{32}. If you use P^{32}, tissues can be analyzed by both chemical tests and by tracers. Place one jar and plant in a stream of air from a fan and another under a bell jar or plastic cover. Place an open container of water under the bell jar and raise the

bottom of this jar so there is some air circulation. Under these circumstances, the conditions are hard to control. The temperature will probably rise in the bell jar and carbon dioxide may be a limiting factor. After 24 hours, harvest the plants and analyze the uptake chemically or by radiation. If you find no appreciable difference in chemical tests, start the experiments with plants grown for a day or two in a solution deficient in a certain nutrient you wish to study? In this case would the change be more conspicuous? What if the uptake turns out to be about equal in the bell jar and outside it? Will this be contrary to your expectations?

5. **Is growth and the uptake of nutrients affected by temperature?**

   Again it will be difficult to control all the variables, e.g., when the temperature goes up, the humidity may decrease and transpiration may increase. The fresh weight of plants grown under different temperature conditions could be compared. Nutrient solutions could be maintained at constant temperature and the rate of root elongation measured. Care must be taken to prevent drying of the roots while measurements are made. The investigator might try the effect of root temperature on P^{32} uptake by establishing one tomato plant in a jar placed in a water bath at about 27°C, with the thermostat and stirrer. Another jar should be maintained at a relatively low temperature in a bath of running tap water, about 11-15°C, and still another jar maintained at room temperature. In all cases, a stirrer should be included if possible, to insure equal circulation, whether hot or cold.

   Do you predict increased uptake at 27°C? If this does not happen, consider the effects of aeration and respiration.

   - Note: Tomatoes will grow successfully at a constant temperature of 26-27°C but growth is improved if the day temperature is about 26-27°C and the night temperature is reduced to about 17-20°C.

   It would be interesting to explore the sensitivity of algae to day-night temperature fluctuations. The temperature of algal cultures could also be maintained by the use of the water bath.

6. **Is growth and the uptake of nutrients affected by aeration?**

   The basic plant growth system can be used for this study by simply turning off the air in the experimental bottles. In this case there would be considerable circulation in the aerated bottle and mechanical stirrers should be added to the non-aerated bottles, or at least the bottles should be shaken periodically to increase circulation.

   After 24 hours, harvest tissues and analyze. If the results indicate an increased uptake for the aerated plants, would you conclude that nutrient uptake is at least partly due to active transport, a life process? If so, what do you think would happen if the roots were killed by dipping them in hot water for one minute? Remember to protect the leaves from the heat.

Check for dissolved oxygen in a nutrient solution which is being used successfully for plant growth. Does dissolved oxygen increase with increased bubbling?* Can increased bubbling be harmful? Is there a relationship between temperature and oxygen content? These questions can be investigated with the basic plant nutrition system, with bottled gas to supply additional oxygen and bottled nitrogen used in the controls. If necessary, most of the air can be removed from a nutrient solution by heating.

- Note: Avocado will not grow below 0.6 ppm dissolved oxygen. Tomato plants will continue to make gains in growth as the oxygen in the aerating gas is increased up to about 20%. Beyond 20%, growth in tomatoes will be depressed.

Erickson (1946) found that the growth of aerated and non-aerated plants was about the same for the first two weeks, but by the end of the third week the aerated plants almost doubled the weight of the non-aerated plants. The leaves and stems of non-aerated plants were smaller and the roots were shorter, with the uppermost ones projecting above the level of the nutrient solution and producing a large number of root hairs in the moist atmosphere between the solution and the cover.

Does the size of the bubbles affect the optimum rate of aeration? Does the temperature of the solution affect optimum aeration? What effect does pH have on aeration and vice versa? What effect does changing the composition of the air have on aeration rates, i.e., more or less oxygen, carbon dioxide, nitrogen or sulfur dioxide?

7. Is growth and the uptake of nutrients affected by pH?

Check the uptake of plants in jars having pHs of 4.0, 5.0, 6.0, 7.0, 8.0, adjusting the low pHs with dilute nitric acid and the higher pHs with ammonium hydroxide. At what pH is uptake of P^{32} the greatest?

- Note: The pHs tend to change toward 6.0, perhaps due to the presence of the plants. If buffered solutions were used the various pHs would be stabilized. For a description of various buffer systems see Machils and Torrey (1956). The buffers must be very dilute to avoid an unfavorable osmotic pressure in the nutrient solution. Arnon and Johnson (1942) studied the effects of pH of the nutrient solution on growth. They adjusted the pH of the solution with NaOH and made adjustments to insure that the nutrients needed for growth were supplied.

Arnon and Johnson (1942) studied the effects of pH of the nutrient solution on growth. They adjusted the pH of the solution with NaOH and made adjustments to insure that the nutrients need for growth were supplied (Table 6).

* See Appendix (page 44) for construction of airbreaker.

Table 6. The growth of tomatoes & lettuce in varying pH

Average fresh weight in grams				
External pH	Tomato Shoots	Tomato Roots	Lettuce Shoots	Lettuce Roots
3	—	—	—	—
4	22.3	13.0	85.1	26.0
5	83.2	23.5	285.4	50.8
6	92.7	19.1	292.6	44.7
7	80.9	19.4	162.8	28.1
8	49.1	15.4	165.1	36.5
9	4.3	2.7	8.2	5.3

For preliminary work in this area, limit the investigations to a range of pH 5 to pH 7 due to difficulties encountered with precipitation of salts at higher or lower pH. Chelates such as EDTA will help to prevent precipitation of cations.

8. **How does the nutritional quality of an aqueous soil extract compare with that of a complete nutrient solution?**

   Stir a soil sample thoroughly in water and filter. Analyze a sample of the filtrate. Use the filtrate as a nutrient solution and compare the results with the complete nutrient. Compare the nutrient quality per pound of clay, sand, and loam.

   The filtrate does not necessarily represent the full nutrient quality of the soil sample. Plants are capable of releasing adsorbed ions by the production of hydrogen ions, and the soil may hold ions quite tightly, releasing them only as they are needed by the plants. How could the process of extracting minerals from soil be altered to increase the amount of mineral nutrient released (Table 2)?

9. **Do various plants have various nutrient requirements?**

   A sensible guess would be that plants do vary in their nutrient requirements. The possibilities for investigating this question are endless, but the investigator could study weeds as compared with cultivated plants, and could compare those plants characteristic of different habitats, such as mountain and plain, northern and southern latitudes, wet and dry locations. It might be rewarding to investigate the mineral requirements of a group of different plants living together. There might be significant differences in the mineral requirements of some of the larger plant groups, i.e., algae, mosses and liverworts, ferns and the seed-bearing plants.

10. Can mineral nutrients be toxic?

To investigate this question, increase the concentration of selected minerals in the nutrient solution. Don't forget to control the concentration of the whole solution so that osmotic pressure is not a limiting factor (Table 2 and Table 7).

- Note: most plants will grow normally with 1-5ppm of iron but 10 ppm will probably be toxic for many (Investigation #7).

Table 7. Tentative General Key to Foliar Symptoms of Mineral Toxicities in Plants*

I. Initial injury on mature foliage

A. Site of general injury

1. Necrosis of tissue

a. Leaves become slightly darker green, slightly smaller; sometimes abnormal rolling and curling of young leaves occur; in advanced stages growing tips wilt and die, especially in bright weather. Toxicity due to Magnesium.

b. General yellowing of leaves, older leaf tip and margins later become yellowish to brownish, followed by colored necrotic spots; leaf abscission developes (similar to potassium deficiency in some plants and nitrogen excess in others). Toxicity due to Phosphorus.

2. No necrosis of tissue

a. General hardening of plant; dull green, small leaf, hard stems; some plants have purplish-brown spots on older leaves follwed by leaf drop. Toxicity due to Chloride.

b. General hardening of plant; bluish-green color of leaf, hard stems, later leaves may become curled inward and pimpled, leaf margins brown and terminal growth becomes pale yellow. Toxicity due to Sulfate.

c. Early stages: slender growth, longer internodes, light green leaves. Later stages: stunted growth in general, leaves develop mosaic-like mottling, followed by dull colored spots; leaf wilting and abscission occurs. Toxicity due to Potassium.

B. Site of Injury Localized

1. Necrosis of tissue

a. Marginal chlorosis of leaves develops, which extends inward between veins, followed by brown necrosis and curling of leaf edge; leaf abscission (injury similar to potassium deficiency in some plants and iron deficiency in others—a terminal chlorosis). Toxicity due to Nitrogen (NO_3 and NH_4).

* This table was complied from a resume of available literature and practical observations. (This table is by no means complete and final; it is to be construed only as a preliminary attempt to classify available information.)

b. Chlorosis of leaf margins and tips, chlorosis extends inward, particularly between the veins until the whole leaf becomes pale yellow or whitish; marginal burning and necrosis with crinkling of leaf edges; leaf abscission. Toxicity due to Boron.

c. Water-soaked areas develop along main veins which remain green in leaf of some plants; areas become transparent; intervenal chlorosis develops also, later turning brown, and when entire leaf is brown defoliation occurs. (Also see zinc toxicity below). Toxicity due to Zinc.

d. Chlorosis of lower leaves followed by brown spots, then defoliation. (Also see copper toxicity below). Toxicity due to Copper.

II. Initial Injury on Immature Foliage

A. Site of Injury General

1. Necrosis of tissue

a. Chlorosis of leaves, young leaves become quite yellow; terminal buds die; also older leaves may droop without wilting, veins become colored, red or black; leaf abscission (injury in early stages similar to iron deficiency). Toxicity due to Zinc.

B. Site of Injury Localized

1. Necrosis of tissue

a. Intervenal chlorosis of young leaves, become yellow or whitish with dark brown or nearly white necrotic spots; leaf becomes distorted and crinkled (this is main difference from deficiency); plants like corn have whitish streaks in older leaves. Toxicity due to Manganese.

2. No necrosis of tissue

a. Intervenal chlorosis of young leaves, veins remain green, later entire leaf becomes yellow or whitish (similar to a deficiency). Toxicity due to Iron.

b. Chlorosis of young leaves, veins remain green. Toxicity due to Copper.

11. What mineral nutrients do commercial fertilizers contain? Do commercial fertilizers provide a complete nutrient medium?

Make a water extract of various commercial fertilizers, dilute within the range of the testing kit and analyze. Culture tomato plants in the extract and observe for deficiency symptoms.

12. Can plants absorb nutrients through their leaves?

The basic culture procedures can be used in this investigation by adding a membrane around the stem to keep nutrients from running down to the roots when applied to the leaves. Knowledge of deficiency symptoms, chemical tissue analysis and tracer techniques can be used here. The roots should be suspended in solutions deficient in the mineral being applied to the leaves. The experimental solutions can be applied to the leaves with an atomizer, brush or sponge. The required concentration of the experimental solution and the required frequency of application are studies in themselves. Investigation #13 is related to #12.

13. Do plants translocate nutrients in any special pattern?

The procedures of investigation #12 can be modified for this investigation by limiting the application of nutrients to certain leaves or other parts and checking the translocation, if any. Translocation can be checked by observing deficiency symptoms, by tissue analysis, or by radioactive tracers. To applying nutrients selectively to the upper part of a plant, cut off the tip of a leaf and immerse the leaf in a vial of the nutrient solution (Figure 8). Make the cut underwater with a clean, sharp razor blade. Renew the cut daily.

n Note: All mineral nutrients are not necessarily translocated to the same extent or to the same places.

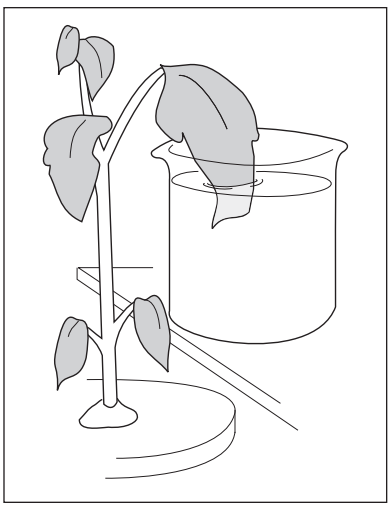

Figure 8: Leaf tip in nutrient solution.

14. In terms of translocation what is the relationship between the roots of a plant and the shoot?

What would happen if half the roots of a plant were in a deficient solution and half in complete solution? Or half the required nutrients were available on one side of the plant and half on the other? Tomato plants are suitable for the "split root" technique. Simply divide the base of the stem in two, longitudinally, with a clean, sharp razor blade and separate the roots as equally as possible.* Can half the roots nourish the whole plant? Will some minerals be translocated and others not?

The split-root technique is applicable to a wide range of investigations: the effects due to unilateral uptake and distribution of nutrient ions to the upper parts of the plant can be demonstrated and studied. The main consideration in selecting plants for this type of study is the suitability of the root system for splitting into two or more approximately equal portions. Rinne and

* See Appendix (page 47) for suggested apparatus.

Langston (1960) used a mint, Mentha piperita, taking advantage of its square stem todivide it into four sections. They used plastic refrigerator containers for the solutions. Other members of the mint family, for example Coleus, should also be amenable to this technique.

Preliminary studies should be limited to two different solutions to avoid undue complexity in the interpretation of results. Try a deficient versus a complete nutrient.

15. Can nitrogen-fixing organisms replace inorganic nitrogen compounds in nutrient solutions?

The useable nitrogen compounds in the soil come almost exclusively from biological processes which utilize molecular nitrogen from
the air and which decompose nitrogenous organic matter. These are the processes of
nitrogen fixation and nitrification (Wilson, 1967). These processes are vital because nitrogen deficiency is the most common limitation to plant growth. The legumes are well known for symbiotic nitrogen fixation, but some other types of plants also fix
nitrogen and form root nodules.

Symbiotic nitrogen fixation is carried out by certain bacteria of the genus Rhizobium living in nodules on the roots of legumes. Each type of legume supports a certain strain of bacteria. Thus, if you work with soybeans, peas, beans, alfalfa or clover, you must see the type of bacterium specifically adapted to that kind of plant. The bacteria for an investigation using soybeans should be collected from the nodules on the roots of soybeans, etc. Bacteria of appropriate types can be obtained from seed dealers.

To answer Question 15, set up young clover plants or other leguminous plants in three groups: Group 1 in complete nutrient, Group 2 in minus nitrogen nutrient, and Group 3 in minus nitrogen nutrient plus nitrogen-fixing bacteria. The bacteria can be collected by crushing nodules of the clover or other legumes in minus nitrogen nutrient so that there is a dense suspension of bacteria. Then soak the roots of the seedlings for Group 3 in the suspension for about one hour and grow the plants in minus nitrogen solution. The pH should be close to 7. If this can be done in aerated solution rather than in sand, the growth of nodules can be checked without disturbing the plants and the investigator can be confident of the composition of the minus nitrogen medium. The manufacture of nitrates by the bacteria will be shown by the freedom of Group 3 from minus nitrogen symptoms, which should be exhibited by Group 2. Group 3 can also be started with inoculated seeds.

The nitrate content of plants can sometimes be observed by making sections of leaf petioles, applying diphenylamine reagent, and observing under the microscope.*

* See Appendix (page 46) Nitrate “Quick Test”.

In addition to nitrogen fixation, another rich source of nitrogen for plant nutrition is the process of nitrification, whereby the ammonia gas (NH_3) from decaying plants and animals is converted to useable nitrate. The bacteria and fungi that bring about decay are common in the soil, as are the bacteria that convert ammonia to nitrite, and nitrite to nitrate (Wilson 1967). The presence of these organisms can be demonstrated by the following experiment. In the bottom of a glass or plastic tube of about 100 mL insert a rubber stopper with a short delivery tube. On top of this, place a small wad of glass wool and fill the cylinder with dampened fertile soil (Figure 9). The soil should be sufficiently sandy so that water will pass through it quite readily. Leave a space at the top of the tube to add water. At the top of the tube add slowly about 50 mL of 0.2% ammonium sulfate solution [$(NH_4)_2SO_4$]. Collect the fluid that comes through the soil and test for nitrate, nitrite and ammonia.*

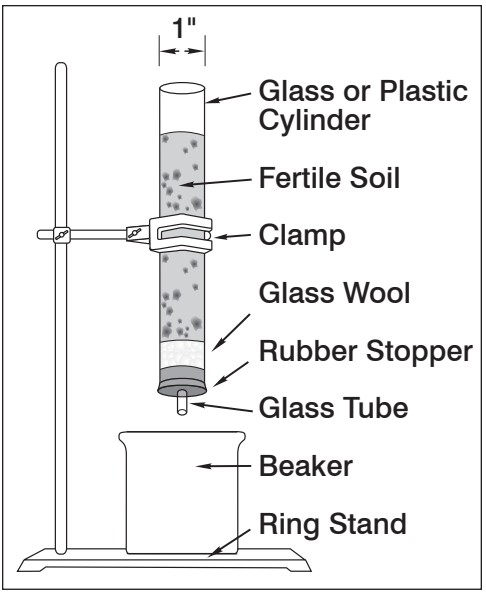

Figure 9. Apparatus for study of nitrification.

Now continue adding the ammonium sulfate solution until tests for nitrite and nitrate are negative. Cover the cylinder and keep moist with distilled water. After a week, wash the column of soil with distilled water until an extract drips from the bottom. Test the extract for nitrite, nitrate and ammonia. This experiment and useful accompanying text can be found in Machils and Torrey (1956).

■ Note: Nitrification is impaired at low pH.

16. To what extent does mineral nutrition affect pigment synthesis in plants?

The basic nutrition experiments done with green plants indicate that chlorosis is a common symptom of deficiency of several minerals, notably iron and nitrogen. What would happen to dark-grown plants brought into the light in various nutritive solutions?

17. What would happen to the pigmentation of variegated plants such as

* See Appendix (page 44) for suggested apparatus.

Coleus under various nutritive conditions?

Be sure to include control plants grown in complete nutrient solution and under the same conditions of light, temperature and humidity. The only factor that should vary is the composition of the nutrient solutions.

- Note: Coleus plants are readily propagated by cuttings and are suitable for the "split-root" technique. (Investigation #14)

18. What is the effect of mineral deficiencies on the growth of microorganisms?

Yeasts and fungi generally require media containing carbohydrates such as sugar. It may be that these organisms also would benefit from certain mineral nutrients. Try a 5-10% solution of sucrose (Reagent grade) in distilled water plus 1 g of peptone in 500 mL of the medium. To this add one-half package of dried yeast. Prepare the same culture using complete mineral nutrient rather than distilled water. Compare growth by counting cells or by measuring the carbon dioxide given off.

19. What minerals are added to the soil by the application of organic fertilizers such as manure, compost, or sewage sludge?

To one-half of a sample of sandy loam add manure or compost or sewage sludge and mix thoroughly. You will then have two mixtures —the plain soil and the soil plus organic matter. Extract the two mixtures with distilled water, filter, and use the filtrates as nutrient solutions and/or analyze the extracts chemically and compare with an extract of the organic fertilizers alone. It may be that the soil has an effect on the availability of the fertilizers. Variations of this study could include analysis of runoff water from heavily fertilized fields, cattle feedlots, landfill areas and sewage treatment plants.

20. Do plants compete for mineral nutrients?

An approach to this question could be the culture of a diverse group of plants together and separately in complete nutrient. If deficiency symptoms develop, they may be caused by differential nutrient absorption or by the secretion of inhibitors by some of the plants. Do not allow light and air to be limiting factors. A plastic dishpan can be fitted with a painted ½" plywood top with holes for plants. Aeration and circulation must be thorough.

21. Do plant tissues vary in mineral uptake?

Tissue analysis by chemical and radiation techniques are described on pages 18-20. It may be advantageous to apply the chemical tests to thin sections and observe under the microscope.

22. What mineral nutrients do seeds contribute to the growth of plants?

A table found in Hewitt (1966) showing the concentrations (in ppm) of

twelve essential elements in tomato seeds from normal or deficient plants grown in sand culture is given on the following page. Many investigations should be suggested by this data.

Table 8. Essential Elements in Tomato Seeds

(ppm)	Concentration Element					
	N	Mg	B	K	Ca	S
Complete	52000	3500	10.5	7000	720	700
Deficient	41000	2800	6.8	4500	380	350

(ppm)	Concentration Element					
	Zn	Cu	P	Mn	Fe	Mo
Complete	56	7	7000	55	800	2.5
Deficient	20	1.8	1500	10	40	0.09

Do varieties of tomato seeds vary in their content of these elements? What concentrations do other plant species show? What treatment of the seeds is best for determining the content of essential elements? What concentrations of the element must be present in the nutrient solution to allow the plant to bear fruit and yet show a deficiency for the element in the seeds? The data was for sand culture—does it hold for water culture?

It is suggested that initial work should be with the major nutrients (N, K, Ca, S, P) where the change appears to be rather large. Establish baseline data for the seeds that are used as tests with the LaMotte kit will not be as quantitative as the study above.

23. How well do domesticated plants compete with wild or native plants?

(Related to investigation #20) Set up a competitive culture of roadside plants and garden or field plants together in complete nutrient and the same kinds of plants separately in complete nutrient. The container with plywood top suggested in investigation #20 could also be used here.

24. What mineral nutrients are found in the rainwater from various locations?

Collect rainwater in clean containers from city, town and country locations. It may be necessary to concentrate solutions so that readings will be within the range covered by the tests. Variations of this study could include collection of samples at various times of the day or week, or collection in proximity to factories, powerplants, highways, seashores, mountain tops, etc.

25. What is the mineral content of demineralized water? Distilled water?

Use the chemical test kits. It may be that the mineral content, if any, is so dilute that it cannot be detected. To check this possibility reduce the volume of the sample a measured amount by boiling.

26. Can sodium ions (Na^+) be substituted for potassium ions (K^+)?

Replace the potassium compounds of the complete nutrient with sodium compounds of the same molarity (Table 3). Do salt marsh plants have a greater tolerance for sodium ions than upland plants? Use brackish water from a salt marsh or make up nutrient solutions of various concentrations using "instant ocean" salts* with complete nutrient.

Spartina has an unusually high requirement for iron and does not do well where soluble iron is deficient. Oxidation of iron sulfide (insoluble) into iron oxide (soluble) takes place within the plant. Spartina roots and stems have a central air chamber that contains the oxygen needed for the conversion of iron sulfide to iron oxide. Root nematodes may help in the conversion.

Seed germination of most marsh plants can take place only during spring rains when the salinity of the salt marsh is temporarily lessened. Otherwise the salt content of the water prevents germination. An exception: Salicornia seeds will germinate in water that is saltier than seawater.

Ahi and Power (1938) studied the effects of concentrations of seawater at various temperatures. Their results for alfalfa grown in cold (55°F) and warm (70°F) greenhouses with NaCl added to the nutrient solution is shown in Table 9.

Table 9. Effects of Sea Water on Growth of Alfalfa

	Weight of dry matter in grams	
	Cold (55°F)	Warm (70°F)
Complete nutrient solution	15.0	13.0
Complete nutrient solution plus 350 ppm NaCl	13.5	11.9
Complete nutrient solution plus 750 ppm NaCl	11.8	9.4
Complete nutrient solution plus 1400 ppm NaCl	4.0	2.6

Reference is made to the work of Bancroft (1918) indicating the concentration of salts that killed bean plants (Table 10).

Table 10. Salt concentrations lethal to bean plants

$MgCl_2$	—	2,640 ppm
Na_2CO_3	—	2,710 ppm
$NaNO_3$	—	3,700 ppm
NaCl	—	3,600 ppm
Na_2SO_4	—	6,510 ppm
$NaHCO_3$	—	12,300 ppm

This information should be useful as ball park figures for further investigations of temperature-salt concentration relations in plants grown in nutrient solutions.

* See Appendix (page 48) for source.

27. Do day-night temperature variations affect mineral uptake and plant growth?

See investigation #5.

28. Is genetically-controlled pigmentation related to mineral uptake in plants?

Biological supply houses sell packets of soybean seed which will produce seedlings exhibiting ¼ homozygous dominant green, ½ heterozygous yellow-green and ¼ homozygous recessive yellow. Would the pigmentation vary in different nutrients? Do the three genotypes vary in mineral uptake? Similar questions could be asked about variegated plants or about plants with albino mutants.

29. What mineral nutrients are needed by lower plants such as mosses, ferns, lichens, algae, fungi, etc.?

Forman (1965) discusses techniques for working with mosses and also suggests several additional investigations. The culture of algae is discussed on page 44. For suggestions for culturing plants see Morholt (1958).

30. How is nitrogen utilized in the nutrition of insectivorous plants?

Do insectivorous plants utilize the nitrogenous material of the insects they capture? Are insects essential to insectivorous plants?

31. What are the sulfur requirements of Chlorella?

Chlorella pyrenoidosa is an excellent experimental organism. It is easily grown in pure culture and could be used for many of the investigations suggested. To study the sulfur requirements Kellough and Zweig (1965) have suggested a sulfur-free medium (Table 11).

Table 11. Sulfur-free Chlorella medium

KNO_3	1.21 Grams
$MgCl_2 \cdot 6H_20$	2.03 Grams
KH_2PO_4	1.23 Grams
FeEDTA*	2 mL
Micronutrients	1 mL
Distilled Water	1.0 liter

Micronutrients:

H_3BO_3	2.86 Grams
$MnCl_2 \cdot 4H_2O$	1.81 Grams
$ZnCl_2$	0.105 Grams
$CuCl_2 \cdot 2H_20$	0.055 Grams
Distilled Water	1.0 Liter

As with the preparation of other nutrient solutions the chemicals should

* See Appendix (page 45) for preparation.

be dissolved in a large quantity of distilled water (300-500 mL) to prevent precipitation, then more distilled water added to bring the total volume to 1.0 liter. The solution should be tested for sulfur, since some of the chemicals could contain sulfur as an impurity. Kellough and Zweig (1965) suggest adding 0.02% concentrations of various sulfur-containing sodium salts (Na_2SO_4, Na_2SO_3, $Na_2S_2O_3$, Na_2S). This procedure allows you to investigate the ability of Chlorella to utilize sulfur from different sources.

Modifications of this procedure could extend the investigation to other essential nutrients and to other species of algae or higher plants.*

32. What are the effects of metals on transport in Elodea canadensis?

An interesting study of this question is described by Lowenhaupt (1963). Consider the possibility of using Elodea or other aquatic plants in many of the suggested investigations. Lemna, or duckweed, also has some advantage in nutrition investigations because its growth can be determined by simply counting the plants.

33. What are the nutrient requirements of isolated leaf tissue?

Leaf disks floated on nutrient solution under a bell jar will sink when the air is evacuated. When illuminated, they will rise as oxygen is produced. Oxygen production may vary in relation to the quality of the medium upon which the leaves are sustained. Disks from dark-grown leaves will synthesize chlorophyll when floated on a 1.0% sucrose solution and illuminated. Variations of the nutrient medium may affect the rate of pigment synthesis. Without sugar, chlorophyll probably will not be synthesized.

34. What effect does varying concentrations of some minerals have on the availability of other minerals?

For a discussion of ion antagonism see Bonner (1952). Plants may grow better in balanced solutions containing both monovalent and divalent cations than they will in the presence of either type of cation alone. Even when a nutrient solution is complete, growth may be decreased if there is a certain excess of one type of cation. It may be that these effects are due to variations in membrane permeability.

Beets or other pigmented roots can be used to demonstrate the effects of certain cations on permeability. When beet slices are placed in distilled water, very little diffusion of pigment occurs. However, in dilute sodium chloride, diffusion increases, and when calcium chloride is added to the sodium chloride solution, permeability again decreases.

* See Appendix (page 42) for preparation.

35. Does the type of compounds used for nutrition have an effect on the availability of the minerals to the plant?

According to Berger (1965) ammonia is especially toxic to potatoes and tomatoes. Symptoms were observed in plants grown in water culture if supplied with ammonia or ammonium salts. However, if both ammonium and nitrate salts were added the plants grew normally.

A series of investigations could be built around this observation. Are certain ammonium salts more toxic than others? Do the symptoms of ammonia toxicity appear more rapidly with some salts? Does the concentration of other nutrients affect ammonia toxicity? What ammonia/nitrate ratio will allow normal growth? Do plants other than tomato (bean, soybean, native species, etc.) show the same symptoms of ammonia toxicity? With the same rapidity? Can the ammonia in plants showing toxicity symptoms be determined with the ammonium-nitrogen tests?

If ammonium nitrate (NH_4NO_3) is used in the nutrient solution, is one ion selectively absorbed? How could this be determined? Does the level of one or the other remain closer to the initial level after the plant has grown in the solution for a period of time?

Even though a plant absorbs nitrogen as nitrate it must be transformed into ammonia before it is used to build amino acids. Is there enough of this ammonia in the plant to detect, using the ammonia-nitrogen test? If so, which parts of the plant give the highest tests?

Ignatieff and Page (1958) state that in solution cultures the ammonium ion restricts the uptake of calcium, potassium and magnesium, whereas the nitrate ion favors their uptake. What additional investigations does this statement suggest? What effect do differing ammonium/nitrate ratios have on the calcium, potassium and magnesium content of the nutrient solution? Of the plant?

UNESCO (1969) states that ammonium salts, in contrast to nitrates, render the soil more acid. Is this true in nutrient solutions? Is the difference in pH responsible for the restricted uptake of calcium, potassium and magnesium?

36. Is iron more available in some compounds than in others? Do chelates increase availability?

Establish one set of young plants in a complete nutrient solution with ferric chloride, $FeCl_3$, and another set with FeEDTA, iron held in the "claws" of an organic compound, ethylenediaminetetraacetic acid.* Grow the plants for two weeks. Maintain the water level with distilled water. If there are no discernible effects, continue the experiment for two more weeks.

* See Appendix (page 45) for preparation of FeEDTA and $FeCl_3$ solutions.

37. Can blue-green algae grow in nitrogen-free solutions? Can blue-green algae supply the nitrogen necessary for plants to grow in nitrogen-deficient solutions?

Several investigators have shown that the water fern, *Azolla*, with its algal symbiont, *Anabaena azollae*, is capable of growing in a nitrogen-free medium. Experimental work has also been done with nitrogen-fixing blue-green algae and rice culture. Seeding with the proper blue-green algae significantly increases the rice yield. UNESCO (1969) attributes the large increase in rice yield in India to the activity of nitrogen-fixing blue-green algae and expects even wider use of selected algae as inocula for rice fields.

A first step in the investigation would be to grow blue-green algae in nitrogen-free solutions and determine if the nitrogen content of the solution increases. Not all blue-green algae fix nitrogen. Most species known to fix nitrogen contain heterocysts and, with few exceptions, are able to grow in mineral media. Some species of *Anabaena*, *Cylindrospermum* and *Nostoc* are known to be nitrogen-fixers. Species of these genre can be obtained from many of the biological supply houses. *Azolla* also is available from many of the same companies. Small aquariums or large culture dishes will make good containers for *Azolla*.

38. What minerals do algae remove from nutrient solutions?

Burlew (1964) refers to work with *Scenedesmus obliquus* on nutrient uptake in which the available nitrate was completely utilized in a 21-day period. The initial and twenty-first day concentrations in milliequivalents per liter were: 2.9 and 0.1 for nitrate, 2.9 and 1.8 for calcium, and 2.0 and 0.3 for phosphate. The cultures were grown in 300 liter polyethylene-lined jars under 1500 ft-c of illumination and with a 5% level of CO_2 in the solution.

A suggested modification would be to grow the algae for specified lengths of time. After the selected growth period the algae can be separated from the solution by filtering or centrifuging. Tests can then be made on the clear solution and the results compared with the initial nutrient solution.

Are there any significant differences between species of algae in terms of nutrient removal? Is there a correlation between the weight of algae produced and the removal of nutrients? Do factors other than nutrient uptake influence the amount of algae produced?

39. What effect would algal growth in a nutrient solution have on the higher plants growing in the same solution?

Expose the plant roots to the same intensity of light as the tops of the plants. Accelerated growth of algae in the solution can be attained by inoculating the solution with algae and making sure the pH of the solution is between 6.5 and 7.0. If the solution is too acid the growth of the algae may be retarded. Plants should be grown in complete nutrient solutions and observed closely for deficiency signs as the algal growth becomes more prolific.

What deficiency symptoms appear? Do algae grow on the roots of the plant? Is root growth inhibited by the presence of the algae? Can the nutrient solution be made acid enough to prevent algal growth and still allow plant growth? What is the effect of adding copper sulfate to the solution? On the plant? On the algae? Do blue-green algae produce the same effect as green algae? Do different species of green algae produce varying effects?

40. Will seeds from plants grown in deficient solutions differ in mineral content from those developed by plants grown in complete solutions?

Choose plants that mature quickly, e.g., beans or peas, and grow them in large containers with aeration and a light intensity of no less than 700 footcandles. When seeds are mature, chop them in a blender with distilled water, filter and analyze the filtrate.

41. Will mineral deficiencies affect the flowering, fruiting and seed production of plants?

Specific information on the effects of mineral deficiencies can be found in the literature. However, most of the information concerns economic crops grown under field conditions. Berger (1965) indicates that slight boron deficiencies permit flowering but no seed production, and more severe deficiencies cause blossoms to drop. In tomatoes, nitrogen deficiencies cause the flower buds to yellow and drop, and copper or manganese deficiencies may completely prevent flower formation.

Growing plants in nutrient solution allows regulation of the concentration of minerals and vary the degree of deficiency. Sensitivity of plants to photoperiod must be considered in the selection of experimental subjects. A desirable plant would be one in which flowering occurs in a reasonable length of time and is not affected by photoperiod. Plants that are to be grown to maturity will most likely require larger containers and continuous replenishment of the essential nutrients. Periodic testing of the solutions is suggested to assure that the only deficiency is the one under examination. The pH of the solution may also require adjustment during the period of growth.

42. Do herbicides in nutrient solutions have an effect on plant growth?

Prepare 12 bottles of seedlings in complete nutrient and, leaving one bottle untreated, introduce increasing concentrations of 0.2% aqueous 2,4-D, starting with two drops and four drops, etc. Do the same with 0.4% aqueous solution of maleic hydrazide.

- Note: 2,4-D is an effective killer of broadleaved plants but has little effect on monocots, such as corn, grass and wheat. Does this suggest another investigation?

Maleic hydrazide can be expected to retard the overall growth of plants. Do the herbicides affect mineral uptake?

43. What would be the effect of increased carbon dioxide content in the nutrient solution on the growth of algae?

In mass culture of algae the CO_2 content of the medium is usually increased by stirring, shaking or bubbling of a CO_2-air mixture through the medium. Some investigators suggest that a CO_2 content in excess of 10% may inhibit growth, while others found CO_2 levels above 5% produced toxic effects. Consider these possibilities: Does one culture method enhance the growth of the culture to a greater extent than the others? One could subject different cultures to conditions of: (1) no disturbance of the medium, (2) stirring of the medium, (3) shaking of the medium—either intermittent or continuous, (4) bubbling of ambient air through the medium, (5) bubbling of different CO_2-air mixtures through the medium, and (6) bubbling pure CO_2 through the medium. Growth rates of the different cultures could then be compared. Other variables, such as temperature and light, must be kept constant for all of the cultures.

- Note: Carbon dioxide can be purchased in small gas bottles or it can be generated by combining hydrochloric acid and marble chips, baking soda and vinegar, etc. Bubble through water to remove other gases.

44. Do algae and higher plants use the same sources of nitrogen?

Many algae can use nitrogen from either nitrate or ammonium compounds, although investigations indicate that the ammonia is more readily absorbed. According to Round (1965), Eugiena gracilis and other chlorophyll-containing flagellates cannot utilize nitrates. It is also stated that in solutions containing NH_4NO_3, the ammonia is preferentially absorbed.

The above information should suggest many avenues for investigation. Does altering the complete nutrient solution to include ammonium compounds increase the growth of algae? Can the preferential absorption of ammonia from NH_4NO_3 be detected? Do different algae show this preference? Does the pH of the solution change? If so, is the change related to the form of nitrogen absorbed?

45. What effect does magnesium deficiency have on Chlorella?

Round (1965) states that magnesium deficiency interrupts cell division in Chlorella, resulting in abnormally large cells. Cultures of Chlorella could be grown in complete nutrient solution and in magnesium-deficient solution and cells from the two cultures compared microscopically. The use of an ocular micrometer with the microscope would aid in measurement of the cell size. To allow for individual cell variations within the cultures, at least ten cells should be measured and the average used for comparison. If an ocular micrometer is not available, estimates of cell size in relation to the high-power field of the microscope must be made. In this case, a large number of measurements should be used to minimize variation due to estimations. In those microscopes having a pointer in the eyepiece, the pointer length and/or width can be used for estimating size. Another possibility is the use of a microprojector. Since we are concerned with comparative cell size, rather than actual cell size, the cells can be measured by projecting them onto a screen and using a ruler to measure the projected image.

For further investigation consider the following questions. Are other species of algae affected in the same way? Does variation in magnesium deficiency cause variation in cell size? Do any other deficiencies affect cell size? Do other factors influence the effect of magnesium deficiency? Does the culture produce cells of normal size if magnesium is added to the magnesium-deficient medium? If so, how quickly does this occur? How does the growth rate compare in the two cultures?

46. What effect does sodium have on the growth of blue-green algae?*

Sodium is not required for the growth of many algae but does appear to be a requirement for the blue-greens. We also know that a high concentration of sodium will inhibit the growth of many organisms. What concentration of sodium produces optimum growth of blue-green algae? Does a high concentration of sodium inhibit growth? Are green algae more sensitive to the inhibitory effects of sodium than the blue-greens? Does the optimum concentration of sodium vary for different species of blue-green algae? Does the concentration of other nutrients affect the optimum level of sodium for the growth of blue-green algae? Does the sodium compound used affect the concentration needed for optimum growth?

47. Will increasing the CO_2 available to plant top or root affect the uptake of minerals and the growth?

An increase of CO_2 content of the air up to about 1.5% can be expected to result in an increase of about 50% in dry weight. (Hewitt and Cutting, 1968)

* See Appendix (page 44) for media containing sodium.

48. Do plants produce inhibitory substances?

It is known that many plants secrete materials into the substrate and that these materials may have inhibitory effects on other organisms. Does this phenomenon take place in nutrient solution growth of plants? How would one design an experiment to test this? What controls would be necessary? What would happen if two different species of plant were grown in one jar of nutrient solution? Suppose the cell sap is squeezed from one plant and added to the nutrient solution in which another species of plant is growing? Would "split-root" technique be useful (Investigation #14)?

Many soil fungi produce inhibitory substances (antibiotics). What effect do these have on plant growth? The same line of investigation could be pursued using algae as the experimental organisms.

49. Do fertilizers leach through soils?

The apparatus shown in Figure 9 could be used. Soil is placed in the column allowing several inches of free space at the top. A measured quantity of fertilizer is placed on the soil surface and water is then poured onto the surface. Collect the extract and test for the minerals it contains. Do some components of the fertilizer leach more readily than others? Will varying the depth of soil in the column affect the results? Do different soils produce different results? Try sand, clay and loam soils. Does the amount of fertilizer used affect the results? How does the extract compare with the soil-water extract in investigation #8? You could grow higher plants and/or algae in the two solutions. Collect the same amount of solution from both and grow the plants under the same conditions of light, temperature, aeration, etc. Be sure the soil and soil plus fertilize extracts are from the same type of soil.

50. What effect does continuous renewal of nutrient solution have on the growth of higher plants and/or algae?

Equipment for investigations of this type may be quite elaborate. The simplest type would be a constant-drip apparatus. This involves a reservoir containing nutrient solution, which is fed into the growth solution at a constant rate, usually several drops per minute. The container in which the plants are growing should be equipped with a constant level device. This allows you to maintain the same volume of solution. The overflow from this container should not be recycled. Control plants grown in the normal way can be used for comparison.

Appendix

Culture of Algae

Algae have not been widely used in secondary school investigations. With some attention to technique and careful selection of species this need not be the case. The algae recommended for the suggested investigations in this publication (and for general use) are: *Euglena gracilis*, *Chlorella pyrenoidosa*, *Scenedesmus sp.*, *Anabaena sp.* and *Cylindrospermum sp*. These algae have been found to be easy to maintain and culture. They have the added advantage of having been widely used in research studies and extensive information on them is available.

Obtain unialgal cultures of the organisms selected and, if available, the bacteria-free cultures. These are readily available from most biological supply houses. Containers for the growth of algal cultures can vary in size and shape, but should be made of glass. The containers suggested for the plant nutrition studies are suitable. Finger bowls, Erlenmeyer flasks and culture tubes can also be used. The containers used should be cleaned in the same manner as recommended for plant nutrition use.

In media that support the growth of bacteria and fungi, these organisms usually grow more rapidly than algae and may completely dominate the culture. Therefore, care should be taken to minimize contamination and sterile conditions maintained whenever possible. In completely inorganic media bacteria and fungi are not as serious a problem.

Algae can be grown in the media given on pages 45-47 and in the complete nutrient medium given on page 10.* *Chlorella* and *Euglena* grow well in Media C and D and Medium D has been used to grow both in complete darkness, although growth is not as rapid as in the light. *Euglena* becomes colorless when grown for some time in the dark. Revised Modified Chu #10 has been used to grow the blue-greens.†

Chlorella can be maintained very well on solid media. Prepare separate containers of agar-agar and Medium D. The agar-agar is dispensed into culture tubes, 5 to 10 mL/tube. Plug the tubes with nonabsorbent cotton and autoclave for 20 minutes at 15 psi. Medium D is prepared and sterilized under the same conditions. While agar-agar and the Medium D are still warm, add 1.0-2.0 mL of Medium D to each of the agar-agar tubes. Cold Medium D will cause some of the agar-agar to solidify and prevent thorough mixing. After the tubes have been rolled between the hands to mix, place them on a slant to solidify. *Chlorella* can then be streaked on the surface of the slant using an inoculating loop. If this is done under sterile conditions the *Chlorella* should remain free of bacterial contamination. Medium D should always be sterilized before use since the tryptone it contains will support a heavy growth of bacteria.

Sterilization can be accomplished by autoclaving or by passing the medium through a Millipore filter into a sterile container. The Millipore system* has

* See Appendix for source.

† Experimentation will permit you to select the best medium for the selected algae under your growth conditions.

other applications. It is especially useful if an autoclave is not available or if the composition of the medium would be altered by the heat of sterilization. Millipore filters can also be used to concentrate and separate the algae from the liquid medium it is growing in. The material used for plant or algal tissue tests can also be filtered in this way.

The growth of algae can be estimated in several ways. If a colorimeter or spectrophotometer is available it can be used to measure the light absorbance or percent transmittance of an algal suspension. One can also determine the fresh weight or dry weight of the algae. To have an appreciable weight of algae requires either a very dense culture or a very large one. The algae in the culture can be concentrated by centrifuging or filtering. A third method is to count the algal cells in a sample of the culture. The most accurate method of counting involves the use of a hemocytometer.

If none of the above equipment is available, approximate comparisons of cultures can be made in the following way. Place 0.1 mL (or one drop) of the culture on a slide and carefully place a cover slip over the drop. The liquid should not extend beyond the edges of the cover slip. Count the number of cells in a high power field.

Repeat this for a total of ten fields. If one moves the slide to a new field without looking into the microscope there will be less tendency to select "good fields" to count. Divide the total cell count by ten to obtain the "average number of cells per high power field." Note that this figure in no way indicates the number of cells in the sample or the culture, but is simply used to compare with the number obtained from a different culture. To assure as great a degree of accuracy as possible the slides and cover slips should be clean and the same size and weight cover slips used.

Additional Nutrient Media

Medium C (Needham, 1937)	
NH_4NO_3	1.0 g/L
KH_2PO_4	0.2 g/L
$MgSO_4$	0.2 g/L
KCl	0.2 g/L
*$FeCl_3$	Trace

Medium D (Needham, 1937)	
Tryptone	2.0 g/L
KH_2PO_4	0.25 g/L
$MgSO_4$	0.25 g/L
KCl	0.25 g/L
*$FeCl_3$	Trace
Sodium acetate	2.0 g/L

* See Appendix (page 48) for source.

Bristol's Basic Medium

Prepare stock solutions of each salt by adding to 400 mL of distilled water.

$NaNO_3$	10.0 g
$CaCl_2$	1.0 g
$MgSO_4 \cdot 7H_2O$	3.0 g
K_2HPO_4	3.0 g
KH_2PO_4	7.0 g
NaCl	1.0 g

Add 10 mL of each stock solution to 940 mL of distilled water. To this add 1.0 mL FeEDTA stock solution and 2.0 mL of the trace element stock solution made up as follows:

$ZnSO_4 \cdot 7H_2O$	0.1 g
H_3BO_3	0.1 g
$MnSO_4 \cdot 4H_2O$	0.15 g
$CuSO_4 \cdot 5H_2O$	0.03 g
Distilled water	1.0 L

Revised Modified Chu #10 Medium and Trace Elements

In approximately 900 mL of distilled water, dissolve the indicated amounts of the following salts and dilute to 1 liter with distilled water.

$Ca(NO_3)_2 \cdot 4H_2O$	0.2 g/L
K_2HPO_4	1.0 g/L
$NaNO_3$	16.6 g/L
$MgSO_4 \cdot 7H_2O$	2.5 g/L
Ferric citrate	0.35 g/L
Citric acid	0.35 g/L

Trace Elements:

$CuCl_2 \cdot 2H_2O$	0.00001 g/L
$ZnCl_2$	0.04 g/L
H_3BO_3	0.24 g/L
$CoCl_2 \cdot 6H_2O$	0.002 g/L
$MnCl_2 \cdot 4H_2O$	0.14 g/L

* Note: 1/mL of FeEDTA stock solution can be used instead of $FeCl_3$. For $FeCl_3$ trace use two drops of a 1% $FeCl_3$ solution..

Concentration (Parts Per Million) of Nutrient Solutions (shown on page 10)

Macronutrients

	Ion					
Solution	Ca^{+2}	Mg^{+2}	K^{+}	NO_3^{-}	PO_4^{-2}	SO_4^{-2}
Complete	200	49	235	210	31	64
Minus Ca	—	49	235	70	31	64
Minus Mg	160	—	391	196	31	48
Minus K	221	49	—	140	31	64
Minus N	101	49	196	—	31	208
Minus P	160	49	235	196	—	64
Minus S	160	49	274	252	31	—
Minus Trace	200	49	235	210	31	64
Minus Fe	200	121	117	168	31	160

Micronutrients*

Ion						
Fe	Mn	Cu	Zn	B	Mo	Cl
5.0†	0.5	0.02	0.05	0.5	0.001	0.6

Iron EDTA

FeEDTA can be prepared by dissolving 26.1 grams of EDTA in 286 milliliters of 1M potassium hydroxide (KOH) and then adding 24.9 grams of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and diluting to 1.0 liter. This solution is then aerated for a minimum of twelve hours (it can be left overnight) to produce the FeEDTA complex.

One milliliter of this solution added to one liter of nutrient solution provides 5 ppm of iron.

$FeCl_3$

As used in nutrient solutions, a trace of $FeCl_3$ is one or two drops per liter of a 1% solution. Dissolve 1.0 g of $FeCl_3$ in 100 mL of distilled water to make a 1% solution. One or two drops should be added once or twice a week.

Nitrate "Quick Test"

The presence of nitrates can be determined by using diphenylamine reagent. [Dissolve 1.0 grams of diphenylamine in 100 mL of concentrated sulfuric acid.] Several slices of plant tissue or drops of the solution to be tested are placed in one of the depressions of a spot plate and several drops of the diphenylamine reagent added. A blue color indicates the presence of nitrate — the darker the color, the higher the nitrate content.

CAUTION: The test solution contains concentrated acid.

Nitrite "Quick Test"

The nitrite test uses sulfanilic acid reagent. Heat distilled water to boiling and then allow it to cool. To 135 mL of this water, carefully add 15 mL of glacial acetic acid. Dissolve 0.5 g of sulfanilic acid in this solution. Using the same procedure as in the nitrate test add several drops of the sulfanilic acid reagent. A red color should develop within two to three minutes if nitrite is present.

CAUTION: Highly acidic solution.

Airbreaker

A plastic airbreaker should be used in aeration of the nutrient solutions since a regular airstone may interact with the solutions. To make a plastic airbreaker use a small plastic vial (approximately 5 cm or less) with a tightly fitting cap. First make a hole in the cap just large enough for a short piece of glass tubing. (Plastic vials with dropper tops are useful since the tip of the dropper may be cut off, leaving a hole for the glass tube.) Make several tiny holes in the sides of the vial by heating a piece of wire until it glows and then pushing it through the side. Use the thinnest piece of wire available and make the holes as uniform in size as possible. Pack the vial with glass wool, put the glass tubing through the hole in the top and then connect it to a piece of plastic tubing leading from the air pump.

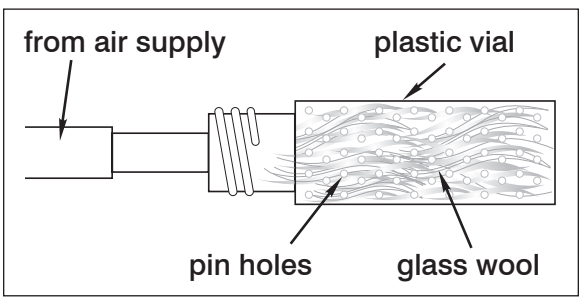

Figure 10. Airline filter

Stock Solution Bottle

The measurement and dispensing of stock solutions is facilitated by modification of a dispensing bottle as shown in Figure 11. A calibrated syringe is modified by cutting a hole through the piston and replacing the solid piston rod with a length of glass tubing. A length of polyethylene or Tygon tubing connects the glass tubing of the syringe to the outlet of the dispensing bottle. A pinch clamp is used on the tubing to control the flow of liquid.

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* The concentrations of micronutrients is the same for all solutions with the exception of the mius iron which has 0.00 ppm Fe, and the minus Trace where the readings for Mn, Cu, Zn, B, Mo, and Cl should be 0.00 ppm.

† Using 1.0 mL of the FeEDTA stock solution per liter.

In use, the piston is withdrawn to the calibration mark of the number of milliliters of stock solution required. If the syringe is then held below the level of the liquid in the stock bottle and the pinch clamp released, the syringe will fill with solution. The pinch clamp is then tightened and the liquid in the syringe dispensed into a container by depressing the piston.

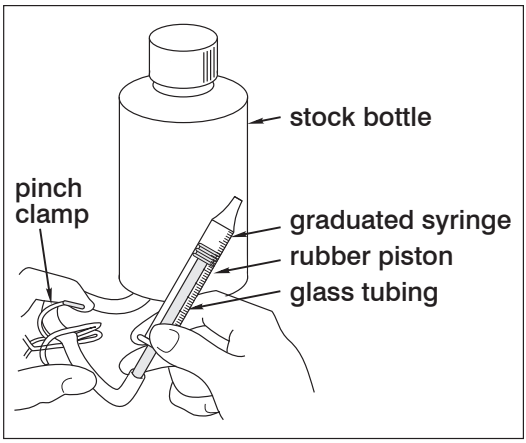

Figure 11. Apparatus for dispensing stock solutions

Split-Root Apparatus

For the "split-root" technique plastic refrigerator containers can be used. If containers are of the same size and shape with straight sides they are easier to hold together and a tight cover can be more easily fitted. The cover can be of plastic or painted plywood. If a hole is made large enough to be fitted with one of the flat corks the handling of the plants is made easier. If a thin plastic cover is used the cork will aid in support of the plant. Figure 12 illustrates the suggested apparatus.

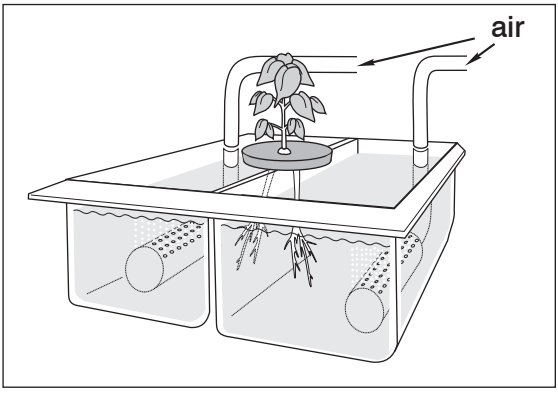

Figure 12. Split-root apparatus

Sources of Equipment & Supplies

This is a list not intended to be comprehensive nor to indicate preference for one source over another. Check equipment and supply catalogs for additional sources.

Bottles

Bottles used in the plant nutrition set-up described are wide-mouth, 5-3/4" high, 2-7/8" in diameter (70 mm), capacity 16oz.

Carolina Biological Supply Company
2700 York Road
Burlington, North Carolina 27215-3398

Phone: 800-334-5551
FAX: 800-222-7112
www.carolina.com

Kits - For Chemical Investigations

Code 3561-01	Hydroponics Test Kit
Code 5940	Plant Nutrition Chemical Package, supplies 10 nutrient stock solutions
Code 1596	“Plant Nutrition Studies” by Dr. Robert Stegner
Code 5860-01	Dissolved Oxygen Test Kit
Code 7297-DR-01	Carbon Dioxide Test Kit
Code 3352-01	Nitrite in Water Test Kit

A wide selection of soil analysis test equipment is also available.

LaMotte Company
802 Washington Avenue
Chestertown, Maryland 21620

Phone: 800-344-3100
www.lamotte.com

Corks

Corks to fit the above bottles should be tapered, flat corks, 2-1/2" diameter, 5/8" long.

Sargent-Welch Scientific Company
PO Box 5229
911Commerce Court
Buffalo Grove, Illinois 60089-5229

Phone: 800-727-4368
www.sargentwelch.com

EDTA

Available from most chemical supply companies, listed as EDTA or Ethylenediaminetradcetic Acid.

"Instant Ocean" Synthetic Sea Salts

Aquarium Systems, Inc.
8141 Tyler Blvd. Mentar, Ohio 44060

Phone: 800-822-1100
www.aquariumsystems.com

Millipore Supplies and Kits

Millipore Corporation
80 Ashby Road
Bedford, Massachusetts 01730

Phone: 800-MILLIPORE
www.millipore.com

Bibliography

Ahi, S.M., and W.L. Powers, 1938, "Salt Tolerance of Plants at Various Temperatures", *Plant Physiology*, 13: 767.

Allen, M.B., and Arnon D.I., 1955, "Studies on Nitrogen-Fixing Blue-Green Algae, I. Growth and Nitrogen Fixation by *Anabaena cylindrica* Lemm", *Plant Physiology*, 30: 366.

Allison, R.V., 1923, "Studies on the Relation of Aeration and Continuous Renewal of Nutrient Solutions to the Growth of Soybeans in Artificial Culture", *American Journal of Botany*, 10: 554.

Arnon, D.I., and Johnson C.M., 1942, "Influence of Hydrogen Ion Concentration on Growth of Higher Plants Under Controlled Conditions", *Plant Physiology*, 17: 525.

Arnon, D.I., and Hoagland D.R., 1940, "Crop Production in Artificial Culture Solutions and in Soils with Special Reference to Factors Influencing Yields and Absorption of Inorganic Nutrients", *Soil Science*, 50: 463.

Bancroft, R.L., 1918, "The Alkali Soils of Iowa", *Iowa State Bulletin*, 177.

Berger, Kermit C., 1965, *Introductory Soils*, The Macmillan Company, Collier-Macmillan Limited, London.

Bonner, James, and Galston Arthur W., 1952, *Principles of Plant Physiology*, W.H. Freeman and Company, San Francisco.

Brady, Nyle C., ed., 1967, *Agriculture and the Quality of Our Environment*, American Association for the Advancement of Science, Washington, DC.

Burlew, John S., 1964, *Algal Culture from Laboratory to Pilot Plant*, Carnegie Institution of Washington, Publication 600, Washington, DC.

Chang, H.T., and Loomis W.E., 1945, "Effect of CO2 on Absorption of Water and Nutrients by Roots", *Plant Physiology*, 20:221.

Clark, H.E., and Shive J.W., 1932, "Influence of Continuous Aeration Upon the Growth of Tomato Plants in Solution Cultures", *Soil Science*, 34:37.

Clark, H.E., and Shive J.W., 1934, "The Influence of the pH of a Culture Solution on the Rates of Absorption of Ammonium and Nitrate Nitrogen by the Tomato Plant", *Soil Science*, 37: 203.

Crafts, A.S, 1961, *Translocation in Plants*, Holt, Rinehart and Winston, New York.

Crafts, A.S., and Yanaguchi S., 1960, "Absorption of Herbicides by Roots", *American Journal of Botany*, 47: 248.

Daubenmire, R.F., 1959, *Plants and Environment: A Textbook of Plant Autecology*, John Wiley and Sons, Inc., New York.

Durell, W.D., 1941, "The Effect of Aeration on the Growth of the Tomato in Nutrient Solution", *Plant Physiology*, 16: 327.

Ellis, Carleton, and N.W. Swaney, (revised and enlarged by Tom Eastwood), 1947, *Soilless Growth of Plants*, Reinhold Publishing Corp., New York.

Erickson, L.C., 1946, "Growth of Tomato Roots as Influenced by Oxygen in the Nutrient Solution", *American Journal of Botany*, 33: 551.

Fogg, G.E., 1966, *Algal Cultures and Phytoplankton Ecology*, The University of Wisconsin Press, Madison.

Fogg, G.E., 1956, "Nitrogen Fixation by Photosynthetic Organisms", *Annual Review of Plant Physiology*, Vol. 14, Annual Reviews, Inc., Palo Alto, California.

Forman, Richard T.T., 1965, "The Physiological Ecology of Mosses", Biological Sciences Curriculum Study, *Research Problems in Biology: Investigations for Students*, Series 4, Anchor Books, Doubleday & Co., Inc., Garden City, New York.

Fried, M., and Broeshart H., 1967, *The Soil-Plant System in Relation to Inorganic Nutrition*, Academic Press, Inc., New York.

Foth, Henry D., 1967, *A Study of Soil Science*, LaMotte Company, Chestertown, Maryland.

Hewitt, E.J., 1966, *Sand and Water Culture Methods Used in the Study of Plant Nutrition*, (2nd edition revised), Commonwealth Agricultural Bureaux, Farnham Royal, England.

Hewitt, E.J., and Cutting C.V., editors, 1968, *Recent Aspects of Nitrogen Metabolism in Plants*, Academic Press.

Ignatieff, Vladimir, and Page H.J., editors, 1958, *Efficient Use of Fertilizers*, (2nd edition), Food and Agriculture Organization of the U.N., Rome, Italy.

Jackson, Daniel F., 1964, *Algae and Man*, Plenum Press, New York.

Janick, Jules, Schery Robert W., Woods Frank W. and Ruttan Vernon W., 1969, *Plant Science: An Introduction to World Crops*, W.H. Freedman and Company, San Francisco.

Johnson, E.S., and Hoagland D.R., 1929, "Minimum Potassium Level Required by Tomato Plants Grown in Water Cultures", *Soil Science*, 27: 86.

Kellough, Richard D., and Zweig Gunter, 1965, "A Study of the Sulfur Requirements of *Chlorella*", In Biological Sciences Curriculum Study, *Research Problems in Biology: Investigations for Students*, Series 3, Anchor Books, Doubleday & Co., Inc., Garden City, New York.

Kirkby, E.A., 1968, "Influence of Ammonium and Nitrate Nutrition on the Catioanion Balance and Nitrogen and Carbohydrate Metabolism of White Mustard Plants Grown in Dilute Nutrient Solutions", *Soil Science*, 105: 133.

Kirkby, E.A., and Mengel K., 1967, "Ionic Balance in Different Tissues of the Tomato Plant in Relation to Nitrate, Urea or Ammonium Nutrition", *Plant Physiology*, 46: 6.

Kitchen, H.B., editor, 1948, *Diagnostic Technique for Soils and Crops*, American Potash Institute, Washington, DC.

Kramer, Paul J., 1969, *Plant and Soil Water Relationships: A Moden Synthesis*, McGraw-Hill Book Company, Inc., New York.

Kurtz, E.B., and Robert S. Mellor, 1966, *Plant Physiology: Laboratory Exercises*, Burgess Publishing Company, Minneapolis.

LaMotte Company, 1970, *The LaMotte Soil Handbook*, LaMotte Company, Chestertown, Maryland.

Loomis, Walter E., editor, 1953, *Growth and Differentiation in Plants*, Iowa State College Press, Ames, Iowa.

Loomis, Walter E., and Charles A. Shull, 1939, *Experiments in Plant Physiology*, McGraw-Hill Book Company, Inc., New York.

Lowenhaupt, Benjamin, 1963, "Effects of Metals on Transport in Elodea canadensis" In: American Institute of Biological Sciences, *Research Problems in Biology: Investigations for Students*, Series 1, Anchor Books, Doubleday & Co., Inc., Garden City, New York.

Machilis, Leonard, and Torrey John G., 1956, *Plants in Action: A Laboratory Manual of Plant Physiology*, W.H. Freeman and Company, San Francisco.

Mayer, William V., 1970, *Radiation and Its Use in Biology: A Laboratory Block*, Educational Programs Improvement Corporation, Boulder, Colorado.

Meyer, Bernard S., Anderson D.B., and Swanson C.A., 1955, *Laboratory Plant Physiology*, (3rd edition), D. van Nostrand Company, Inc., Princeton, New Jersey.

Morholt, Evelyn, Brandewein Paul F., and Joseph Alexander, 1958, *A Sourcebook for the Biological Sciences*, Harcourt, Brace and Company, New York.

Muenscher, W.C., 1922, "The Effect of Transpiration on the Absorption of Salts by Plants", *American Journal of Botany*, 9: 311.

Myers, J., 1946, "Culture Conditions and the Development of the Photosynthetic Mechanism, III, Influence of Light Intensity on Cellular Characteristics of *Chlorella*", *Journal of General Physiology*, 29: 419.

Needham, James G., et al, 1959, C. 1937, *Culture Methods for Invertebrate Animals*, Dover Publications, Inc., New York.

Olsen, C., 1950, "The Significance of Concentration for the Rate of Ion Absorption by Higher Plants in Water Culture", *Physiol. Plant.*, 3: 152.

Pringsheim, E.G., 1949, *Pure Cultures of Algae: Their Preparation and Maintenance*, Cambridge University Press, Cambridge.

Rinne, R.W. and Langston R.G., 1960, "Studies on Lateral Movement of Phosphorus 32 in Peppermint", *Plant Physiology*, 35: 216.

Rorison, I.H., editor, 1969, *Ecological Aspects of the Mineral Nutrition of Plants*, Blackwell Scientific Publications, Oxford, England.

Roseu, Walter G., 1965, "The Role of Nitrogen in the Nutrition of Insectivorous Plants", In Biological Sciences Curriculum Study, *Research Problems in Biology: Investigations for Students*, Series 4, Anchor Books, Doubleday & Co., Inc., Garden City, New York.

Round, F.E., 1965, *The Biology of the Algae*, Edward Arnold Limited, London, England.

Sprague, Howard B., 1964, *Hunger Signs in Crops*, (3rd edition), David McKay Company, Inc., New York.

Stout, P.R., and Hoaglund D.R., 1939, "Upward and Lateral Movement of Salt in Certain Plants as Indicated by Radioactive Isotopes of Potassium, Sodium and Phosphorus Absorbed by Roots", *American Journal of Botany*, 26: 320.

Sutcliffe, J.F., 1962, *Mineral Salts Absorption in Plants*, Pergamon Press, New York.

Truog, Emil, editor, 1951, *Mineral Nutrition of Plants*, University of Wisconsin Press, Madison.

Ulrich, J.M., and McLaren A.D., 1965, "The Absorption and Translocation of C^{14} Labeled Proteins in Young Tomato Plants", *American Journal of Botany*, 52: 120.

UNESCO, 1969, Soil Biology, UNESCO, Paris, France. Wallace, T., 1951, *Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms* (2nd edition), Her Majesty's Stationery Office, London, England.

Went, F., 1957, *The Experimental Control of Plant Growth*, Ronald, New York.

Wilson, Carl L., and Loomis W.E., 1967, *Botany* (4th edition), Holt, Rinehart and Winston, New York.

Wolken, Jerome J., 1961, *Euglena: An Experimental Organism for Biochemical and Biophysical Studies*, Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey.

Other Environmental Science Handbooks

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Dr. Charles E. Renn, Professor of Environment Engineering Science, Johns Hopkins University

This text covers the factors that determine the quality of water. It studies the "life cycle" of water, beginning with water in its purest form found in nature, observing it as it is altered by its contact with the atmosphere and the Earth, and continuing the study through the basic treatments involved in the production of high quality water suitable for use in industry and in the home. The problems of scale formation, corrosion and staining are discussed, as are the objectionable characteristics of taste, color and turbidity in water.

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Investigating Water Problems · 1589

Staff, LaMotte Company

This booklet has been prepared expressly for teachers and students that are interested in investigating the quality of water supplies. The intent of this publication is to provide technical support and background information concerning the water quality factors and to give basic information on field and laboratory water testing techniques. It is assumed that the reader is not an experienced chemist but is an inquirer seeking the essential information that is needed for meaningful interpretation of the results of field studies of aquatic environments.

A Study of Soil Science · Code 1530

Dr. Henry D. Foth, Professor of Soil Science, Michigan State University

This illustrated booklet deals with the subjects of soil formation, variations in soil composition, the plant cycle, soil reaction (pH), and the major, minor and trace elements. It also includes information on soil as a water reservoir, movement of water in soil and soil as a filter media for ground water. A glossary of soil science terms is included.

The LaMotte Soil Handbook · 1504

Staff, LaMotte Company

This booklet discusses the composition and nature of soils, the major and minor plant nutrients, soil pH, and includes an extensive listing for the pH preferences of a wide variety of plants. Valuable information and tables are provided to give a meaningful interpretation of soil tests. A glossary of terms is included.

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