SOME PHYSIOLOGICAL EFFECTS INDUCED IN THE COWPEA, *VIGNA SINENSIS* (L.) SAVI, BY TWO STRAINS OF ALFALFA MOSAIC VIRUS

by

IRA KELLY MILLS

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1956

APPROVED:

Redacted for Privacy

Associate Professor of Botany

In Charge of Major

Redacted for Privacy

Chairman of Department of Botany

Redacted for Privacy

Chairman of School Graduate Committee

Redacted for Privacy

Dean of Graduate School

Date Thesis is presented May 5, 1956

Typed by Della Ruth Mills

ACKNOWLEDGMENTS

The writer wishes to thank Dr. R. O. Belkengren for his patient counsel during the research for this thesis and for his constructive criticism of the manuscript.

The writer's sincere appreciation is also expressed to Dr. J. A. Milbrath for his cooperation.

The assistance of Mr. H. H. Millsap and of Mr. Gordon Snow in the preparation of the photographs and plates is gratefully acknowledged.

To Della Mills, the writer's wife, is due special appreciation for her encouragement and invaluable assistance during the course of the work.

TABLE OF CONTENTS

SOME PHYSIOLOGICAL EFFECTS INDUCED IN THE COWPEA, *VIGNA SINENSIS* (L.) SAVI, BY TWO STRAINS OF ALFALFA MOSAIC VIRUS

INTRODUCTION

The symptoms of virus diseases on plants have probably been observed for centuries, but it was not until the late 19th Century that the importance of the plant viruses in the biological scheme began to be recognized. Although Adolf Mayer (27) discovered the infectious nature of tobacco mosaic virus in 1886 and Ivanowski (19) in 1892 demonstrated the filterability of the infectious principle, it was left to Beijerinck (3) to coin the term *virus*. Beijerinck in 1898 formulated a theory of *contagium vivum fluidum* to explain how an apparently sterile plant filtrate could induce a disease in healthy plants. The crystallization of tobacco mosaic virus by Stanley (38) in 1935 was an historical milestone in the investigation of the nature of virus particles but it did little to explain the effects of virus in the living biological system.

Most of the investigational work carried on by the plant virologist has, in the past, dealt primarily with the identification, host range, transmission, and relationships of plant viruses. Investigations into the effects of virus infection upon the physiology of the host plant have been limited largely to gross respiration

studies and to conventional analytical studies of, for example, carbohydrate and nitrogen relations. Two fields of the physiology of virus diseases for which there is little available information include those dealing with the details of intermediary metabolism and the specific effects of related viruses upon the host plant.

Through the cooperation of Dr. J. A. Milbrath, several related viruses capable of inducing differential responses in the cowpea plant were available, and it was thought that a study of the effects of related virus strains on the physiology of the cowpea would be of interest. Since respiration rate often provides one of the best indications of metabolic activity or changes of metabolic activity, the respiration rates of diseased and healthy plants were studied. Preliminary investigations had indicated disturbed phosphate relationships in other plant virus diseases. Since phosphate is related to energy transfer from respiration, and since the phosphate metabolism in virus diseases has only been sketchily studied, an investigation of certain aspects of the phosphate metabolism of diseased plants appeared to be a natural adjunct to respiration studies.

Inasmuch as some conflicting reports are found in the literature concerning the occurrence of a particular effect as a result of virus infection, it was thought that

the presence or absence of such effects might depend upon the time after inoculation or infection of the plant that the effect is measured. Consequently, a time-sequence study of respiration rates and nitrogen contents was performed on the inoculated leaves and plant parts other than the inoculated leaves at a time after inoculation when the virus could have become systemic.

LITERATURE REVIEW

Several aspects of the effects of viruses upon the physiology of host plants have been studied in some detail for specific virus-host plant complexes. Bawden and Pirie (2, p.180) point out that no invariable response is obtained by infections of a particular host genotype with a given virus, since the effects may be determined, to a degree, by the physiological condition of the host.

Much of the research work seeking to describe and explain the disturbed metabolism of virus-diseased plants has dealt with the investigation of carbohydrate content, nitrogen content, and respiration rate of infected plants as compared with healthy plants. In one of the early investigations of carbohydrate relations in virus-diseased plants, the tops of spinach plants infected with spinach blight were found by True and Hawkins (40, pp.381-384) to contain both starch and sucrose in markedly greater quantities than were present in healthy plants. However, the quantity of reducing sugars was lower in blighted tops. Practically no differences were found in the amounts of sucrose, starch, and reducing sugars in the roots of blighted and healthy plants.

The total carbohydrate of the above-ground parts of leaf-roll potatoes was investigated by Campbell

(7, pp.427-430) who found that the diseased plants had a higher dry weight and a greater percentage of carbohydrate than healthy plants. In contrast, Brewer, Kendrick, and Gardner (5, p.847) observed that tomato plants infected with mosaic, compared with healthy plants, were characterized by a reduction in total weight and by a reduction in carbohydrates.

Carbohydrates were found by Rosa (35, p.168) to accumulate in all parts of tomato plants infected with western yellow blight to a greater degree than in healthy plants but a greater accumulation occurred in leaves than in stems and roots.

Dunlap (13, pp.350-351) in an investigation of a number of host-virus complexes found that, in general, mosaic viruses differed from those of the yellows type in their effects upon the nitrogen and carbohydrate metabolism of the host plant. Carbohydrates were found to increase and total nitrogen to decrease in leaves in such virus diseases as yellows of peach, plum, and asters, and in leaf-curl of raspberry. In contrast, mosaic-type diseases on such plants as tobacco, tomato, pokeweed, cucumber, and raspberry were characterized by a decrease of carbohydrate and an increase of total nitrogen. The ratio carbohydrate/nitrogen in the yellows type of disease would thus be larger than for a mosaic-type disease. Bawden and Pirie

(2, p.181) divided the plant viruses into two types on the basis of carbohydrate/nitrogen ratio, the yellows diseases in which the ratio is increased, and the mosaic diseases in which the ratio is decreased. O'Reilly (30) found that Western X disease virus caused a carbohydrate accumulation but a reduced nitrogen content in the leaves of peach. A decrease in the rate of growth in the early stages of the disease after inoculation, accompanied by a rapid accumulation of carbohydrate and an increase in total nitrogen, was also found by Shapalov (36) in tomatoes infected with yellows.

A point which is not frequently brought out in the literature is that any accumulation of carbohydrate automatically results in a lowered percentage of nitrogen. On the other hand, anything that interferes with carbohydrate accumulation brings about a low relative carbohydrate content and a higher percentage of nitrogen. An understanding of these relationships is of importance, especially in evaluating the older literature where the results of analyses were usually based on dry weight.

To supplement his previous findings, Dunlap (14, p.334) studied the influence of tobacco mosaic virus infection upon the amounts of each of several carbohydrate materials in tobacco leaves. Reduced amounts of reducing sugars, disaccharides, dextrins, starch, and pentosans

were found in mosaic-diseased plants in comparison with healthy plants. Although Dunlap (14, p.336) reported starch to be more readily converted into simpler compounds in mosaic than in healthy plants, Cordingley, Pearsoll, and Wright (10, p.81) observed the opposite phenomenon to be the case.

Jodidi, Moulton, and Markley (21, p.1068) investigated the effects of mosaic disease of spinach upon nitrogenous substances of this plant. The tops of infected plants were found to have a lower percentage of total nitrogen, nitrate, acid amide, "monoamino" and "diamino" nitrogen but a larger percentage of ammonia than healthy plants. Nitrous acid, normally not a constituent of plants, was found only in the diseased plants. A similar study (20, pp.1888-1889) on a mosaic disease of cabbage gave similar results, with the diseased cabbage leaves having a smaller proportion of total nitrogen, nitrate, acid amide, "monoamino" and "diamino" nitrogen. Again, nitrite was found in diseased but not in healthy plants. These investigators concluded that the lower levels of nitrogen in diseased plants were due to the occurrence of nitrite which, in the presence of amino nitrogen, could bring about a chemical reaction resulting in the elimination of elemental nitrogen.

Potato plants infected with leaf-roll were found by Campbell (7) to have a higher percentage of total nitrogen when calculated on the basis of fresh weight, but the percentage of total nitrogen in diseased plants was found to be about the same as in healthy on a dry weight basis. The accumulation of starch in leaves of plants infected with potato leaf-roll probably accounts for this difference in results. Brewer and his colleagues (5, pp.847-848) observed no reduction in total nitrogen content of tomato plants infected with mosaic. Tomato plants infected with western yellow blight showed a decreased total nitrogen content in leaves, but an increased nitrogen content in the stems and roots (35, p.168). In view of this, Rosa (35) suggested that the inability of infected plants to translocate nitrogen or to synthesize higher nitrogen compounds, rather than nitrogen starvation, might be the effect of the virus infection.

Cordingley, Pearsoll, and Wright (10, pp.80-84) investigated the effects of mosaic disease on certain metabolic products of tobacco. The results of this investigation indicated that diseased leaves had higher nitrogen but lower carbohydrate contents. The yellow areas of infected leaves were found to show more pronounced effects than green areas of the same leaves. Higher protein nitrogen and lower amino acid nitrogen

contents in diseased leaves led these investigators to the conclusion that protein breakdown was retarded in infected leaves.

Commoner and Dietz (9, p.856) investigated the variations of non-protein nitrogen with time after inoculation of tobacco leaves with tobacco mosaic virus. Infected leaf discs cultured in water contained less non-protein nitrogen than healthy discs during the time the virus was being synthesized, but this effect disappeared when virus formation ceased in the discs. This difference in non-protein nitrogen between healthy and infected tissue was considered to be comparable in magnitude to the amount of virus synthesized.

The respiration rates of leaves from healthy plants have been shown to decrease with age (29, pp.222-223). Kidd, West, and Briggs (23, p.376) found that the respiration rate based on dry weight of entire Helianthus annuus plants decreased continuously from the time of germination of the seed to maturity of the plant. These investigators suggested that the observed decrease in respiration with age may be due to a reduction in the effective amount of respiring cell matter per unit dry weight as more and more non-living structural material is accumulated during the life of the plant.

Hover and Gustafson (18, p.39) state that as a cell increases in size and age, the amount of protoplasm present probably remains the same while the weight of dry matter increases. Therefore, the respiration rate of an old leaf calculated on the basis of dry weight would be less than that of a young leaf, whereas the respiration per unit weight of protoplasm might be the same in both cases. These workers suggest that respiration rates based upon the amount of protoplasm present would be more accurate than those based on dry weight and that the amino nitrogen or total nitrogen could be used as a basis for calculation of respiration rates.

A considerable amount of work has been directed toward the investigation of the influences of virus infection upon the respiration rate. Dunlap (13, pp.352-353) measured the respiration rates of different plant species infected with either yellows or mosaic types of virus diseases. The respiration rate, as measured by the evolution of carbon dioxide, was higher in young diseased leaves than in young healthy leaves. With older leaves, however, the respiration rate was greater in the healthy leaves. Caldwell (6, p.214), studying the effects of aucuba mosaic on tomato, found that the carbon dioxide output of diseased leaves was greater than that of healthy ones whether the

respiratory rate was based on fresh weight, dry weight, or nitrogen.

Whitehead (45, pp.71-72) measured the carbon dioxide evolved by healthy and leaf-roll-infected potato plants at successive stages in the life cycle from dormant tubers to vigorously growing plants. His results indicated that the respiration rate of the diseased plants is always higher than that of healthy ones except for a short period from the end of dormancy in the tuber to the first unfolding of the leaves.

The respiration rate, based on either fresh weight or total nitrogen, of peach foliage infected with Western X disease virus was found to be higher than that of healthy leaves and, further, respiration rates were higher with increasing symptom severity (30).

The effects of tobacco mosaic virus upon the respiration rate of the tobacco plant has received considerable attention, with various and sometimes apparently conflicting results being reported. Dufrenoy (11, pp.293-294) reported that the mature leaves of mosaic-infected tobacco plants have a higher respiration rate than comparable healthy leaves. However, young buds infected with mosaic were found to respire at a lower rate than buds from healthy plants. In a physiological and cytological study of leaf tissue infected with tobacco mosaic, Dufrenoy and

Dufrenoy (12, p.603) observed that oxygen uptake by the light-green areas of mosaic leaves was greater than that of dark-green areas of the same leaves, while tissue from dark-green areas of healthy leaves used less oxygen than either of these.

In contrast to the preceding report, Lemmon (24) maintained leaves under various regimes of light and darkness and, without exception, found that healthy tobacco leaves gave higher respiration rates than leaves infected with tobacco mosaic virus. In another study, Takahashi (39, pp.496-500) measured the oxygen uptake, at intervals of time after inoculation, of sections of healthy and mosaic-infected tobacco leaves cultured in distilled water and exposed to either light or darkness. The respiration rate of virus-infected leaf tissue tended to be slightly lower than that of the uninfected controls, whether subjected to light or to dark periods. In contrast to these findings which showed a decreased respiration rate in mosaic-infected tobacco leaves, Kempner (22, p.610) was unable to find any appreciable difference between the respiration rates of healthy and diseased leaves unless the cells of the diseased leaves had reached the stage of necrosis.

Glasstone (16, pp.273-274) investigated the respiration rates of entire healthy and mosaic-infected tobacco

plants and determined that the respiration rates of diseased and healthy plants were about the same for some time after inoculation, but that the respiration rates of the diseased plants rose suddenly over that of the healthy ones. This rise was then followed by a decrease of the respiration of diseased plants to a rate about equal to that of healthy plants. Glasstone (16, p.275) suggested that energy produced by the increased respiration rate is related to the increase of virus and its movement within the plant since the increased respiration came at the time when the virus became systemic.

Woods and DuBuy (46, pp.297-300), who estimated respiratory activity on the basis of the rate of protoplasmic streaming and the rate of oxygen uptake, reported that respiration rates of tobacco mosaic virus-infected leaves began to increase over that of healthy leaves within about 90 hours after inoculation. Multiplication of virus in inoculated tissues, however, began within 30 hours and had almost reached the maximum titer by the time diseased-tissue respiration rate increased over healthy.

The respiration rates of mosaic-infected and of healthy Burley tobacco leaves were measured and compared at intervals after inoculation by Wynd (49, p.95). Following inoculation on the first leaf, a maximum increase in the oxygen consumption of the first, second, and third

leaves from inoculated plants over that of similar leaves from healthy plants was observed on the fourth day after inoculation. At this time the oxygen uptake of the first, second, and third leaves from inoculated plants was, respectively, about 40%, 25%, and 18% greater than that of comparable healthy leaves. Subsequently, the respiration rates of diseased leaves fell below those of healthy leaves and remained so until the termination of the experiment 21 days after inoculation. Transmissible virus was not generally present in any leaf other than the inoculated leaf until about 14 days after inoculation. Wynd concluded that the metabolic changes in the diseased tissue were cellular in nature and not due to the presence of virus material, since the greatest enhancement of oxygen consumption occurred simultaneously in all leaves of inoculated plants and at a time when no transmissible virus was present in any but the inoculated leaves.

Despite the numerous observations that virus infection may influence the respiration rate, very little attention has been given to the effects of virus disease on the phosphorous metabolism of the host plant, even though the importance of phosphorous in respiration is well known. Some of the earlier investigations of phosphorous in virus-diseased plants were based upon the total phosphorous in the ash of incinerated leaves or other

plant parts. Freiberg (15, p.185) reported that there was no appreciable difference in the phosphorous content of the ash from healthy or mosaic-infected leaves of tobacco. True, Black, and Kelly (41, pp.371-372) studied the ash content in tops and roots of normal and blighted spinach plants. More total phosphorous, expressed on a dry weight basis, was present in the tops of healthy plants than in diseased. In roots, however, the opposite was found.

Significant changes in the protein phosphorous in tobacco plants infected with tobacco mosaic, but no appreciable changes in the lipid phosphorous, were reported by Rischkov (34, p.951). In infected plants the amount of phosphorous in soluble proteins increased, while the phosphorous in the insoluble proteins decreased, when the values were expressed as fractions on the basis of dry weight. More organic phosphorous was reported to be present in diseased than in healthy plants.

The influence of tobacco mosaic virus on the nitrogen and phosphorous in tobacco was investigated by Holden and Tracey (17, pp.151-156) and pronounced systemic effects were reported in infected plants. In diseased compared with healthy plants, the total phosphorous per plant decreased while the total phosphorous expressed as a percentage of dry matter increased. The total amount of nitrogen per plant was about the same in both diseased and

healthy plants, but an increase in the total nitrogen as a percentage of the dry matter was observed.

Vayonis (43, pp.692-694) carried out a time-sequence study in which he separated and determined the quantities of total phosphorous, inorganic phosphorous, and phosphate esters in the leaves of healthy and of tobacco-mosaic-infected tobacco leaves at daily intervals after inoculation. From the time of inoculation up until the fifth day afterward, there appeared to be no consistent changes in total phosphorous in diseased and healthy leaves. However, when visible symptoms became apparent on the fifth day, the total phosphorous, as a percent of dry matter, in diseased leaves tended to fall slightly below that of the controls. The inorganic phosphorous content was at first about the same in diseased and healthy leaves, but there was a tendency toward increased amounts of inorganic phosphorous with disease development. Inorganic phosphorous content of diseased leaves increased over that of healthy leaves up to the fifth day, and then fell to a lower level than was found in healthy leaves. There was a higher percentage of phosphate esters present in diseased tissues for the first two to three days after inoculation, at which time this fraction fell below the healthy controls until the fifth day, when the phosphate ester content of diseased leaves again increased.

MATERIALS AND METHODS

EXPERIMENTAL:

Several experimental approaches were used in the investigation of some physiological effects induced in host plants by virus infection. Respiration measurements based both on fresh weight and nitrogen content were performed on the first foliar leaves of seedlings at intervals of time subsequent to inoculation with virus to obtain a generalized picture of the effects of the virus in a primary infection. Further investigations included the study of the systemic effects on respiration rates and nitrogen contents of later appearing leaves and the roots of seedlings from previously inoculated plants. The changes in acid-extractable organic and inorganic phosphate of leaves after light or dark periods were also investigated. To provide a basis of comparison, healthy and diseased plant parts were studied concurrently.

The plant chosen for use in this investigation was the cowpea, Vigna sinensis (L.) Savi, variety Ramshorn, the seeds for which were obtained from Burpee Seed Company. The selection of this host plant was governed primarily by three considerations: (1) ease and speed of propagation, (2) reasonable uniformity of growth, and (3) susceptibility to infection by two or more related viruses.

The foliar development of the cowpea seedling is characterized by the appearance, soon after germination, of the first epicotyledonary leaves, called primary leaves in this investigation. The primary leaves are simple whereas the leaves formed subsequently are trifoliate.

The viruses used in the investigation, alfalfa mosaic virus 20 (AM 20) and alfalfa mosaic virus 39 (AM 39), were selected from a number of alfalfa mosaic virus strains because they show different symptom expression and patterns of infection in the cowpea. The characteristics of these virus strains on cowpea, according to Milbrath[1], are as follows:

> "AM 20 on black cowpea: Local lesions essentially chlorotic, always diffused, never marginate. When tested on nine different varieties of cowpeas, the symptoms were quite variable, consisting of pinpoint necrotic spots with chlorotic halos, necrotic rings with green centers, or necrotic arcs. This strain of alfalfa mosaic virus became systemic on all varieties of cowpea, causing a bright golden mottle and rather severe stunting of the entire plant.
>
> "AM 39 on black cowpea: Local lesions strongly marginate, usually large with purple margins and brown or purple centers, or black necrotic spots. These symptoms occurred consistently on nine different varieties of cowpea. This strain of the virus did not become systemic on any variety except that occasionally the infection moved down the leaf petiole and caused severe necrotic streaks in the stem."

---

[1]Milbrath, J. A., Department of Botany and Plant Pathology, Oregon State College. Personal communication.

The characteristics of these viruses on cowpea are illustrated in Figures 1 and 2.

METHODS:

1. Propagation of Plants

A "paper-doll" technique was employed for germination of seeds in order to obtain cowpea seedlings sufficiently straight for mounting in corks or milk-bottle caps. A loosely rolled core of paper towelling about 1 inch in diameter and 9 inches long was prepared and rolled in one end of a four-layered strip of cheesecloth approximately 9 inches wide and 36 inches long laid flat on a table. A single thickness of paper towelling was then placed on the cheesecloth, moistened with water, and cowpea seeds laid in a lengthwise row about 1½ cm. from the top of the paper with the hila toward the bottom edge. Another thickness of paper towelling was then wetted and laid on top of the seeds, after which the bundle was carefully rolled and a rubber band placed around the bundle. About 25 seeds were germinated in each roll.

The bundles so prepared were soaked in tap water, the lower end immersed in about four inches of water in a battery jar, and the jar with bundles placed in a dark cabinet at room temperature. Four or five days later, the jars were removed and placed under fluorescent lights set

FIGURE 1. From left - Healthy, AM 20- and AM 39-Inoculated Cowpea Plants, Seven Days after Inoculation on Primary Leaves.

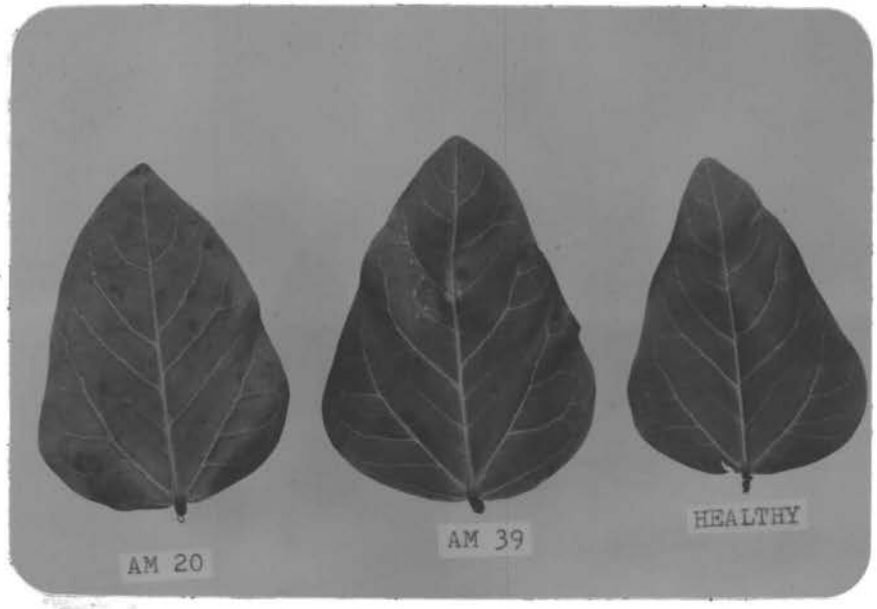


FIGURE 2. AM 20-Inoculated, AM 39-Inoculated, and Healthy Primary Leaves of Cowpea, Seven Days after Inoculation.

for alternating twelve-hour light and dark periods. The seedlings were grown under these conditions until large enough to transplant into the culture solution.

Young seedlings, selected for uniformity of size, were transferred to inorganic culture solution from the paper-dolls. The culture solution used is known as Shive's R5S2 (28, p.243) and is a three salt solution. Stock solutions were made up as follows: KH_2PO_4, 98 g. per liter; $Ca(NO_3)_2 \cdot 4H_2O$, 49 g. per liter; and $MgSO_4 \cdot 7H_2O$, 148 g. per liter. Trace elements were made up in a solution containing 0.6 g. H_3BO_4, 0.4 g. $MnSO_4$, 0.05 g. $ZnSO_4$, 0.05 g. $CuSO_4$, 0.024 g. $(NH_4)Mo_7O_{24} \cdot 4H_2O$, and 0.025 g. $CoCl_2$ in one liter of water. For use, 25 ml. of each of the three major salt solutions and 1 ml. of the trace element solution were mixed with tap water and diluted to 4 liters. This makes a solution one-quarter strength of Shive's R5S2. Iron was added as a mixture of equal parts of sodium ferric ethylenediaminetetraacetate and ethylenediaminetetraacetic acid, about 0.1 g. of the mixture per 4 liters of culture solution.

In Experiment 1, glass tumblers were used for growing plants, but in later experiments half-pint milk bottles were employed. When plants were grown in tumblers, the seedlings were mounted one plant per vessel in waxed corks by wrapping the hypocotyl of the plant with glass wool,

then inserting the plant root first through a hole in the cork, and pulling down until the plant was held in place by the pressure of the glass wool on the sides of the hole. Some difficulty was experienced in using the corks, especially in putting lateral roots through the hole without breakage. This difficulty was overcome by using half-pint milk bottles as the culture vessel and mounting the plants in cardboard bottle caps.

Each bottle cap was prepared by cutting two slits, in the shape of a cross, into the cap, one long slit from the edge to just beyond the center of the cap, and another slit at right angles to the first and intersecting it at the approximate center of the cap. A rod was then pushed upward through the point of intersection so that the four free corners projected upward, leaving about a 5 mm. hole in the center. The cap was then dipped in a mixture of melted paraffin and beeswax which served to reduce fungus growth and to stiffen the corners. Seedlings were mounted in the caps simply by wrapping the hypocotyl with glass wool, spreading the cap open at the slit and slipping it over the glass wool, then closing the slit. Seedlings so mounted were held in place when the caps were pressed into the mouth of the milk bottles.

After transplanting to nutrient solution, the plants were placed under artificial lights. The source of

artificial light was a bank of eight 40-watt, soft white fluorescent tubes mounted 22 inches above the bench, and giving an average light intensity of about 500 foot-candles at the benchtop. The growth area under the light bank was approximately 26 inches by 50 inches. An electrical Inter-Matic switch, set to give a daily 6:00 a.m. to 6:00 p.m. photoperiod, was used to control the light and dark periods.

2. Inoculation Technique

Diseased plants were obtained by inoculating primary leaves of seedlings with the appropriate virus strain by the carborundum-rub technique of Rawlins and Tompkins (32). All inoculations were made at the stage in the growth of the plants when the primary leaves were well expanded and the first trifoliate leaves were just beginning to appear. This was between three and five days after the plants were placed in the nutrient culture.

In Experiment 1, where AM 20 was used alone, the inoculum source was systemically infected cowpea foliage. In all other experiments, leaves from systemically infected Kentucky 56 tobacco plants were the sources of inocula. Inoculum was prepared by grinding tissue from young infected leaves in a mortar with a small quantity of distilled water. Leaves to be inoculated were dusted lightly

on the upper surface with carborundum powder and rubbed with a forefinger previously dipped in the inoculum. The leaves were rinsed with distilled water after rubbing. In some experiments, both primary leaves of the plants were inoculated; in other experiments, only one primary leaf was inoculated. In the latter case, the inoculated leaf was marked for identification by punching out a small section along the margin.

Leaves used to provide healthy material for study were rubbed similarly with carborundum, but distilled water was used rather than ground infected tissue.

3. Respiration Studies

In Experiments 1 and 2, both primary leaves of each seedling in the diseased groups were inoculated. Diseased leaf tissue samples for respiration studies were obtained by removing three 12 mm. discs with a punch from one-half of each primary leaf on three inoculated plants; a duplicate diseased sample was then obtained by punching discs from the other half of the same leaves. Duplicate samples of healthy tissue similarly were taken from three uninoculated plants.

In Experiment 3, only one primary leaf of a seedling was inoculated, and the opposite leaf was left uninoculated to provide healthy material for comparison. In this

experiment, a sample of diseased primary leaf tissue was obtained by punching three discs from one-half of each inoculated leaf on six plants. A duplicate sample was then taken by punching discs from the opposite halves of the same leaves. Duplicate samples of healthy tissue similarly were collected by punching the uninoculated leaves on the same six plants. When this sampling technique was employed with primary leaves, duplicate samples of both diseased and healthy tissues came from the same plants.

Three groups of plants were used in Experiment 4 to compare the respiration rates of trifoliate leaves from healthy plants with those of similar leaves from plants inoculated with either of the two virus strains. One group was inoculated on one primary leaf with AM 20, another group was similarly inoculated with AM 39, and the third group was left uninoculated. The plants were then grown under artificial illumination until three trifoliate leaves had been developed. Samples of leaflets were selected from the first, second, and third trifoliate leaves of each group. A given sample was composed of one lateral leaflet from the appropriate trifoliate leaf from each of six plants. Leaflets in each sample were matched as closely as possible for similarity of size within the sample but the size varied somewhat between leaflet samples of the same age.

Duplicate root samples for respiration and nitrogen studies were collected from healthy plants and from plants previously inoculated with AM 20 or AM 39. The terminal 8 to 9 cm. portions of the main roots, plus the attached lateral roots, from six plants were included in each sample. The excess solution was blotted from the roots with paper towels prior to weighing.

All respiration determinations were made by the "direct method" of Warburg (42, pp.12-15) in a Warburg respirometer at a temperature of 30° C. Samples for respiration studies were selected, weighed, and immediately placed in the main compartment of the Warburg flasks. In Experiments 1 and 2, 2 ml. of distilled water were delivered to the main compartment of the vessels, but in other experiments, no water was used. Three ml. of Shive's nutrient solution were added to the main compartment of the flasks when respiration of roots was measured. The edges of the center well were greased, then 0.2 ml. of 5% KOH was placed in the center well of the vessel and a three-fourths-inch square of filter paper was pleated and inserted into the well to increase the absorbing area. The vessels were attached to the manometers and these, in turn, were mounted on the shaking mechanism with the vessels immersed in the water bath to which a dye had been added to prevent photosynthesis by the tissue. An equilibration

period of from 20 to 60 minutes was allowed before the manometer stopcocks were closed and readings started. Readings were taken at intervals over a two-hour or three-hour period, the longer reading time being used with the shorter equilibration periods.

The oxygen uptake for each reading interval was calculated according to the method of Umbreit, Burris, and Stauffer (42, pp.7-8), the results plotted, and the respiration rate, determined from the slope of the line, expressed as microliters oxygen uptake per gram fresh weight of tissue per unit time, or as microliters oxygen uptake per milligram nitrogen per unit time.

4. Recovery of Virus from Uninoculated Primary Leaves and from Roots

The possibility exists that when one primary leaf of a cowpea plant is inoculated, the virus could be translocated into the opposite uninoculated leaf. In Experiment 3, in which the uninoculated leaves provided the healthy material, tests were made to determine whether transmissible virus was present in the uninoculated leaves by the time the respiration studies were concluded.

Soon after the removal of the disc samples on the fifth and seventh days, the remaining parts of the uninoculated leaves from AM 20- and AM 39-inoculated plants

were ground separately in mortars and a little distilled water added. Four young cowpea seedlings were used as test plants for each virus with one primary leaf on each plant being sprinkled with carborundum, then rubbed with the juice extracted from the ground leaves. The opposite primary leaf on two of the test plants in each group was inoculated with juice from tobacco infected with the appropriate virus to insure that the test plants were susceptible to the virus. These test plants were later examined for local lesions.

A similar recovery test was performed in Experiment 5. In this case, the terminal 8 to 9 cm. portions of main roots from three plants of each of the healthy, AM 20-, and AM 39-inoculated groups supplied the separate inocula.

5. Nitrogen Determinations

The microkjeldahl method of the Association of Official Agricultural Chemists (25, pp.745-747) was used, with slight modification, to determine total nitrogen. After determining the rates of respiration, the tissue samples were transferred to 10 ml. digestion flasks and dried in an oven at 110° C. Root samples were rinsed twice in distilled water prior to drying. Two ml. of concentrated sulfuric acid were added to each sample and the flasks again placed in the oven for from 24 to 48 hours,

after which approximately 1.30 g. of potassium sulfate and 55 mg. of anhydrous $HgSO_4$ were added. The samples were digested over a Bunsen flame until clear, strongly heated an additional 45 minutes, and then transferred to a micro-kjeldahl distillation apparatus. The ammonia was released by addition of 8 ml. of sodium hydroxide-sodium thiosulfate solution and steam distilled into 5 ml. of a 4% solution of boric acid. The ammonia was then titrated with HCl using methyl red-methylene blue indicator. A blank of the reagents was carried through with each set of samples. Total nitrogen was expressed as milligrams nitrogen per gram fresh weight of tissue.

6. Phosphate Determinations

Leaf samples for phosphate analyses were collected at the appropriate times, weighed, placed in glass vials, and stored frozen at about -25° C. until the analyses could be performed. Primary leaf samples were obtained from plants inoculated on one primary leaf and diseased tissue samples from these leaves were taken on the day following the first appearance of local lesions. The samples of healthy tissue were taken from the opposite primary leaf which had not been inoculated. Duplicate samples were obtained by cutting diseased or healthy leaves of six plants

longitudinally along both sides of the midrib, and placing opposite halves from each leaf into two separate samples.

Duplicate samples of trifoliate leaves for phosphate studies were obtained from the opposite lateral leaflets of the first trifoliate leaves from six diseased or six healthy plants.

Samples to be used for phosphate determinations after light periods were collected between 5:30 p.m. and 6:00 p.m. Samples for phosphate determinations on leaves following a dark period were collected the following morning between 6:00 a.m. and 7:00 a.m. with the laboratory darkened except for sufficient light to prepare the samples.

Extracts for determination of acid soluble phosphate were prepared from leaf samples by a technique devised by Dr. R. O. Belkengren, Department of Botany and Plant Pathology, Oregon State College. This involved grinding the leaf samples in a mortar with 5 ml. of ice-cold 0.75 M trichloroacetic acid (TCA). After grinding, an additional quantity of cold TCA solution was added to bring the phosphate concentration of the sample to within the range of a standard curve. The sample then was transferred to a 42 ml. centrifuge tube, the tube placed in an ice bath, and the contents stirred mechanically for 15 minutes. The sample was centrifuged for two to three minutes at 3,000

r.p.m. to settle the plant debris, and the supernatant filtered under vacuum, through approximately 1 mm. of specially prepared filter-aid in a Pyrex sintered funnel, into an eight-inch test tube inserted in a one-liter filter flask containing cracked ice and water. The samples were kept below 5° C. throughout the extraction procedure.

The method of Pons, Stansbury, and Hoffpauir (31, p.495) was used for all phosphate analyses. Inorganic and total acid-extractable phosphate were determined on the TCA extract, and the acid-extractable organic phosphate calculated as the difference between these two values. Analysis for the acid-extractable inorganic phosphate was made immediately after preparation of the TCA extract by the following procedure (31, p.495):

1. An aliquot of TCA extract is delivered into a separatory funnel marked at a level of 20 ml.
2. Five ml. of ammonium molybdate solution (50 g. in 400 ml. 10 N H_2SO_4, made to 1 liter with distilled water) are added, and the solution is made up to the 20 ml. mark with distilled water.
3. Ten ml. of isobutyl alcohol are delivered into the funnel which then is stoppered and vigorously shaken for two minutes. The two layers are allowed to separate and the lower (aqueous) phase is drawn off and discarded.

4. Ten ml. of approximately 1 N H_2SO_4 are added to the alcohol layer in the funnel and the system shaken for one-half minute. The liquid layers again are allowed to separate and again the aqueous layer is discarded.
5. Fifteen ml. of stannous chloride solution, freshly prepared by diluting 1 ml. of a stock solution of stannous chloride (10 g. in 25 ml. concentrated hydrochloric acid) to 200 ml. with 1 N sulfuric acid, is added to the isobutyl alcohol in the funnel, and the system shaken for one minute. The aqueous phase is discarded after separation of the layers.
6. The isobutyl alcohol layer is washed into a 50 ml. volumetric flask and made to volume with 95% ethanol.
7. The sample is allowed to stand for 40 minutes after which the spectral absorption at 730 mμ is determined with a Beckman Model DU Quartz Spectrophotometer.

A standard curve was determined from three standards and a reagent blank with each group of four samples. The amount of inorganic phosphate in the aliquot of TCA extract was determined by reference to this curve. The quantity of acid-extractable inorganic phosphate in the

original sample was calculated and the results expressed as micrograms of inorganic phosphate per gram fresh weight of tissue.

Total acid-extractable phosphate was determined by delivering an aliquot of TCA extract to a 10 ml. digestion flask and evaporating to dryness in an oven. One ml. of concentrated H_2SO_4 was then added and the flask replaced in the oven for 12 to 24 hours. Two or three drops of 30% H_2O_2 were added and the digest was boiled over a low flame until colorless, after which the sample was transferred to a volumetric flask and made up almost to volume. The sample was allowed to stand at room temperature for about two days or else was kept in the oven overnight to allow for hydrolysis of pyrophosphate. For analysis, the solutions of samples and standards were made to volume, aliquots transferred to the separatory funnels, and the determination carried out as previously described. In this case the standard curve was determined from standards made up in an appropriate dilution of sulfuric acid rather than in TCA.

EXPERIMENTAL RESULTS

1. Investigation of Rates of Respiration and Nitrogen Contents

The rate of respiration and nitrogen content are considered together inasmuch as respiration rates in primary leaves and in roots are expressed in terms of total nitrogen as well as in terms of fresh weight. The respiration rates and total nitrogen contents were determined for healthy and inoculated primary leaves at daily intervals after inoculation. These same factors were also investigated for trifoliate leaves of different ages and for the main roots from healthy and inoculated plants.

a. Variation of Respiration Rates and Nitrogen Contents in Primary Leaves after Inoculation

The respiration rate and the nitrogen content of healthy and diseased primary leaves were studied in three different experiments. The effects of AM 20 and of AM 39 infections compared with healthy plants were investigated in separate experiments. Finally, the effects of AM 20 and of AM 39 on respiration and nitrogen content of primary leaves of plants grown under similar conditions were studied in a single experiment.

In Experiment 1, respiration measurements were started one day following inoculation with AM 20 and were continued

at daily intervals for a period of five days. Local lesions were present on the inoculated leaves the second day after inoculation as pinpoint lesions visible when the leaves were viewed in transmitted light.

The average respiration rates of healthy and diseased leaves based on fresh weight are given in Table 1 and compared graphically in Figure 3, and based on nitrogen content in Table 2 and Figure 4.

The graphs, Figure 3 and Figure 4, show that the respiration rate of diseased leaves was higher than that of healthy leaves by the first day after inoculation. The greatest differences of respiration rates between diseased and healthy leaves were observed on the third day after inoculation, one day after the first appearance of local lesions. At this time, the diseased leaf respiration rate was 45% higher on a fresh weight basis and 20% higher on the basis of nitrogen content than the respiration rate of comparable healthy leaves. Respiration rates of healthy and diseased leaves approached each other on the final two days of the experiment.

The diseased leaves, in most cases, show a slightly greater nitrogen content. This elevation in nitrogen content is not, however, as great as the elevation in rate of respiration of diseased leaves since respiration

TABLE 1

Respiration Rates Based on Fresh Weight of Healthy and AM 20-Infected Primary Leaves of Cowpea on Successive Days after Inoculation.

Days after inoculation	Microliters oxygen uptake per gram fresh weight per hour	
	Healthy	Diseased
1	215	246
2	202	253
3	185	269
4	273	288
5	204	214

FIGURE 3

Respiration Rates of AM 20-Infected Primary Leaves on Fresh Weight Basis Expressed as Percent of Healthy Respiration Rates on Successive Days after Inoculation.

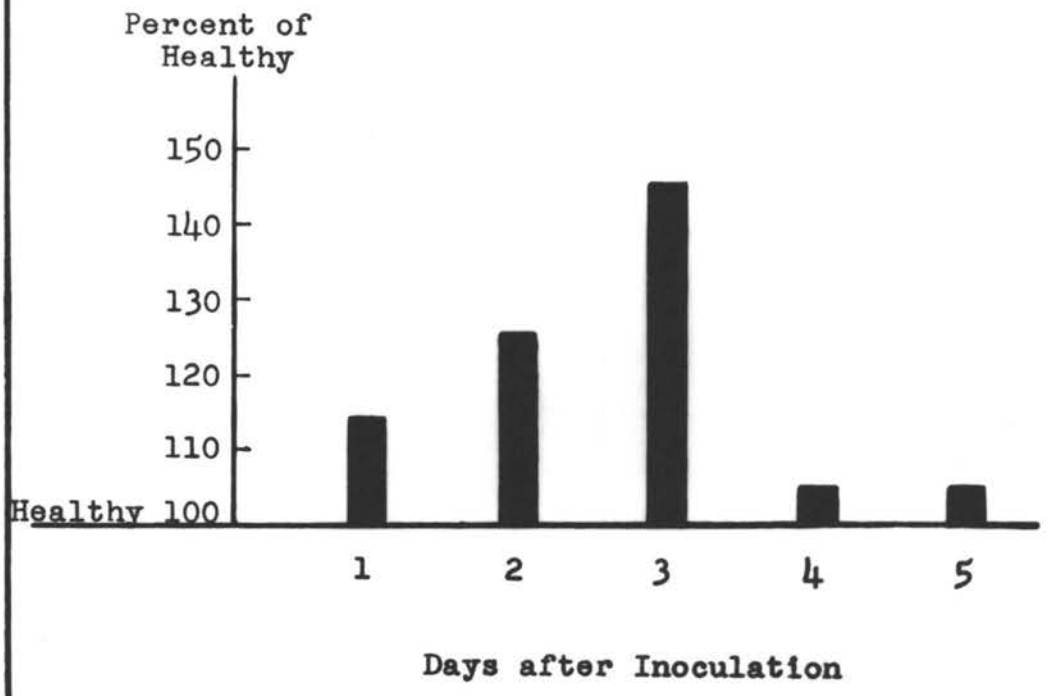


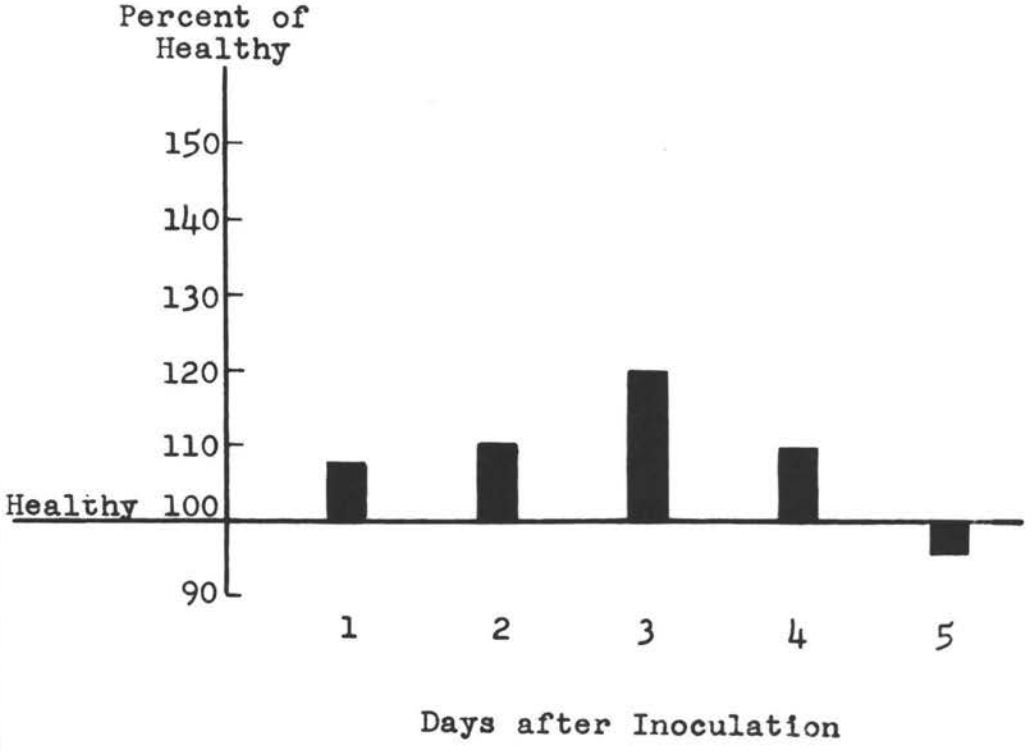
TABLE 2

Nitrogen Contents and Respiration Rates Based on Nitrogen Content of Healthy and AM 20-Infected Primary Leaves of Cowpea on Successive Days after Inoculation.

Days after inoculation	Milligrams nitrogen per gram fresh weight		Microliters oxygen uptake per milligram nitrogen per hour		
	Healthy	Diseased	Healthy	Diseased	Percent difference
1	4.25	4.51	51	55	7.9
2	4.10	4.66	49	54	10.2
3	3.40	4.10	55	66	20.0
4	3.76	3.59	73	80	9.6
5	2.94	3.27	69	66	- 4.4

FIGURE 4

Respiration Rates of AM 20-Infected Primary Leaves Expressed as Percent of Healthy Respiration Rates per Milligram Nitrogen on Successive Days after Inoculation.



differences of up to 20% were found when the respiration rate was based on nitrogen content.

Experiment 2 was conducted in the same manner as Experiment 1 except that AM 39 was used as the inoculum instead of AM 20. Measurements of respiration rates were made at daily intervals for a period of five days after inoculation. The first small local lesions were visible by transmitted light on the third day after inoculation and later developed into the characteristic reddish-brown lesions.

The respiration data on a fresh weight basis for healthy and AM 39-infected primary leaves are given in Table 3 and respiration rates of diseased leaves as percent of healthy rates are illustrated in Figure 5. The nitrogen content and the rate of respiration based on nitrogen content for healthy and diseased leaves are presented in Table 4. Figure 6 illustrates the rates of respiration based on nitrogen content of diseased leaves as percent of that of healthy leaves.

The respiration rates of primary leaves infected with AM 39 were almost the same as those of healthy leaves for the first three days following inoculation. On the fourth day, one day after local lesions first appeared, the greatest difference between healthy and diseased leaves was observed. This difference amounted to about 32% based on

TABLE 3

Respiration Rates Based on Fresh Weight of Healthy and AM 39-Infected Primary Leaves of Cowpea on Successive Days after Inoculation.

Days after inoculation	Microliters oxygen uptake per gram fresh weight per hour	
	Healthy	Diseased
1	236	248
2	250	246
3	241	253
4	272	358
5	341	386

FIGURE 5

Respiration Rates on Fresh Weight Basis
of AM 39-Infected Primary Leaves
Expressed as Percent of Healthy Respiration Rates
on Successive Days after Inoculation.

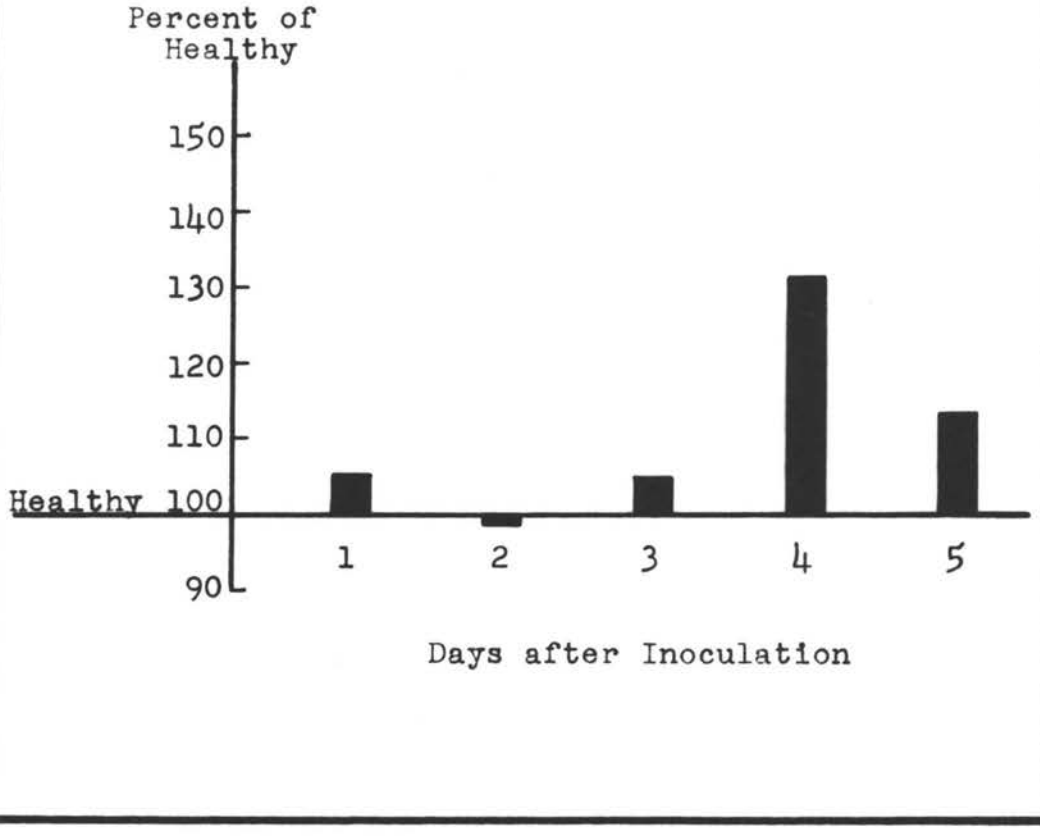


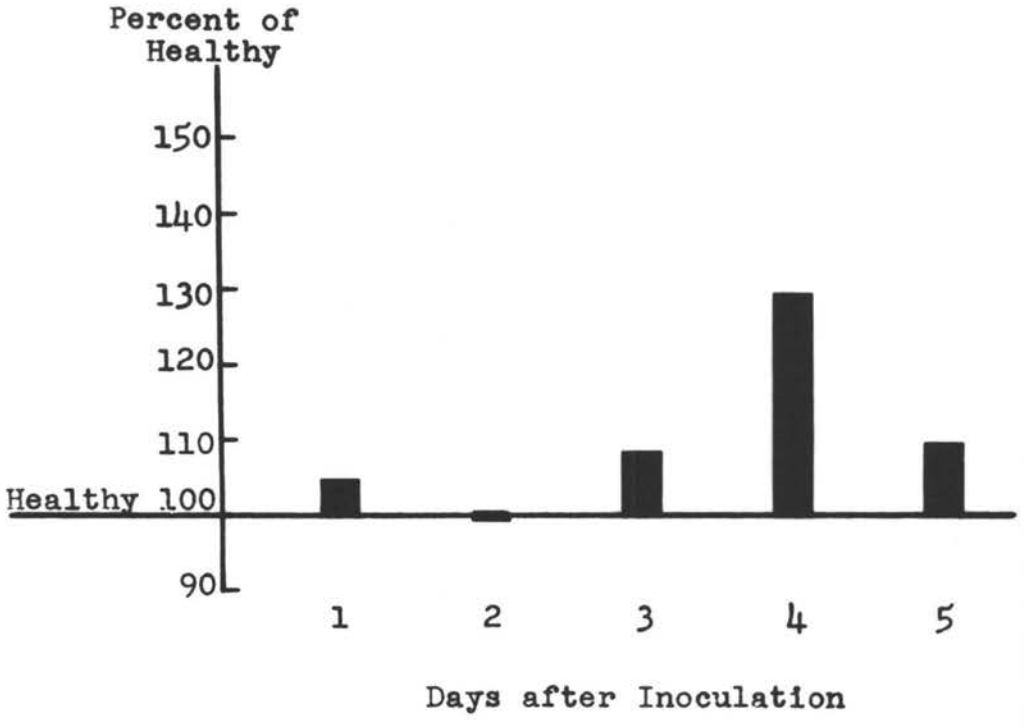
TABLE 4

Nitrogen Contents and Respiration Rates Based on Nitrogen Content of Healthy and AM 39-Infected Primary Leaves of Cowpea on Successive Days after Inoculation.

Days after inoculation	Milligrams nitrogen per gram fresh weight		Microliters oxygen uptake per milligram nitrogen per hour		
	Healthy	Diseased	Healthy	Diseased	Percent difference
1	5.56	5.53	43	45	4.8
2	5.71	5.65	44	44	0.0
3	5.02	4.85	48	52	8.3
4	4.73	4.79	58	75	29.3
5	4.08	4.18	84	92	9.5

FIGURE 6

Respiration Rates of AM 39-Infected Primary Leaves Expressed as Percent of Healthy Respiration Rates per Milligram Nitrogen on Successive Days after Inoculation.



fresh weight and to about 30% when respiration rate was calculated on the basis of nitrogen content. A smaller difference between healthy and diseased respiration rates was found on the fifth and final day of the experiment.

Only slight differences in nitrogen content were found between healthy and diseased leaves. In contrast to the AM 20-infected plants in Experiment 1, there appeared to be no tendency for the diseased leaves to have a higher nitrogen content than healthy leaves.

In Experiment 3, the respiration rates of healthy, AM 20-, and AM 39-inoculated primary leaves from plants grown at the same time were measured at intervals over a seven-day period. Leaves inoculated with AM 20 first showed local lesions on the third day whereas local lesions appeared on AM 39-inoculated leaves on the second day after inoculation. The average respiration rates found for healthy and diseased leaves on a fresh weight basis are summarized in Table 5 and the diseased leaf respiration rates as a percent of healthy tissue respiration rates are graphically illustrated in Figure 7. The results of nitrogen analyses of healthy and diseased leaves used in Experiment 3 are presented in Table 6. Table 7 gives the oxygen uptake per milligram nitrogen content of the leaves, and the diseased leaf respiration rates relative to those of comparable healthy leaves are illustrated in Figure 8.

TABLE 5

Respiration Rates Based on Fresh Weight of Healthy, AM 20-, and AM 39-Infected Primary Leaves of Cowpea on Different Days after Inoculation.

Days after inoculation	Microliters oxygen uptake per gram fresh weight per hour			
	AM 20		AM 39	
	Healthy	Diseased	Healthy	Diseased
1	290	305	295	304
2	281	323	306	323
3	310	370	331	395
4	368	468	376	423
5	302	321	329	353
7	305	305	293	311

FIGURE 7

Respiration Rates Based on Fresh Weight of Healthy, AM 20-, and AM 39-Infected Primary Leaves of Cowpea on Successive Days after Inoculation.

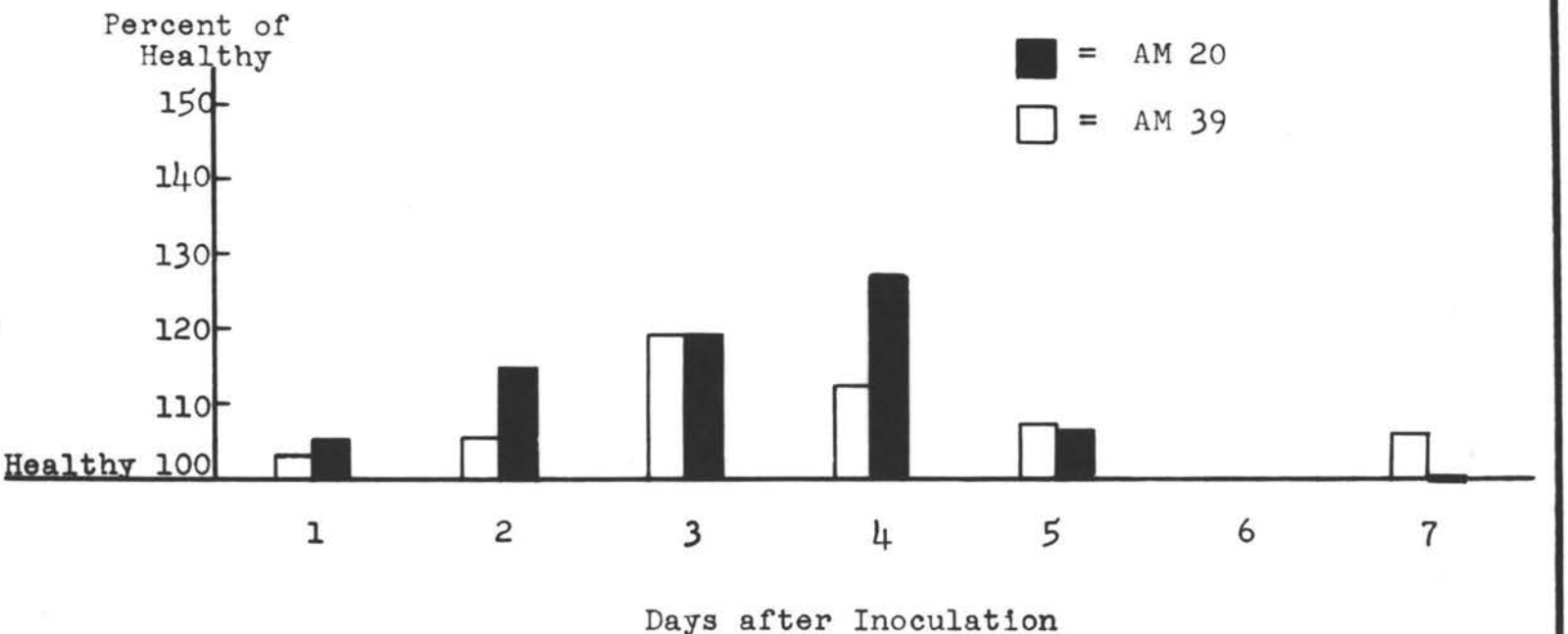


TABLE 6

Nitrogen Contents of Healthy, AM 20-, and AM 39-Infected Primary Leaves of Cowpea on Different Days after Inoculation.

Days after inoculation	Milligrams nitrogen per gram fresh weight			
	AM 20		AM 39	
	Healthy	Diseased	Healthy	Diseased
1	5.60	5.46	5.73	5.74
2	5.80	5.89	5.94	5.85
3	5.64	5.48	5.30	5.42
4	4.64	4.59	4.21	4.32
5	3.24	3.51	3.82	3.79
7	3.43	3.34	3.38	3.20

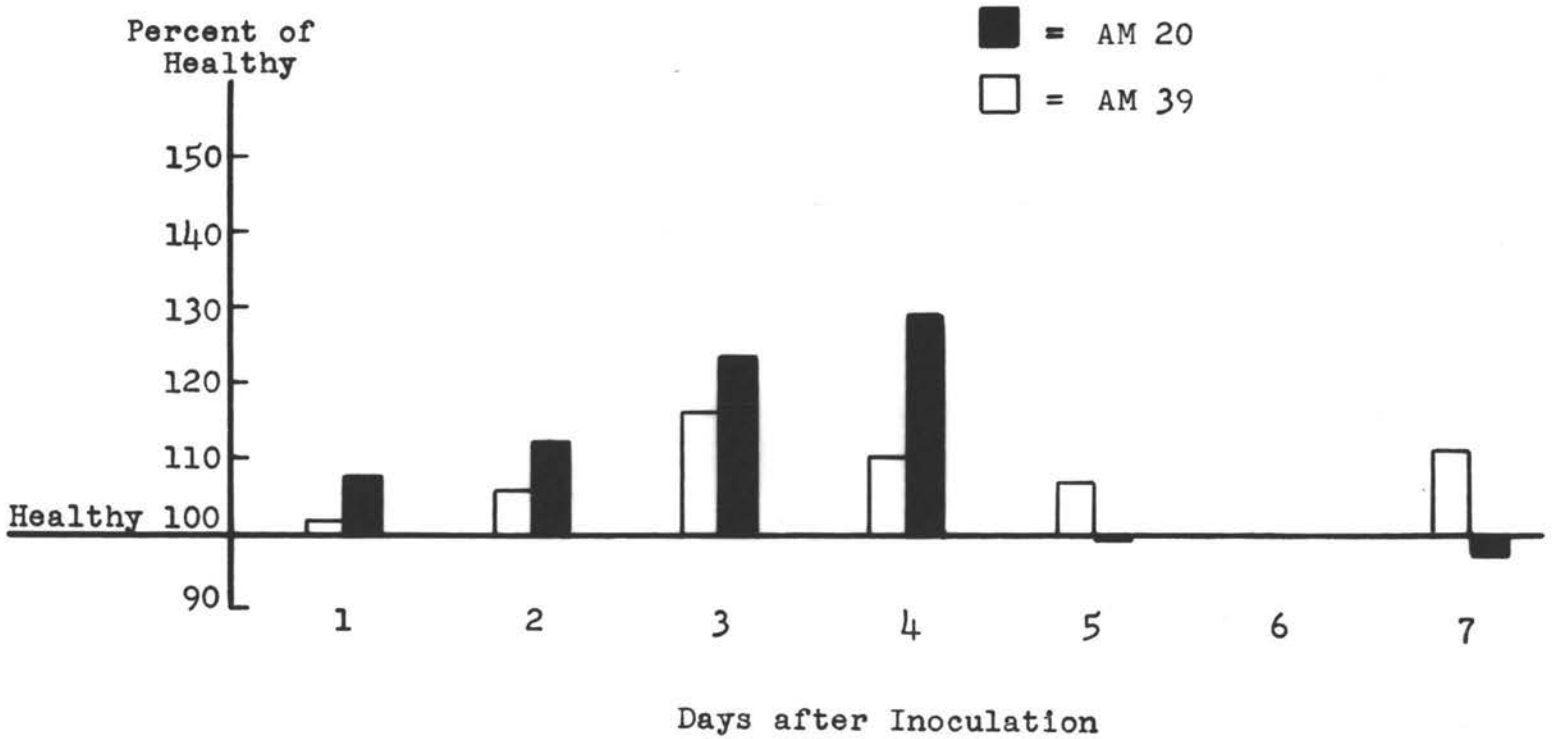
TABLE 7

Respiration Rates Based on Nitrogen Content of Healthy, AM 20-, and AM 39-Infected Primary Leaves of Cowpea on Different Days after Inoculation.

Days after inoculation	AM 20			AM 39		
	µl. O_2/mg. N/hr.		Percent difference	µl. O_2/mg. N/hr.		Percent difference
	Healthy	Diseased		Healthy	Diseased	
1	52	56	7.7	52	53	1.9
2	49	55	12.2	52	55	5.8
3	55	68	23.6	63	73	15.9
4	79	102	29.2	89	98	10.1
5	93	92	- 0.7	86	93	6.8
7	92	89	- 3.2	87	97	11.5

FIGURE 8

Respiration Rates Based on Nitrogen Content of AM 20- and AM 39-Infected Primary Leaves Expressed as Percent of Healthy Leaf Respiration Rates on Successive Days after Inoculation.



Attempts to recover virus from the uninoculated leaves by transmission to other plants gave negative results at both five days and seven days, except that one plant of the four inoculated from the seven-day, AM 20 group showed some small lesions. It was questionable whether these were local lesions or due to inoculation injury. The test plants were capable of being infected since leaves rubbed with inocula taken from tobacco produced local lesions.

The results of Experiment 3 show that primary leaves inoculated with either virus strain had respiration rates above those of comparable healthy leaves, whether the respiration is expressed in terms of fresh weight or nitrogen content. As in the two preceding experiments, the greatest respiration differences between the healthy and diseased leaves occurred on the day following the appearance of local lesions. Subsequently, the respiration rates of AM 20-infected leaves and healthy leaves were almost the same whereas respiration rates of the AM 39 leaves remained slightly above those of the healthy leaves for the duration of the experiment. In plants inoculated with either virus, the respiration rates based on fresh weight gradually increased up to the fourth day of the experiment and then declined. Nitrogen-based respiration rates showed similar trends.

Nitrogen content in leaves infected with either virus declined gradually over the seven days of the experiment, but no marked trends toward consistent nitrogen differences between diseased and healthy leaves is apparent.

b. Respiration Rates and Nitrogen Contents of Trifoliate Leaves

The respiration rates and nitrogen contents of trifoliate leaves of different sizes and ages were measured in Experiment 4. The leaflet samples were collected ten days after inoculation of the experimental plants with the two viruses, at which time the third trifoliate leaves had barely unfolded and the trifoliate leaves on AM 20-infected plants were showing chlorotic symptoms. The third (youngest) trifoliate leaves on some AM 20-infected plants were becoming withered and necrotic; however, plants showing obvious necrosis were avoided in sampling since necrotic tissue would give no respiration and introduce errors into weights and nitrogen analyses.

The results of the respiration measurements on a fresh weight basis are given in Table 8. Because the leaflet sizes in the different samples varied the respiration data are plotted in Figure 9 as average leaflet weight versus microliters of oxygen taken up per gram fresh weight per hour, to provide a comparison of the

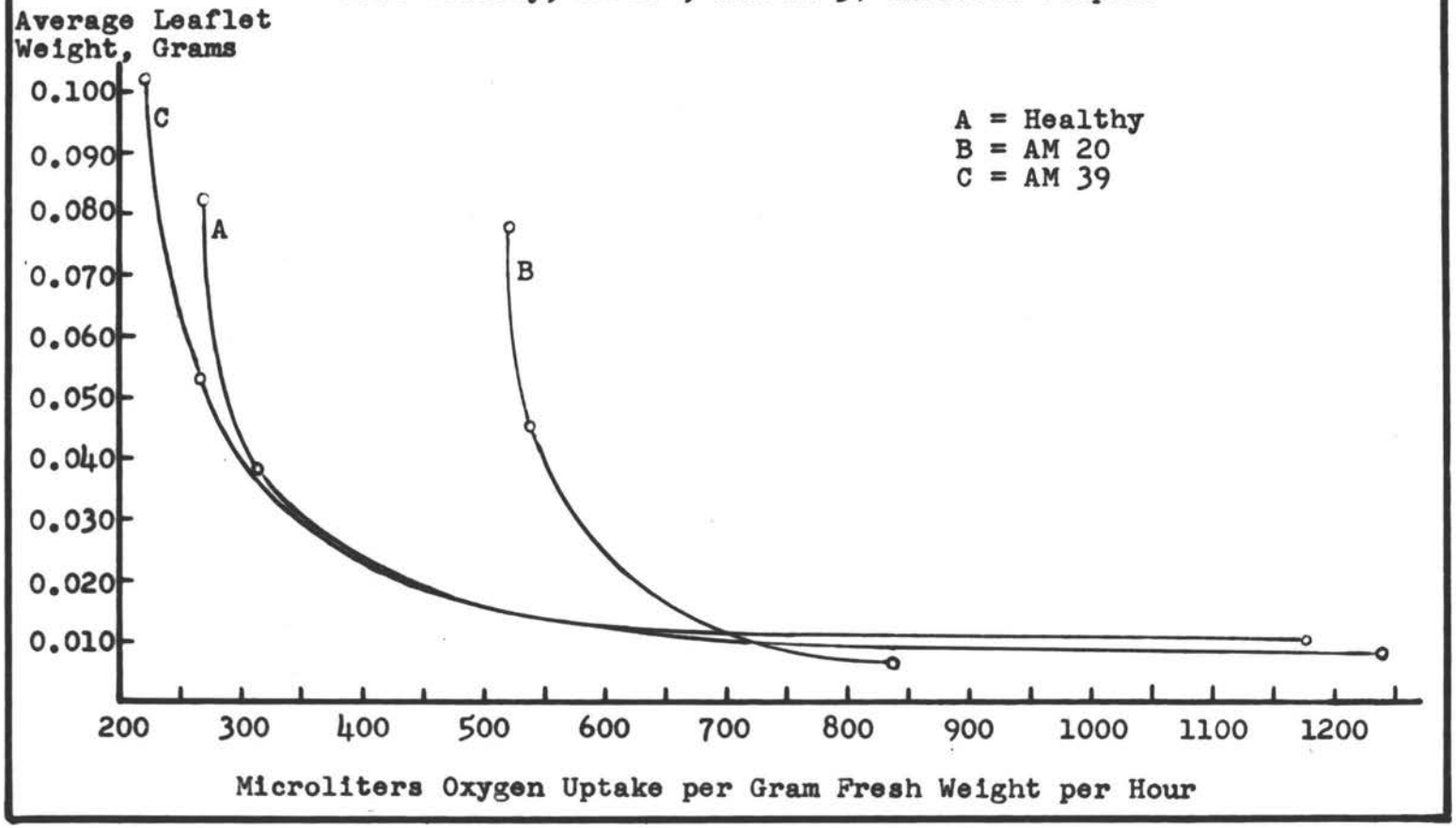
TABLE 8

Respiration Rates Based on Fresh Weight
of First, Second, and Third Trifoliate Leaves
from Healthy, AM 20-, and AM 39-Infected Cowpeas.

Leaf class	Healthy		AM 20		AM 39	
	Average leaflet weight	µl. O_2/g. fr. wt./hr.	Average leaflet weight	µl. O_2/g. fr. wt./hr.	Average leaflet weight	µl. O_2/g. fr. wt./hr.
First	0.082 g.	269	0.077 g.	521	0.102 g.	221
Second	0.038	312	0.045	536	0.053	266
Third	0.008	1,240	0.006	837	0.010	1,175

FIGURE 9

Respiration Rates Based on Fresh Weight
of First, Second, and Third Trifoliate Leaves
from Healthy, AM 20-, and AM 39-Infected Cowpeas



changes of respiration with size in the trifoliate leaves of diseased and healthy plants. The nitrogen contents and respiration rates based on nitrogen contents of these leaves are given in Table 9 and compared in Figures 10 and 11.

Figure 9 shows that the respiration rate in trifoliate leaves declines rapidly with increasing size. After a certain size is attained, the decline is slower. The respiration rate of leaflets from plants inoculated with AM 39 was not appreciably different from that of trifoliate leaves of similar size from healthy plants. However, leaflets from first and second trifoliate leaves from plants inoculated with AM 20 showed respiration rates up to about 100% greater than healthy leaves of similar size. The respiration rate of the smallest and youngest leaflets from AM 20 plants was below that of healthy leaflets.

The nitrogen content also was lower with increasing leaf size. Further, the nitrogen contents in leaflets of similar size from the three different classes of plants, healthy, AM 20, and AM 39, were quite close except for the smallest leaflets from AM 20 plants in which less nitrogen per gram fresh weight was found. See Figure 10.

TABLE 9

Nitrogen Contents and Respiration Rates Based on Nitrogen Content of First, Second, and Third Trifoliate Leaves from Healthy, AM 20-, and AM 39-Infected Cowpeas.

Leaf class	Healthy		AM 20		AM 39	
	Mg. N/g. fr. wt.	µl. O_2/ mg. N/hr.	Mg. N/g. fr. wt.	µl. O_2/ mg. N/hr.	Mg. N/g. fr. wt.	µl. O_2/ mg. N/hr.
First	7.24	37	6.64	78	6.52	34
Second	7.64	41	6.90	78	7.03	37
Third	13.99	89	9.73	86	13.33	88

FIGURE 10

Variation of Nitrogen Content with Leaf Size in Trifoliate Leaves from Healthy, AM 20-, and AM 39-Infected Cowpeas.

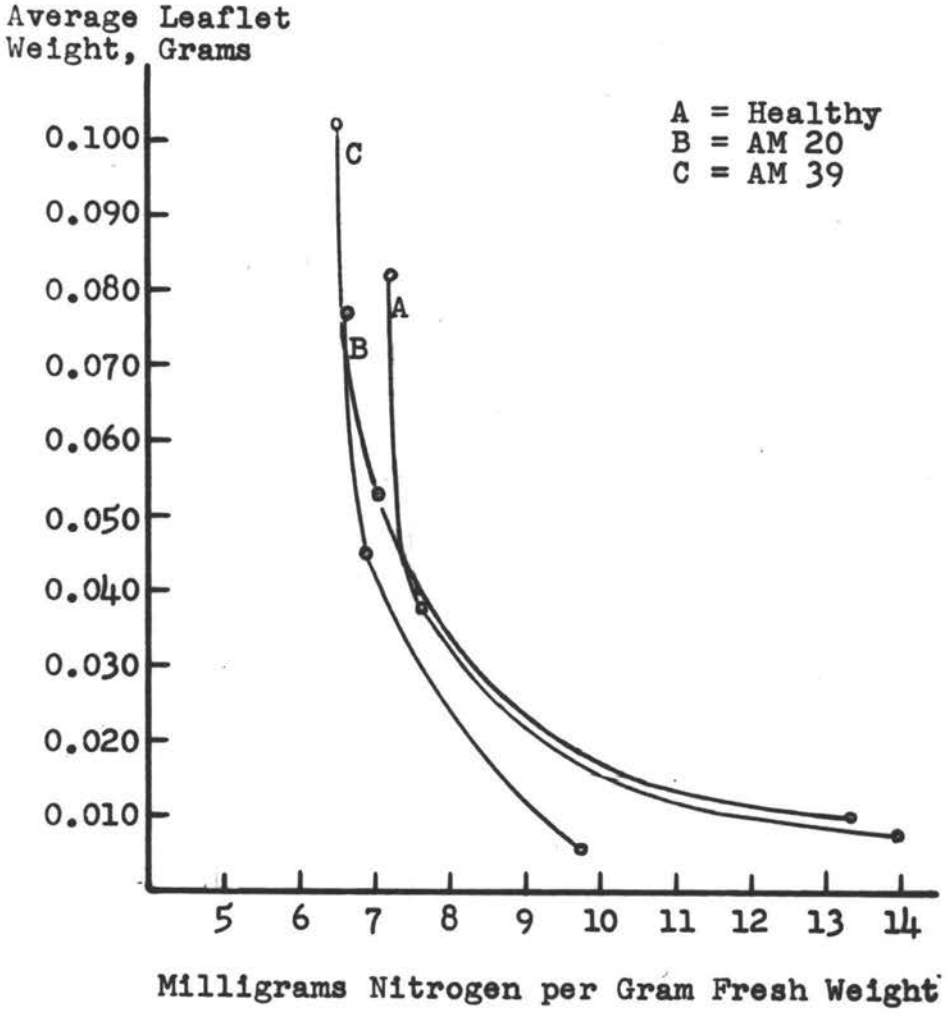
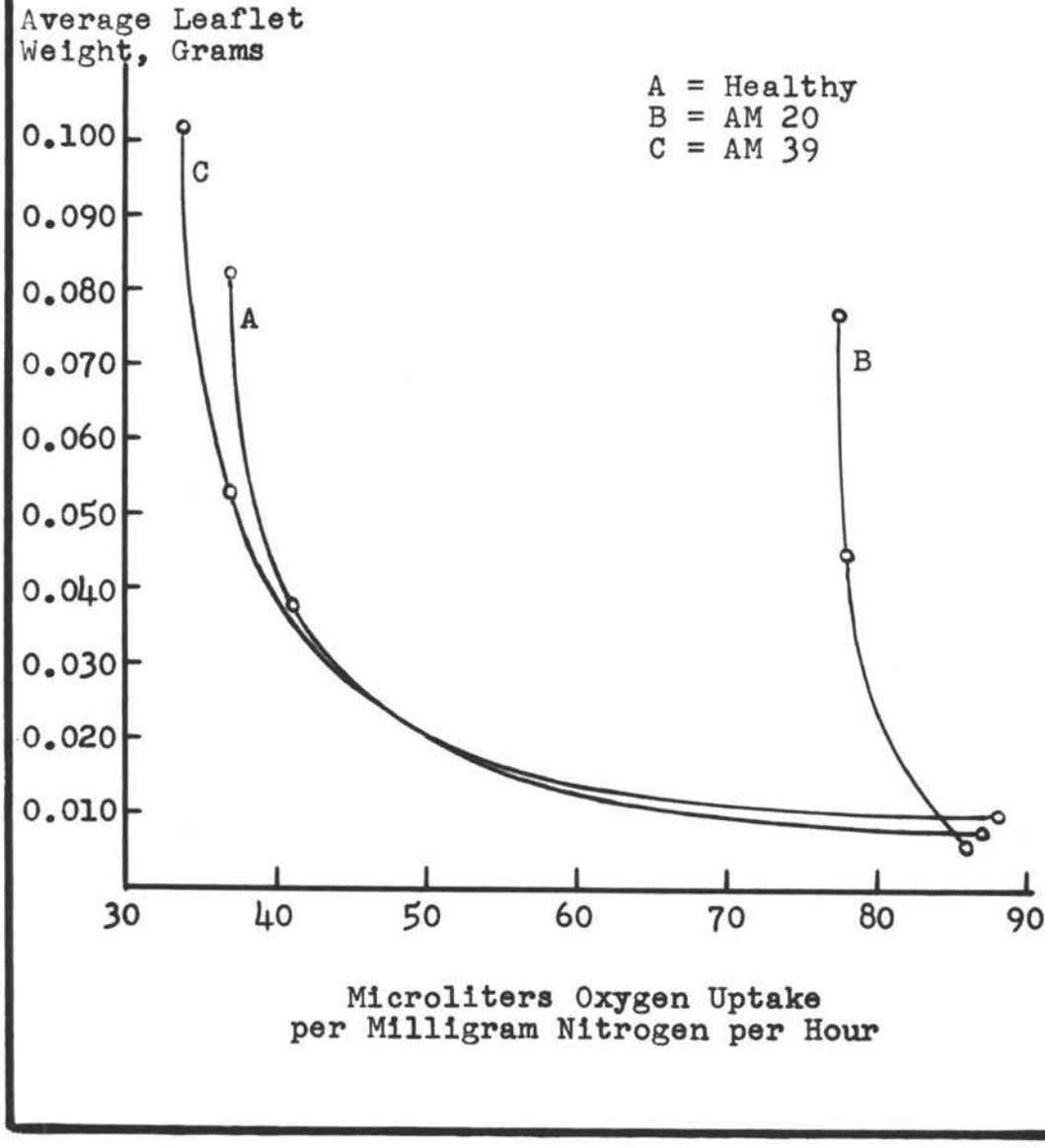


FIGURE 11

Respiration Rates Based on Nitrogen Content of First, Second, and Third Trifoliate Leaves from Healthy, AM 20-, and AM 39-Inoculated Cowpeas



c. Respiration Rates and Nitrogen Contents of Roots

Root samples for respiration and nitrogen measurements in Experiment 5 were drawn from plants inoculated ten days previously with AM 20 or AM 39 and from healthy plants of the same age. At the time of sampling, the trifoliate leaves of AM 20 plants showed systemic chlorotic symptoms, but there were no noticeable differences between trifoliate leaves of healthy and AM 39 plants. No visible differences were evident in the roots of any of the three groups of plants.

Table 10 gives the average results for nitrogen content, and respiration rates based on nitrogen content and on fresh weight for the diseased and healthy root samples.

In a test to recover virus from the roots of the three types of plants, healthy, AM 20-, and AM 39-inoculated, transmission was obtained only with the AM 20 plants. All leaves of the four test plants which were rubbed with inoculum prepared from the roots of plants inoculated ten days previously with AM 20 developed local lesions, while similar tests of roots from healthy and from AM 39-inoculated plants were negative for transmissible virus.

The respiration rates on a fresh weight basis observed in these roots are quite low in comparison with those

TABLE 10

Respiration Rates and Nitrogen Contents of Roots from Healthy, AM 20-, and AM 39-Infected Cowpeas.

Plants	Respiration rate, fr. wt. µl. O_2/g./hr.	Respiration rate, fr. wt. % difference from healthy	Mg. N/gram fr. wt.	Respiration rate, nitrogen µl. O_2/mg. N/hr.	Respiration rate, nitrogen % difference from healthy
Healthy	141	--	1.28	111	--
AM 20	92	- 34.8	1.27	72	- 35.0
AM 39	126	- 10.6	1.21	104	- 6.3

previously found for primary leaves. However, when the respiration is expressed in terms of nitrogen content, the rate is found to be higher in roots than in primary leaves from the healthy plants. This last is due to the comparatively low levels of nitrogen found in the roots. Very little difference was found in nitrogen content of the roots from different plant groups.

The roots from AM 20-infected plants respired at a rate of about 35% below healthy roots on both nitrogen content and fresh weight bases, while the respiration of AM 39 roots was only slightly less than that of healthy roots.

2. Investigation of Phosphate Content

Experiments were performed to determine the acid-extractable inorganic, organic, and total phosphate in primary and trifoliate leaves after light and dark periods.

a. Phosphate Studies on Healthy and Diseased Primary Leaves

Samples of healthy and diseased primary leaves were collected from plants that had been inoculated on one primary leaf, with the opposite, uninoculated leaf supplying the healthy material. The samples obtained after a light period were collected in the evening of the day on

which local lesions became evident. The dark-period samples were taken the following morning.

The average results of phosphate determinations on AM 20-infected primary leaves are given in Table 11 while those for AM 39-diseased leaves are given in Table 12. The percentage of the total phosphate which is present as organic phosphate is calculated to provide a relative comparison of the phosphate distribution in healthy and diseased tissue after light or dark periods.

These results show that more acid-extractable phosphate is present in diseased than in healthy tissue in the phosphate fractions determined, in both AM 20- and AM 39-diseased primary leaves. This was found also whether the plants were subjected to light or to dark periods; however, considerably less phosphate was found in primary leaves after exposure to a dark period than was present after a period of illumination.

Using the relative amounts of organic phosphate present in each sample as a basis of comparison for the phosphate distribution in healthy and diseased leaves after light or dark periods, the data show that the relative amounts of organic phosphate in diseased and the comparable healthy leaves are quite similar after illumination. For AM 20-infected and the comparable healthy leaves, the organic phosphate represented 29.4% and 31.0%,

TABLE 11

The Amount and Distribution of Acid-Extractable Phosphate Present in Healthy and AM 20-Infected Primary Leaves after Periods of Illumination and Darkness.

After period of	Leaves	Phosphate Micrograms per gram fresh weight Total	Inorganic	Organic	Percent as organic
Light	Healthy	1,738	1,198	540	31.0
	Diseased	2,103	1,473	630	29.8
Dark	Healthy	1,125	1,020	105	9.3
	Diseased	1,578	1,381	197	12.5

TABLE 12

The Amount and Distribution of Acid-Extractable Phosphate Present in Healthy and AM 39-Infected Primary Leaves after Periods of Illumination and Darkness.

After period of	Leaves	Phosphate Micrograms per gram fresh weight Total	Inorganic	Organic	Percent as organic
Light	Healthy	1,599	1,167	432	27.1
	Diseased	2,045	1,507	538	26.3
Dark	Healthy	1,269	1,127	142	11.2
	Diseased	1,375	1,195	180	13.1

respectively, of the total phosphate present after a light period. Following exposure to a dark period, the relative amounts of organic phosphate dropped to 12.5% and 9.3%, respectively, for AM 20-infected and healthy leaves.

Similar changes were observed in the relative amounts of organic phosphate in AM 39-infected and the comparable healthy leaves. The organic phosphate in AM 39-infected leaves declined from 26.3% after illumination to 13.1% in the dark while the healthy leaves showed a drop from 27.1% to 11.2% under the same conditions.

b. Phosphate Studies in Healthy and Diseased Trifoliate Leaves

Duplicate samples of the first trifoliate leaves from healthy, AM 20-, and AM 39-inoculated plants were collected after periods of light and of dark. Light samples were taken on the evening of the ninth day after inoculation and the dark samples were obtained the following morning. At the time of sampling, the trifoliate leaves of AM 20 plants were showing definite systemic symptoms.

The average results of phosphate analyses on trifoliate leaves are presented in Table 13.

The acid-extractable phosphate content of trifoliate leaves is much lower than was previously found for primary

TABLE 13

The Amount and Distribution of Acid-Extractable Phosphate Present in Trifoliate Leaves from Healthy, AM 20-, and AM 39-Infected Cowpeas after Periods of Illumination and Darkness.

Plants	Treatment	Phosphate Micrograms per gram fresh weight			Percent as organic
		Total	Inorganic	Organic	
Healthy	Light	536	439	97	18.2
	Dark	570	482	88	15.4
AM 20	Light	521	403	118	22.6
	Dark	529	390	139	26.3
AM 39	Light	519	420	99	19.1
	Dark	499	426	73	14.6

leaves. No marked differences in total phosphate content between light and dark samples were observed. The percentages of organic phosphate were quite similar for trifoliate leaves from healthy and AM 39-inoculated plants, a lower relative amount of organic phosphate being observed in both after the dark period. AM 20-infected leaves, however, had a slightly higher percent of the total acid-extractable phosphate present in the organic form than was found for healthy leaves, and in contrast to healthy leaves, the relative amount of organic phosphate increased in the dark.

DISCUSSION

Primary leaves are inoculated when they are approximately ten days old. This marks the beginning of the experimental period that ends five to seven days later, shortly before the primary leaves become senescent and wither. The respiration rates of primary leaves, whether diseased or healthy, show a slight rise during each experiment, generally followed by a decline. This rise and subsequent decline are more pronounced when the respiration rates are based upon fresh weight than when they are based upon nitrogen content. Nicolas (29, pp.222-223) demonstrated that the rate of respiration in leaves declines with age. Hover and Gustafson (18, p.39) found that this is true up to a certain age; however, they observed that as the leaves of wheat, corn, sorghum, and oats became still older there was a tendency for respiration rates to increase again. The increased respiration rates observed in the primary leaves of cowpeas as they approach senescence may be related to an active movement of materials out of the primary leaves and into the younger parts of the plant. The energy required for this could result in enhanced rates of respiration.

Both AM 20- and AM 39-infected primary leaves show a similar pattern of respiration rate changes over a period

of time. Although the appearance of the first local lesions on inoculated leaves varies between the second and third days after inoculation in different experiments, the greatest differences in respiration rates between healthy primary leaves and leaves inoculated with either virus are regularly found to occur on the day following the appearance of local lesions. That is, the maximum differences in respiration rate are always observed on the third or fourth day following inoculation. With tobacco mosaic virus, increases of respiration rates have been reported to occur in tobacco leaves at similar times after inoculation. Wynd (49, p.95) observed the respiration rate of infected leaves to reach the maximum difference over healthy leaves on the fourth day, while Vayonis (43, p.695) reported the greatest difference on the third day, after inoculation.

With AM 20-infected leaves, there appears to be a gradual increase in respiration rate of diseased over healthy leaves during the two or three days prior to the peak, after which the respiration rate of the diseased leaves drops fairly abruptly to a level about equal to that of healthy leaves. In contrast, the respiration rate of AM 39-infected primary leaves appears to increase over that of healthy only slightly until about the day local lesions appear, at which time the rise is more abrupt to

reach the greatest observed difference on the following day. Following this peak, the difference between AM 39-diseased and healthy primary leaf respiration rates decreases more gradually than was observed with AM 20-infected leaves.

The percentage increase of the respiration rates of diseased primary leaves over healthy varies in different experiments. For example, in Experiment 1, the highest respiration rate difference for AM 20-infected primary leaves on a fresh weight basis is 45%, while in Experiment 3, with the same virus, the difference is only about 27%. That this may be due to differences in the numbers of local lesions in the different experiments is perhaps indicated by the observation that respiration rates in virus-diseased peach foliage increase with symptom severity (30) and that the light-green parts of tobacco leaves infected with mosaic have higher respiration rates than the dark-green areas (12, p.603).

Respiration rates of the trifoliate leaves of cowpea are found to decrease rapidly with increasing size and age. Trifoliate leaves of comparable size and age from uninoculated plants and those from plants inoculated with AM 39 have quite similar respiration rates based on either nitrogen content or fresh weight. In contrast, comparable leaves from plants inoculated with AM 20 show considerable

differences from healthy leaves. The first and second trifoliate leaves from the latter plants have respiration rates of almost 100% in excess of healthy leaves of similar size, but the respiration rate of the third leaf falls below that of healthy leaves. When respiration rates are based on nitrogen content, the rate found for this leaf is only slightly below that of comparable leaves from healthy plants.

The results with the first two AM 20-infected trifoliate leaves, showing increased respiration rates in leaves with systemic symptoms, are in agreement with the findings of other workers for various host-virus relationships (13, pp.352-353; 30; 46, p.297; and 16, pp.273-274). The diminished respiration rates in the youngest AM 20-infected trifoliate leaves may be comparable to the results obtained by Dufrenoy (11, pp.293-294) who observed that while mature leaves of tobacco plants infected with mosaic respired at a higher rate than healthy leaves, the respiration rate of diseased buds was lower than that of healthy.

The respiration rates of roots from healthy and from AM 39-inoculated plants are within about 10% of each other on a fresh weight basis and even closer together on a nitrogen basis. Roots from AM 20-infected plants have a respiration rate of about 35% lower than healthy roots.

Inasmuch as transmissible virus was obtained from roots of AM 20 plants but not from those of AM 39 plants, it would appear that the virus might have to be present in the roots to exert a physiological effect. The reduced respiration rates of roots, and perhaps of the third trifoliate leaves, of the AM 20 plants may be caused by a partial starvation or by an effect of the virus on mechanisms controlling respiration. If the virus interferes with translocation, these results could easily be explained. A shortage of food could result in a lower respiration rate and in difficulties of water uptake, ion uptake, and translocation of water and ions. A reduced photosynthetic activity could have about the same effect as reduced translocation.

It is possible that average values such as those presented here of respiration rates of diseased leaves may have somewhat less significance than maximum values would have, since maximum values might more closely reflect the limit of the effect of the virus and tend to minimize variations due to lack of uniformity of infection. At the present time it is not possible to obtain uniform infections, and the results obtained by some investigators (12 and 30) indicate higher respiration rates with stronger symptom severity.

In all experiments, a general decline of total nitrogen content per unit of fresh weight with increasing age and size of both the primary and trifoliate leaves is found. In only one experiment was there a consistent trend toward a higher nitrogen content in diseased leaves. In all other cases, the nitrogen contents of comparable healthy and diseased primary leaves are quite uniform. Decreasing nitrogen content with age and growth has been reported previously by Chibnall (8, pp.353-359) in bean leaves and by Richards and Templeman (33, pp.373-376) in barley. The latter investigators suggest that the increase in content of non-protoplasmic material throughout the life history of the leaf continually tends to diminish the internal nitrogen concentration, thus the highest nitrogen contents can be expected in the youngest leaves.

There is considerably less total nitrogen present in the very young third trifoliate leaf of the AM 20 plants than is observed in leaves of similar size from healthy or AM 39-inoculated plants. The reason that similar lowered levels of total nitrogen were not found in the first and second trifoliate leaves from AM 20 plants might be related to the fact that these leaves could have differentiated before the virus became systemic and may even have matured to a point well past cell division. The third leaf was possibly differentiated in the presence of the

stunting virus, which could have affected the number of cells produced as well as the extent to which they developed afterward. This nitrogen deficiency in the third leaf may also be related to reduced nitrate uptake by the roots, to a reduced translocation of nitrate, or, as a result of the stunting induced by the virus, the leaf at this size may be at a considerably more advanced state of maturity. The similarity of nitrogen contents of roots from both diseased and healthy plants would appear to rule out disturbed nitrate uptake as a factor. The possibility that this nitrogen deficiency could arise as a result of synthesis and retention of virus protein in older leaves or in roots is not likely since the nitrogen contents of the older trifoliate leaves and of roots of AM 20-infected plants are not appreciably different from those of healthy plants. A relatively advanced state of maturity in relation to size in the third trifoliate leaves of AM 20-infected plants is possible, because at this length of time after inoculation (ten days) the stunting effect of AM 20 becomes more pronounced and these leaves never attain the size of the first and second trifoliate leaves.

Results indicating no appreciable differences of total nitrogen content between healthy leaves and leaves infected with a mosaic virus were obtained by Caldwell (6, p.214) using tomato and tobacco, and by Brewer,

Kendrick, and Gardner (5, pp.847-848) using tomato. However, both increases (13, p.350 and 10, pp.80-84) and decreases (20, p.1888 and 21, p.1068) of total nitrogen have been reported for mosaic-diseased leaves.

No transmissible virus was definitely found in the uninoculated primary leaf where the opposite primary leaf had been inoculated seven days previously; that is, for the duration of the time-sequence experiment. The similarity between the results from these experiments and those in which the healthy leaves were taken from separate uninoculated plants is in line with this also. In addition, these results strongly suggest that there is little influence of the infected leaves on the respiration or metabolism of other non-infected parts of the plants. This is in contrast to the findings of Wynd (47, p.652 and 49, p.95) who reported disturbed metabolic effects in uninoculated leaves of tobacco plants as long as ten days before transmissible virus was found in these leaves.

Higher levels of acid-extractable phosphate are found in AM 20- and AM 39-infected primary leaves both after periods of light and of darkness than are found in comparable healthy tissues. After exposure to light, the ratio of organic to inorganic phosphate is about the same in infected tissue as in healthy tissue although the total amount of organic phosphate is higher in the

diseased tissue. The small differences found in the organic to inorganic phosphate ratio are probably not very important. After exposure to a period of darkness, the amounts of total phosphate and the relative amounts of organic phosphate found in all three sets of plants are considerably lower. The reasons for this are not known, but Mason and Maskell (26, p.134) have observed both upward and downward translocation of phosphate in the cotton plant and Biddulph (4, p.352) has suggested the possibility of a "daily circulation" of phosphate through the bean plant.

The differences between the amounts of phosphate in primary leaves compared to trifoliate leaves are probably indicative of different patterns of phosphate metabolism in the different kinds of leaves. The relatively large decreases of phosphate content during darkness which are observed in primary leaves do not occur in trifoliate leaves. Further, while larger amounts of phosphate are found in diseased as compared with healthy primary leaves, this is not observed in trifoliate leaves. The relative amounts of organic phosphate in AM 20-infected trifoliate leaves appear to increase in the dark. Contrastingly, similar leaves from healthy and from AM 39-inoculated plants have lower percentages of organic phosphate after a dark period.

The phosphorous relationships found between diseased and healthy primary leaves of cowpea one day after appearance of symptoms are similar in some respects to the results obtained by Vayonis (43, pp.692-694) with mosaic-diseased and healthy tobacco leaves. This investigator observed the acid-extractable inorganic phosphate and the organic phosphate to be present at higher percentages of the dry weight in diseased than in healthy leaves.

The results obtained with AM 20- and AM 39-inoculated plants indicate that where any difference occurs in a diseased plant, this response requires the presence of the virus and/or disease symptoms in the plant part in question. Comparison of the results obtained for plant parts from healthy and from AM 39-inoculated plants shows that the only ways in which these diseased plants differ appreciably from healthy are in the increased respiration rate and increased phosphate content of the inoculated primary leaves. The respiration rates and nitrogen contents of roots and trifoliate leaves, and the phosphate relations of trifoliate leaves from AM 39-inoculated plants do not appear to be greatly different from healthy plants.

In general, the effects of the two viruses are quite similar where they are able to produce an infection in the cowpea. The milder response of this plant to infection

with AM 39 probably is due to the fact that this virus does not spread much beyond the site of inoculation to infect new tissues. Infection of a primary leaf with AM 39 does not alter significantly the rate of respiration or phosphate metabolism in uninfected tissue. In contrast, AM 20, which becomes systemic in cowpea, induces changes of respiration rate in primary leaves, trifoliate leaves, and roots and also gives evidence of disturbed phosphate metabolism in both primary and trifoliate leaves.

SUMMARY

This thesis describes experimental work investigating some effects of two strains of alfalfa mosaic virus on the physiology of cowpea plants. A time-sequence study of rates of respiration and nitrogen contents of inoculated and healthy primary leaves up to seven days after inoculation was performed. Experiments were conducted ten days after inoculation to measure the respiration rates and nitrogen contents of the first, second, and third trifoliate leaves and of roots from healthy, AM 20-, and AM 39-inoculated plants. Analyses for acid-extractable phosphate were performed on samples of healthy and diseased primary leaves collected on the day following appearance of local lesions and on trifoliate leaves from healthy and diseased plants nine days after inoculation.

Pronounced physiological effects due to infection with AM 39 were observed in the inoculated primary leaves, but not in trifoliate leaves or roots. AM 20, on the other hand, induced physiological changes in trifoliate leaves and roots in addition to those in the inoculated primary leaves. These differences between strains are probably due to the fact that AM 20 becomes systemic in the cowpea whereas AM 39 does not.

Higher rates of respiration were found in AM 20- and in AM 39-infected primary leaves than in healthy leaves.

The respiration rates of the first and second trifoliate leaves from AM 20-infected plants were higher, but that of the third leaf was lower, than comparable leaves from healthy plants. AM 20 caused a lower rate of respiration in roots. Respiration rates of trifoliate leaves and of roots from AM 39-inoculated plants were not appreciably different from those of corresponding tissues from healthy plants.

No appreciable differences consistently were found in the nitrogen contents of primary leaves and roots from healthy or diseased plants. With trifoliate leaves, only the third trifoliate leaves from AM 20-infected plants appeared to differ appreciably from comparable healthy leaves in having a lower nitrogen content.

Less acid-extractable phosphate was found in healthy primary leaves than in diseased. The total amount of phosphate declined during a dark period in both healthy and diseased primary leaves, but the relative amount of organic phosphate appeared to decrease more in healthy than in diseased leaves. The total acid-extractable phosphate content was similar for trifoliate leaves from healthy, AM 20-, and AM 39-inoculated plants. In light, a higher relative amount of organic phosphate was present in trifoliate leaves of AM 20 plants than was observed with healthy or AM 39 leaves. The percentage of organic

phosphate increased during dark in AM 20, but decreased in healthy and AM 39 trifoliate leaves.

BIBLIOGRAPHY

1. Bawden, F. C. Plant viruses and virus diseases. 3d ed. Waltham, Chronica botanica, 1950. 335p.

2. Bawden, F. C., and N. W. Pirie. Physiology of virus diseases. Annual review of plant physiology 3:171-188. 1952.

3. Beijerinck, Martinus. Ueber ein contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Verhandelingen der Koninklyke akademie van Wettenschappen te Amsterdam 65:3-21. 1898. (Phytopathological classics, no. 7. 1942)

4. Biddulph, Orlin. Diurnal migration of injected radiophosphorous from bean leaves. American journal of botany 28:348-352. 1941.

5. Brewer, P. H., James B. Kendrick, and Max W. Gardner. Effect of mosaic on carbohydrate and nitrogen content of the tomato plant. Phytopathology 16:843-851. 1926.

6. Caldwell, John. The physiology of virus diseases in plants. VI. Some effects of mosaic on the metabolism of the tomato. Annals of applied biology 21:206-223. 1934.

7. Campbell, Elmer Grant. Potato leaf-roll as affecting the carbohydrate, water, and nitrogen content of the host. Phytopathology 15:427-430. 1925.

8. Chibnall, Albert Charles. Investigations of the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochemical journal 16:344-362. 1922.

9. Commoner, Barry, and Phyllis M. Dietz. Changes in non-protein nitrogen metabolism during tobacco mosaic virus synthesis. Journal of general physiology 35:847-856. 1952.

10. Cordingley, J. Grainger, W. H. Pearsoll, and A. Wright. The effect of mosaic disease upon certain metabolic products in the tobacco plant. Annals of applied biology 21:78-89. 1934.

11. Dufrenoy, Jean. Modifications pathologiques du metabolisme cellulaire chez les tabacs. Annales des Epiphyties 18:259-316. 1932.

12. Dufrenoy, Jean, and M. L. Dufrenoy. Cytology of plant tissues affected by viroses. Phytopathology 24:599-619. 1934.

13. Dunlap, Albert A. The total nitrogen and carbohydrates, and the relative rates of respiration, in virus-infected plants. American journal of botany 17:348-357. 1930.

14. _____. Carbohydrate variations accompanying the mosaic disease of tobacco. American journal of botany 18:328-336. 1931.

15. Freiberg, George W. Studies in the mosaic diseases of plants. Annals of the Missouri botanical gardens 4:175-225. 1917.

16. Glasstone, Violette F. C. Study of respiration in healthy and mosaic-infected tobacco plants. Plant physiology 17:267-277. 1942.

17. Holden, Margaret, and M. V. Tracey. The effect of infection with tobacco-mosaic virus on the levels of nitrogen, phosphorous, protease, and pectase in tobacco leaves and their response to fertilizers. Biochemical journal 43:151-156. 1948.

18. Hover, J. Milton, and Felix G. Gustafson. Rate of respiration as related to age. Journal of general physiology 10:33-39. 1927.

19. Ivanowski, Dmitrii. Ueber die Mosaikkrankheit der Tabakspflanze. Saint Petersbourg imperial academy of science bulletin 35:67-70. 1892. (Phytopathological classics, No. 7. 1942)

20. Jodidi, S. L. A mosaic disease of cabbage as revealed by its nitrogen constituents. Journal of the American chemical society 42:1883-1892. 1920.

21. Jodidi, S. L., S. C. Moulton, and K. S. Markley. The mosaic disease of spinach as characterized by its nitrogen constituents. Journal of the American chemical society 42:1061-1070. 1920.

22. Kempner, Walter. Chemical nature of the oxygen-transferring ferment of respiration in leaves. Plant physiology 11:605-613. 1936.

23. Kidd, Franklin, Cyril West, and G. E. Briggs. A quantitative analysis of the growth of Helianthus annuus. I. The respiration of the plant and of its parts throughout the life cycle. Proceedings of the royal society of London 92:368-384. 1921. (Series B)

24. Lemmon, Paul. Comparative studies on metabolism of healthy and mosaic-infected tobacco leaves. American journal of botany (Abstract) 22:912. 1935.

25. Lepper, Henry A., chairman, editorial board. Official methods of analysis of the association of official agricultural chemists. 7th ed. Washington, Association of official agricultural chemists, 1950. 910p.

26. Mason, T. G., and E. J. Maskell. Further studies on transport in the cotton plant. I. Preliminary observations on the transport of phosphorous, potassium, and calcium. Annals of botany 45:125-173. 1931.

27. Mayer, Adolf. Ueber die Mosaikkrankheit des Tabaks. Die Landwirtschaftlichen Versuchs-Stationen 32:451-467. 1886. (Phytopathological classics, no. 7. 1942)

28. Miller, Edwin C. Plant physiology. 2d ed. New York, McGraw-Hill, 1938. 1201p.

29. Nicolas, M. G. Contribution a l'étude des variations de la respiration des végétaux avec l'age. Revue générale de botanique 30:209-224. 1918.

30. O'Reilly, Harry J. Physiological changes induced in peach foliage by the Western X disease virus. Phytopathology (Abstract) 43:481. 1953.

31. Pons, Walter A., Jr., Mack F. Stansbury, and Carroll L. Hoffpauir. An analytical system for determining phosphorous compounds in plant materials. Journal of the association of official agricultural chemists 36:492-503. 1953.

32. Rawlins, T. E., and W. G. Tomkins. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology 26:578-587. 1936.

33. Richards, F. J., and W. G. Templeman. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Annals of botany 50:367-402. 1936.

34. Rischkov, V. L. The nature of ultra-viruses and their biological activity. Phytopathology 33:950-955. 1943.

35. Rosa, J. T. Chemical changes accompanying the western yellow blight of tomato. Plant physiology 2:163-169. 1927.

36. Shapalov, Michael. The growth rate of tomato plants affected by yellows. Phytopathology (Abstract) 21:106. 1931.

37. Smith, Kenneth M. Recent advances in the study of plant viruses. 2d ed. Philadelphia, Blakiston, 1951. 300p.

38. Stanley, W. M. Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. Science n.s. 81:644-645. 1935.

39. Takahashi, William N. Respiration of virus-infected plant tissue and effect of light on virus multiplication. American journal of botany 34:496-500. 1946.

40. True, Rodney H., and Lon A. Hawkins. Carbohydrate production in healthy and blighted spinach. Journal of agricultural research 18:381-384. 1918.

41. True, Rodney H., Otis F. Black, and James W. Kelly. Ash content in normal and blighted spinach. Journal of agricultural research 15:371-375. 1918.

42. Umbreit, Wayne H., Robert H. Burris, and John H. Stauffer. Manometric techniques and tissue metabolism. 2d ed. Minneapolis, Burgess, 1951. 227p.

43. Vayonis, George Constantine. Phosphorous disturbances in mosaic-virus infected tobacco plants. Physiologia plantarum 7:687-697. 1954.

44. Whitehead, T. Respiration of healthy and leaf roll potatoes. Nature 128:967. 1931.

45. _____. The physiology of potato leaf-roll. I. On the respiration of healthy and leaf-roll infected potatoes. Annals of applied biology 21:48-77. 1934.

46. Woods, Mark W., and H. G. DuBuy. The effect of tobacco mosaic virus on cellular respiration. Phytopathology 32:288-301. 1942.

47. Wynd, F. Lyle. Certain enzymatic activities of normal and mosaic infected tobacco plants. Journal of general physiology 25:649-661. 1942.

48. _____. Metabolic phenomena associated with virus infection in plants. The botanical review 9:395-465. 1943.

49. _____. Respiration of mosaic-infected tobacco plants. Plant physiology 18:90-98. 1943.