# MOSAIC DISEASES OF PLANTS

# Studies Concerning Mosaic Diseases of Plants.

by

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## Introduction.

During recent years the increasing economic importance of the mosaic and other virus diseases of our crop plants has given a great impetus to the study of these obscure troubles. As a result, the known mosaics have been increased from the single one affecting tobacco to numerous mosaics of other Solanaceae, Leguminoseae and Gramineae, with scattered cases in twenty or more additional families of both Monocotyledons and Dicotyledons. Current literature is replete with reports of mosaic diseases on new plants, either wild or cultivated, and studies concerning plant virus diseases are daily entering widely divergent channels of research.

The worker with virus diseases of plants has to contend with factors peculiar to his field of study. He is, in the first place, dealing with a phenomenon which at the present time has not been decisively analyzed. Many facts have been established relative to the behaviour of the causal agent under certain conditions, but its specific nature is as yet only the subject of conjecture. And in the second place the investigator is faced with something that is at times variable. Such complications as the masking of symptoms, failure of the virus to grow on artificial media, the occurrence of complex virus mixtures and the like all contribute to make

the study of these diseases more difficult.

And yet the hope of success is by no means forlorn. With the cooperation of the student engaged in the study of animal viruses, much information will be amassed, and doubtless our knowledge as to cause and control will soon be complete. Until that time no avenue of enquiry from which any knowledge may be gleaned, however unimportant it may appear, can be passed by as not meritting attention.

## Purpose of the Investigations.

Each year witnesses the addition to our present store of a large number of papers and treatises relative to the mosaic and related diseases of plants. This fact alone demonstrates the importance attached to these diseases by scientific workers.

As conditions stand, therefore, and despite much contradictory evidence in some of the results obtained, any addition to the accumulated knowledge we possess is of some value. It was essentially with this in mind that the present investigation was undertaken.

## Scope of the Work.

The studies reported in this paper embrace the following phases of study:

1. Classification of viruses used.

2. Studies to determine the effect of certain cultural treatments on the leaf structure of mosaic tobacco plants.

3. Studies on the catalase content of healthy and mosaic tobacco leaves.

4. Studies to determine the external and internal effects of nitrogen and potassium deficiencies on the leaves of healthy and mosaic tobacco plants.

5. Animal nutrition studies with potato and clover mosaic diseases.

6. Blood and serum reactions with the mosaic diseases of tobacco and potato.

7. Filtration studies with tobacco mosaic.

8. Studies to determine the method of spread of the virus in tobacco leaves.

9. Studies on the effect of mosaic disease on the yield and quality of tobacco.

## Review of the Literature.

The literature of virus diseases has been reviewed in considerable detail by many authors. Allard (1) has reviewed the literature of the tobacco mosaic disease up to 1914. Wakefield (87) and Dickson (21) have classified the literature dealing with the symptoms, methods of transmission, and the theories as to the causal agent of mosaic diseases. Excellent reviews of both theoretical and experimental data in regard to the nature of the filterable virus diseases of animals are given by Simon (80) and MacCollum (48). Kankel (42) summarizes the established facts available up to 1924 as to the nature of mosaic diseases. A very comprehensive general review appears in the work of Goldstein (32).

The present review deals only with the more outstanding contributionsto our knowledge of mosaic diseases, and mainly in so far as they concern those diseases dealt with in this paper. Further citations to the more pertinent literature are dealt with in their proper places throughout the report.

In the following review certain inclusive headings have been adopted. These are as follows:

a. Symptoms and other characteristics of virus diseases.

b. Range of hosts susceptible to virus diseases.

c. Strains of mosaic virus.

d. Methods of disease transmission:

1. Needle inoculations and rubbing methods.

2. Transmission by grafting.

3. Transmission by insects.

e. Carriers of mosaic diseases.

f. Nature of mosaic diseases:

1. Cytological studies.

2. Filtration studies.

3. Serological studies.

(a) Symptoms and other characteristics of virus diseases:

Mayer (52) in 1886 was the first to investigate the tobacco mosaic disease, and to demonstrate its infectious nature.

Beijernick (8) a few years later was the first to fully appreciate the biological significance of this disease. He found that it is propagated as a filterable virus, that a definite leaf mottling resulted from the infection and that diseased leaves are distinctly smaller than healthy ones.

Smith (82) in 1894 described the symptoms of two different diseases of peach trees which he thought definitely related to the virus disease group. Peach yellowswas characterized by a red spotting and abnormally early maturity of the fruit, while trees affected with peach rosette possessed olive green foliage which tended to become compactly bunched, a very conspicuous feature of the disease.

Chapman (15) states that the leaves of mosaic diseased tomato plants are mottled, and appear stiff and badly curled. The light green areas on the tomato fruits become yellowish, and in badly affected plants, purplish red.

Allard (4) describes a mosaic disease of petunia plants in which the leaves are distorted, curled and wrinkled, and show irregular dark green areas along the veins. He also (2) describes pokeweed mosaic as bearing a close resemblance to that of tobacco. McClintock and Smith (53) describe the spinach blight disease as a specific one differing from the mosaic disease of cucumber and tobacco in that the spinach plants are eventually killed by the disease. Doolittle (24) describes the cucumber mosaic as causing a mottling, savoying and wrinkling of the leaves, dwarfing of the stems and petioles and the appearance of dark and yellow-green areas on the fruits.

Brandes (11) describes the symptoms of the mosaic disease of sugar cane and other grasses as a mottling and striping of the leaves. In advanced stages of the disease the plants are dwarfed and yellowed, though seldom killed. Schultz (75) states that distinct mottling of the leaves, distortion of the leaf blade and a general dwarfing of the plant are symptoms of a transmissible mosaic disease of Chinese cabbage, mustard and turnip.

Lyon (47) describes three major diseases of sugar cane, Yellow stripe disease and sugar cane mosaic are said to be identical. "Sereh" is a disease of sugar cane apparent in the

form of bushy tufts of leaves from an arrested growth of the canes. The third, or Fiji disease, is characterized by the appearance of galls or swellings on the under surface of the leaves.

Rand (70) describes the symptoms of pecan rosette as evident in an undersized and more or less crinkled leaf, which upon further development becomes mottled with chlorotic areas between the principal veins. The leaves are often malformed, with the leafblade suppressed, so that a midrib with merely a ragged edge of blade appears, a condition which suggests the frenching of tobacco leaves. Rankin and Hockey (71) describe leaf curl or yellows of raspberry as differing from the mosaic of raspberry. In leaf curl, the leaflets appear darker green than normal, and the midvein arches downward. A similar arching of the lateral veins causes a downward curling of the entire margin of the leaflet.

McKinney (54) describes a rosette and mosaic disease of wheat and rye, in which the mottling consists of irregular streaks in the long axis of the leaves, and in which the plants are often dwarfed and inclined to excessive tillering. Carsner and Stahl (13) describe the curly-top disease of the sugar beet as evident in a curling of the leaves, and the appearance of irregular swellings of the veins on the under surface of affected leaves. Marked phloem necrosis is produced throughout the plant. The mosaic disease of clover, characterized by a general mottling

of the leaf, is described by Elliott (28).

Detailed descriptions of the symptomatology of tobacco mosaic are given by Dickson (22), Allard (2) and Chapman (16) and of the virus diseases effecting the potato by Orton (65), Schultz et al (74), Quanjer (69), Folsom (30), Murphy (59), Smith (84) and Atanasoff (6).

b. Range of hosts susceptible to virus diseases.

Most of our cultivated crop plants, with few exceptions, are susceptible to virus diseases. These diseases are more common on herbaceous than on woody plants, and fortunately most forest trees are free of them. A review of the various plant families, some of whose species are susceptible to attack by viruses, shows that the range of infection of virus diseases is a large one. Thus plants belonging to the following families have been shown to harbor one or more viruses:

DICOTYLEDONS. Ranunculaceae, Cruciferae, Phytolaccaceae, Malvaceae, Leguminosae, Rosaceae, Cucurbitaceae, Umbelliferae, Saxifrageae, Compositae, Solanaceae, Chenopidiaceae, Ericaceae, Urticaceae.

MONOCOTYLEDONS. Iridaceae, Liliaceae, Musaceae, Graminaceae.

Although Mayer (52) early established the infective nature of the tobacco mosaic virus and showed its transmissibility within the species Nicotiana tabacum, it was not until 1907 that Clinton (18) showed interspecific transmission

of the disease to be possible. It has now been shown that this disease can be transmitted to plants which differ widely in their taxonomic position, such as Petunia violacia, Solanum carolminose, Physalis alkekengi, Nicotiana rustica, N. glutinosa, Phaseolus vulgaris and many others. Certain of these species and others not mentioned do not manifest the typical mosaic mottling when inoculated with the tobacco mosaic virus, there being apparently morphological or physiological differences in the hosts which in some way affect the response of the various species to the same virus. In this connection, Nishimura (63) reports Physalis alkekengi as being a masked carrier.

Brandes (11) found that more than one thousand varieties of sugar cane, including all the commercial varieties, are susceptible to sugar cane mosaic. Corn, sorghum, rice, millet, the crab grasses, fox tails and Panicum were all found to be the hosts of what appears to be an identical disease agent. Doolittle and Walker (26) show that cucurbit mosaic is capable of transmission to a wide range of hosts, including milkweed, pepper, pigweed, wild cucumber, catnip and all the species of Cucurbitaceae tested. Kunkel (42) finds that aster yellows can be transmitted through insects to fifty different species of plants in twenty different families. The disease however shows great variation in the symptoms it produces in the different hosts.

A large amount of work has been done with the various virus diseases attacking the potato. Many workers, notably

Johnson (40) have shown the transmissibility of some of these viruses to tobacco,

Elmer (29) finds that he can cross-inoculate mosaic disease between hosts belonging to distinct orders and families. Artificial inoculation appears to be more difficult and the incubation period longer when the cross inoculations are made between families than when the inoculations are made within the same family. For successful cross inoculations he recommends that the plants to be inoculated and the plants furnishing the virus should all be growing vigorously.

### c. Strains of mosaic virus.

The presence of two or more strains of virus native to a single plant, and even capable of producing distinctive sets of reactions within a given host, has been demonstrated in a number of cases. The most notable work on the existence of distinct strains of virus in the potato, and their isolation is that of Schultz and Folsom (76, 77). They distinguish seven distinct and transmissible degenerative diseases of potato, each with its own characteristic symptoms in the growth habit of the plant, in effects on the structure of the tissues of the stem, and in chlorotic leaf patterns. These diseases may be transmitted singly or in various combinations to healthy potato plants. The strains of virus are characterized as: "mild mosaic," "leaf rolling mosaic," "rugose mosaic," "streak

disease," "leaf roll and net necrosis," "spindling tuber" and "unmottled curly dwarf." All these diseases are believed to be due to distinct though similar viruses.

Goss and Peltier (33) confirm the work of Schultz and Folsom as to the existence of distinct strains of virus in potato which run true to type by tuber perpetuation when the possibility of infection with other virus diseases the previous year has been eliminated. Dickson (22) finds that the combination of two mosaic viruses, namely that of potato and that of tomato or tobacco (these two mosaics being considered identical), when inoculated into tomato, produce in the tomato a characteristic disease symptom of streaking or striping of the stem.

Johnson (39) recognizes eight different mosaic diseases that are transmissible to tobacco, and Bennett (9) has shown that three different mosaics attack raspberries. Kunkel (43) notes two distinct mosaics occurring on corn.

Walker (88), working with the mosaics of cucumber, tomato and the cultivated ground cherry, has shown that the properties of a virus from a given plant may be decidedly changed on transferring it to another host. The infective agents in juice from diseased tomato and ground cherry plants in tests on ageing, drying, heating, dilution, and treatment with alcohol reacted in the same manner. The active agent in juice from mosaic cucumber, on the other hand, was incapable of withstanding ageing, drying, heating, and treatment with

alcohol to a degree comparable to that of the agents from the other two plants. But the infective principle in juice from ground cherry plants previously inoculated with the virus from mosaic cucumber plants gave the same reaction as the active principle in juice from ground cherry plants inoculated with virus from mosaic ground cherries. And, in like manner, the properties of the virus from cucumber plants were found to be the same regardless of whether the plants were inoculated from mosaic cucumbers or from mosaic ground cherries. Another example of the modification of a virus by passage through particular host plants is that reported by Carsner (14) for the virus of the curly-top disease of sugar beets when passed through either of the three species Chenopodium murale, Rumux crispus or Suaeda moquini. If carried from these plants back to sugar beet, the virus causes a mild or attenuated form of the curly-top disease. The attenuated form or strain of curly-top occurs naturally in sugar beet fields.

d. Methods of disease transmission.

Although the mosaic and related diseases have been shown to be of an infectious nature in nearly every case, the natural methods of communication of the disease in the field, and the experimentally demonstrated methods of disease trans-

mission are considerably varied. Mayer (52) in 1886 was the first to show the transmissibility of the tobacco mosaic disease. He pressed out juices of diseased plants, filled capillary tubes with them, inserted the tubes into healthy plants, and was able to initiate the infection which showed up in two or three weeks.

1. Needle inoculations and rubbing methods:

In tobacco the mosaic disease is transmitted easily from one plant to another by gently rubbing some juice from a diseased plant on the leaves of a healthy plant, or by inoculation with a needle dipped in virus. In these cases, providing the inoculum is a virulent one, a single inoculation is usually sufficient to bring about re-infection. Johnson (39) reports that a number of inoculations are usually necessary in transmitting the leaf roll mosaic of potatoes.

Allard (5) found that spraying or dropping the virus upon tobacco leaves had no effect. If the leaf is uninjured, no infection will take place. Rubbing the leaves gently with a small piece of cheesecloth or absorbent cotton was sufficient to break the trichomes, thus allowing entrance of the virus. He also finds more than one inoculation, or several at various points, more effective than a single inoculation.

2. Transmission by grafting:

Smith (81), working with peach yellows and peach rosette, found that even seemingly healthy buds from diseased

trees, when grafted upon healthy trees, would produce the disease.

Baur (7) found that the infectious chloroses of certain variegated species of Malvaceae could not be transmitted by means of inoculations of juices obtained from the variegated plants, but only by means of grafting.

Bonquet (10), in work which has since been substantiated by many workers, found that the curly-top disease of sugar beet is transmissible by grafting buds connected with wedge-shaped pieces of root tissues from diseased beets into shoulders of healthy ones.

3. Transmission by insects:

A large amount of work has been done in connection with insect vectors of virus diseases, and various subsidiary related problems.

The numerical relationships of the insects concerned are briefly as follows: Coleoptera and Orthoptera, transmission agents of two or three diseases; Thysanoptera, Tingidae, Hemiptera - Heteroptera one disease only; Capsidae and Coccidae three cases; Hemiptera - Homoptera, Jassidae and Fulgoridae seven cases; Aphididae twenty-seven cases. The aphides are thus found to play by far the largest part in plant virus transmission, and one of these insects is outstanding in its apparent affinity for plant viruses. This is Myzus persicae which is associated with no fewer than fourteen viruses.

At the present time the insects which are known to transmit tobacco mosaic disease are Myzus persicae, Macrosiphum tabaci, Protoparce sexta and Pseudococcus citri. Those transmitting potato mosaic include Myzus persicae, Macrosiphum gei, Aphis rhamin and Mamestra Grassicae.

Smith (85) in a recent article deals very comprehensively and fully with virus diseases of plants and their relationship with insect vectors.

e. Carriers of mosaic diseases.

Nishimura (63) transmitted the mosaic disease of tobacco to Solanum aculeatissimum and to Physalis Alkekengi. In the latter case however, no symptoms developed, though the plant contained the infectious principle as shown by re-inoculation onto tobacco. This species of Physalis acts as a symptomless carrier of the mosaic virus of tobacco.

Johnson (39) reported several new mosaic diseases in tobacco produced by inoculating healthy tobacco plants with the juices of apparently healthy potato plants and tubers. He suggests that these potato plants or tubers may be acting as carriers of several distinct potato viruses.

Schultz (78) has described the appearance of a form of necrosis or streak disease of potato resulting from cross inoculations of juices between healthy potatoes. He suggests that these healthy potato plants were acting as carriers of

the viruses, since they are themselves susceptible to ordinary streak disease of potato and showed no sign of the disease.

### f. Nature of mosaic diseases.

#### 1. Cytological studies:

There is a considerable amount of literature dealing with the histological and cytological aspects of plant virus diseases. This has been reviewed by various writers, notably Goldstein (32) and Smith (83).

Histological modifications occurring in mosaic tissues were first described by Woods (91) in connection with tobacco mosaic. This investigator observed a reduction in length of the palissade cells in the lighter colored areas of the leaf and a decrease in the amount of intercellular space throughout these regions. Iwanowski (38) later confirmed these observations, describing also certain variations in the width of the lamina in the light and dark areas, together with certain changes in the chloroplasts and nuclei.

Dickson (21) made an anatomical study of numerous mosaic diseased plants, and drew the following conclusions:

1. There was a difference between the chlorotic and green area of the leaf, the ratio being about 2:3, due to hypoplasia of both palisade and spongy parenchyma cells in the chlorotic areas.
2. The dark green areas exhibited hyperplasia.

3. There was a regular arrangement of cells in the light area, thus reducing the intercellular spaces.
4. Hypoplasia was accompanied by a degeneration of cell contents.
5. The epidermal cells were smaller in area but deeper over the hypoplastic areas than over the normal.

The presence of cell inclusions in mosaic tissues was first reported by Iwanowski (38) who described bodies of three types occurring in the chlorotic areas of mosaic tobacco leaves: (1) Colorless, crystalline plates, resembling some waxy material but of lower refractive index, and showing cross-striations on treatment with acids; (2) protoplasm-like bodies near, or in close association with the nuclei, and whose appearance suggested parasitic amoebae; (3) granular inclusions, which on staining resembled zoogloea, consisting of very minute short rods usually occurring in the palisade cells and believed to be bacteria.

Inclusions more or less similar to the amoeba-like bodies of Iwanowski have since been described by many workers in association with several different virus diseases of plants. Descriptions of the different cell inclusions occurring in mosaic tobacco are given by Rawlins and Johnson (72) and by Goldstein (32). Smith (83) reports the presence of protoplasmic bodies in the tissues of mosaic tobacco, petunia, Datura stramonium, pokeweed and Euonymus japonicus.

These cell inclusions, or "X-bodies", are to-day generally conceded to arise as a result of the virus disease, and are not thought to be causal agents.

2. Filtration studies:

With the continued failure to find a visible organism as the causal agent of the mosaic and allied diseases, investigators subjected the expressed juices to all manner of experimental procedures in the hope that their results would show the true nature of the causal agent.

Iwanowski (38) in 1892 was the first to demonstrate that the causative agent of a disease could be passed through a bacterial filter, when he found that the juices from diseased tobacco plants after passage through a Chamberland bacterial filter, still retained their infectious properties. He found that the passage of the virus through the filter rendered it less virulent; the finer were the pores of the filter, and the less the pressure, the weaker were the infective qualities of the filtrate. Iwanowski believed this to suggest that the infective particles were actually being held back by mechanical means, and therefore the virus was not of the nature of a fluid.

That there is a limit to the possibilities of passage through bacterial filters by viruses is evident from the studies of Allard (3). He found that filtration of the virus of tobacco mosaic through a normal Berkefeld filter did not deprive the juices of their infective properties, but that there was evidence that the virus had become attenuated and less infectious. When the virus was filtered through a Livingston atmometer cup, the virus was completely removed from the filtrate.

Duggar and Armstrong (27) reported that it was possible to find a filter which, in a given interval of time, at a given pressure, permitted only a relatively small number of the infectious particles to pass through. A standardization of the filters was accomplished by testing their capacity to permit or prevent the passage of colloidal particles of known or approximately known sizes. It was found that the infective particles of mosaic disease of tobacco approximate in size those of a fresh 1% haemoglobin solution, the particles of which are 30 $\mu\mu$ in size.

From the above results it appears that we cannot hope to observe the virus particles by ordinary microscopic methods. Even through the use of the ultramicroscope, until it is possible to separate a virus from the colloids found in plant juices or animal fluids, we cannot hope to detect or distinguish the actual virus particles.

Very recently a short newspaper article appeared in which it was stated that Miss Agnes Quirk of the United States Bureau of Plant Industry had perfected a method whereby virus particles could be observed microscopically. To date, no scientific report of the work has apparently been published.

3. Serological studies:

While serological reactions have been employed for over thirty years in the field of medicine, phytopathologists

have made only a limited use of them in the study of plant diseases.

In 1928, Purdy (67) showed that when rabbits received a series of injections of saline extracts of leaves from tobacco plants affected with the filterable virus of common field mosaic disease, the serum of these animals formed a heavy precipitate when mixed with virus extract and incubated for a suitable period. No precipitate occurred, however, when the virus extract was added to serum drawn from a rabbit previous to injection of the plant juice. Furthemore, precipitin absorption experiments showed that the addition of virus extract to serum from which all the normal plant proteins had been precipitated and drawn off, resulted in the formation of a heavy flocculent precipitate. This was interpreted as evidence of the presence of material in tobacco virus extract not found in normal juice, and antigenic in nature when injected into an animal.

Matsumoto (49) using a similar method of hyper-immunization likewise produced precipitins in rabbits for virus extracts of tobacco affected with mosaic disease.

Purdy (68) also demonstrated that leaf extracts of tomato, pepper and petunia, affected with the virus of common field mosaic disease, reacted with antiserum for virus extract

of tobacco to form specific precipitates.

Matsumoto and Somozawa (50) hyperimmunized rabbits separately for virus extracts of both tobacco and tomato. They then demonstrated by precipitin absorption experiments that all precipitins in either antiserum could be removed by complete absorption with the virus extract of tobacco or tomato, thus proving the identity of the specific antigenic material in the case of both hosts of the virus.

## Classification and source of viruses used.

### Tobacco:

The application of the technique of Johnson (39) to the tobacco mosaic virus involved throughout these studies revealed the fact that on the basis of his method of classification it falls under the title of Tobacco Virus I.

Type: Allard. U.S.D.A. Bul. 20. 1914.

Host Family: Solanaceae.

Differential Hosts: On tobacco: marked mottling, malformation, stunting.
On N. glutinosa: stem and leaf necrosis, no mottling.
On tomato: mottling and stunting, no stem necrosis.
On pokeweed: no symptoms.

Resistance to ageing in vitro: Several years.

Thermal Death-point: 90°C, 10 minutes.

Resistance to chemicals: High.

(60% alcohol or 1:200 $HNO_3$ does not kill in one day.)

The original diseased material was obtained in the summer of 1928 from the Farnham district, Province of Quebec. Subsequent inoculations into plants which were later ground in a meat chopper and squeezed in a small fruit press gave a large quantity of highly infectious juice. Some of this was preserved in the fall of 1930 in a large bottle under a layer of thymol, and in the following experiments either this juice itself or freshly extracted juices from plants previously inoculated with it were used. All extracts, unless otherwise indicated, were passed through a Berkefeld N filter before use.

The host plant throughout was the variety Resistant Havana.

Potato:

The potato mosaic material was obtained in the form of infected tubers of the variety Early Rose from Franklin Centre, Quebec, in the fall of 1931. The parent plants showed almost 100 per cent mild mosaic infection in the field.

In the nutrition experiments reported in this paper, the tubers were fed to the animals as such. In the serological studies, juices were obtained from healthy and diseased tubers and the plants which developed from them in the greenhouse. These juice extractions were made in a similar manner to those

from tobacco, and all samples were filtered through a Berkefeld N filter before use.

Schultz and Folsom (77) classify the particular mosaic involved, on the basis of external symptoms, as Mild Mosaic of Potato.

Clover:

Clover plants, infected with the mosaic disease, were collected from the trial plots at Macdonald College, St. Annes, during the summer of 1931. These plants, together with uninfected ones, were dried out and stored in boxes in a cool room until used in the nutrition studies.

No juice extracts were made, the material being fed to the animals in the form of dried hay.

The disease is described by Elliott (28) as Mild Mosaic of Clover.

## Experiment No. I

### To determine the effect of certain cultural treatments on the leaf structure of mosaic tobacco plants.

Two of the customary practices in the commercial growing of tobacco are firstly to remove approximately the top eight inches of each plant about the time of blooming, and secondly to break off the suckers growing at the point of conjunction of the leaf petioles and the main stem. These treatments, commonly known as "topping" and "suckering," are designed essentially to control the growth and time of the maturity of the plant and to give improved quality to the leaf.

Nelson(61), working with Havana seed tobacco finds that the degree of topping and suckering plays an important part in determining the quality of the cured leaf. He concludes that low topping consistently gives better quality than high topping, and that several suckerings tend toward higher yields but heavier, darker leaves.

It was considered worthy of study to try and determine the effect of certain degrees of topping and suckering on the leaf structure of both healthy and mosaic Resistant Havana tobacco plants. Treatments with healthy plants were conducted at the Central Experimental Farm, Ottawa, and with diseased

plants at Macdonald College, St. Annes. The experiment was started in June, 1931 and the examination of the sectioned leaf material completed in January 1932. Inoculations of the plants from which the diseased leaf tissue was obtained were made shortly after the plants were set out in the field, the inoculum being infectious juice stored under thymol from the previous year. In all cases the symptoms showed very definitely at the time of topping. In the case of the healthy plants, four vigorous, normal plants were used in each treatment, samples being taken in each case from all four. Only two plants were available in each treatment for the mosaic material. For both healthy and diseased plants, topping denotes the removal of the uppermost eight inches of stalk and any blossoms or leaves attached thereto. At the time topping was carried out the blossoms were just opening.

Degrees of topping and suckering for healthy and diseased plants:

(1) Plants topped once (July 31st, 1931) and suckered once two days before the leaf samples were taken at the time of harvesting (August 21st, 1931).

(2) Plants topped once (July 31st, 1931) and suckered threetimes between the time of topping and harvesting (August 21st, 1931). First suckering carried out just before early bloom stage.

(3) Plants allowed to mature and produce seed. No topping

or suckering was carried out. This, the so-called seedpod type, represents the normal growth and development of the plant. It was used as a standard, any deviation from it indicating the extent to which the leaf could be changed anatomically as a result of the various treatments.

In all treatments, leaf material was taken from the bottom three to five leaves, middle eight to ten leaves, and top fourteen to sixteen leaves of the healthy and diseased plants. These samples, which were later sectioned and stained, were cut out of the centre of the leaves to the right of the midrib, great care being taken to avoid injury. As far as possible only the more chlorotic areas of diseased leaves were used.

Samples of leaf tissue were taken at the following stages of growth of the plants:

(1) Bud stage. The top of the plant had elongated to a considerable extent, but the upper stem portion was still succulent. This somewhat immature stage was about three to four days before blossoming commenced and topping was carried out.

(2) Early bloom stage. The first blossom had opened and the stem was more rigid but was still somewhat succulent and could be broken easily. This stage was about two or three

days after topping.

(3) Harvesting stage. The plants were mature and the stems quite woody. At this stage plants treated normally for commercial purposes would be cut down and removed to the curing barns.

Experimental methods.

Sectioning and staining:

The samples of leaf tissue taken at various times were treated alike as to killing and fixing, embedding, staining and sectioning methods. The most satisfactory killing and fixing agent was found to be stock acetic solution Chamberlain (17). This solution, which was used by Smith (83) in her cytological studies, seemed to penetrate rapidly and effectively.

After remaining in the killing and fixing agent for 24 hours, the tissue was washed and after passage through the range of alcohols was embedded in paraffin wax in the normal manner.

All sections were cut at twelve microns, this being apparently the most satisfactory thickness. Thinner sections tended to alter shape somewhat, and thicker ones did not give sufficiently sharp outlines after staining. This was particularly true of the older leaves. Both cross and longitudinal sections were made in all cases.

Of the stains tried, the simples ones were finally

resorted to. Sections were first stained for six hours in alcoholic safranin (Chamberlain pg. 51) followed by gentian violet dissolved in clove oil as a counterstain (Chamberlain pg. 55).

Cell measurements:

Measurements were made of the lengths and widths of no fewer than fifty of the cells in the palisade layer, upper epidermis and lower epidermis. They were also made of the lengths and breadth of closed stomata on the lower surface of the leaf. Owing to their very irregular shape in most instances cells of the spongy parenchymatous tissue were not measured. Other factors which were studied, and which are reported on later in this paper, were intercellular spaces in the spongy parenchyma tissue, crystalline deposits and veination.

In some cases with the mosaic leaf material some of the palisade cells had divided into two. Measurements were made only on the undivided types, since, for comparative purposes, this was considered preferable.

The various measurements recorded in the following tables were made with the use of a Zeiss microscope equipped with a standardized ocular micrometer. A compensating ocular and oil immersion objective (N.A.. 1.30) were used throughout.

In these tables the letters W and L at the heads of the columns refer to width and length of cell respectively. All values given are in microns.

## Table 1

Measurements of leaf cells of healthy tobacco plants topped once and suckered once.

| Stages. | | Epidermal Cells | | | | Palisade | | Stomata | |
|---|---|---|---|---|---|---|---|---|---|
| | | Upper | | Lower | | | | | |
| | | W | L | W | L | W | L | W | L |
| BUD | (Upper leaves | 1.5 | 2.1 | 1.4 | 2.4 | 1.1 | 3.3 | 2.1 | 2.6 |
| | (Middle " | 2.3 | 3.1 | 1.5 | 2.6 | 1.9 | 8.1 | 2.3 | 2.8 |
| | (Lower " | 3.0 | 5.1 | 1.7 | 2.9 | 3.3 | 8.4 | 2.5 | 3.2 |
| EARLY BLOOM | (Upper leaves | 2.0 | 2.5 | 1.3 | 2.2 | 1.2 | 4.3 | 1.8 | 2.3 |
| | (Middle " | 2.1 | 3.0 | 1.5 | 2.5 | 1.2 | 6.0 | 2.0 | 2.4 |
| | (Lower " | 2.3 | 2.9 | 1.6 | 2.5 | 1.3 | 6.5 | 2.1 | 2.5 |
| HARVEST-ING | (Upper leaves | 2.1 | 2.8 | 1.5 | 2.4 | 1.3 | 4.8 | 2.0 | 2.5 |
| | (Middle " | 2.1 | 3.1 | 1.6 | 2.7 | 1.3 | 7.0 | 2.1 | 2.7 |
| | (Lower " | 2.5 | 2.9 | 1.7 | 2.8 | 1.4 | 7.1 | 2.2 | 2.7 |

## TABLE 11

Measurement of leaf cells of healthy tobacco plants topped once and suckered three times.

| Stages | | Epidermal cells. | | | | Palisade | | Stomata | |
|---|---|---|---|---|---|---|---|---|---|
| | | Upper | | Lower | | | | | |
| | | W | L | W | L | W | L | W | L |
| BUD | Upper leaves | 1.5 | 2.1 | 1.4 | 2.4 | 1.1 | 3.3 | 2.1 | 2.6 |
| | Middle " | 2.3 | 3.1 | 1.5 | 2.6 | 1.9 | 8.1 | 2.3 | 2.8 |
| | Lower " | 3.0 | 5.1 | 1.7 | 2.9 | 3.3 | 8.4 | 2.5 | 3.2 |
| EARLY BLOOM | Upper leaves | 2.1 | 2.9 | 1.4 | 2.4 | 1.5 | 4.9 | 2.0 | 2.5 |
| | Middle " | 2.2 | 3.1 | 1.8 | 2.7 | 1.5 | 6.8 | 2.2 | 2.6 |
| | Lower " | 2.4 | 3.2 | 1.8 | 2.8 | 1.6 | 7.0 | 2.2 | 2.7 |
| HARVEST-ING | Upper leaves | 2.1 | 2.5 | 1.4 | 2.3 | 1.3 | 7.0 | 2.1 | 2.8 |
| | Middle " | 2.3 | 3.5 | 1.8 | 2.6 | 1.4 | 7.2 | 2.1 | 2.6 |
| | Lower " | 2.7 | 3.6 | 1.8 | 2.6 | 1.5 | 9.6 | 2.3 | 2.7 |

## TABLE 111

### Measurement of leaf cells of healthy tobacco plants not topped or suckered.

| Stages | | Epidermal Cells | | | | Palisade | | Stomata | |
|---|---|---|---|---|---|---|---|---|---|
| | | Upper | | Lower | | | | | |
| | | W | L | W | L | W | L | W | L |
| BUD | (Upper leaves | 1.5 | 2.1 | 1.4 | 2.4 | 1.1 | 3.3 | 2.1 | 2.6 |
| | (Middle " | 2.3 | 3.1 | 1.5 | 2.6 | 1.9 | 8.1 | 2.3 | 2.8 |
| | (Lower " | 3.0 | 5.1 | 1.7 | 2.9 | 3.3 | 8.4 | 2.5 | 3.2 |
| EARLY BLOOM | (Upper leaves | 2.0 | 2.9 | 1.5 | 2.2 | 1.3 | 4.9 | 2.2 | 2.6 |
| | (Middle " | 2.1 | 3.1 | 1.6 | 2.5 | 1.6 | 7.1 | 2.3 | 2.8 |
| | (Lower " | 2.3 | 3.6 | 1.8 | 2.6 | 1.9 | 7.3 | 2.4 | 2.8 |
| HARVEST-ING. | (Upper leaves | 2.1 | 3.2 | 1.6 | 2.2 | 1.4 | 5.7 | 2.2 | 2.6 |
| | (Middle " | 2.4 | 4.2 | 1.8 | 2.4 | 2.0 | 8.8 | 2.2 | 2.7 |
| | (Lower " | 2.6 | 5.5 | 1.8 | 2.9 | 2.2 | 9.7 | 2.3 | 2.8 |

Before dealing with the corresponding measurements on mosaic leaves, it might be well to make one or two observations in connection with the three tables already presented, and with other features noted during the examination of the tissues.

In the first place, all samples, irrespective of treatment, showed a slight decrease in cell size from the lower to the upper leaves. Longitudinal sections showed also that the so-called vein "islets" in the lower leaves were of a greater area than in the upper. There is, therefore, a general tendency for a dimensional decrease in the leaf components the higher up the plant the tissue is taken.

The upper leaves generally speaking would appear to show a somewhat erratic development, and the effect of the two treatments seems to be felt here quite definitely. It may be that the process of topping which retards seed formation and activates the plant to vegetative growth may have its major effect in the upper leaves.

The palisade cells are noticeably longer in the untopped unsuckered plants than in the topped and suckered once types, and to a lesser extent in the other treatment.

Both cross and longitudinal sections showed that the older and more mature theleaf the greater were the intercellular spaces in the parenchyma tissue.

The results for mosaic plants follow:

## TABLE 1V

### Measurements of leaf cells of mosaic tobacco plants topped once and suckered once.

| Stages. | | Epidermal Cells | | | | Palisade | | Stomata | |
|---|---|---|---|---|---|---|---|---|---|
| | | Upper | | Lower | | | | | |
| | | W | L | W | L | W | L | W | L |
| BUD | Upper leaves | 1.5 | 1.9 | 1.4 | 2.0 | 1.5 | 4.7 | 2.5 | 2.6 |
| | Middle " | 2.4 | 3.0 | 1.5 | 2.4 | 1.9 | 6.3 | 2.6 | 2.8 |
| | Lower " | 2.6 | 5.0 | 1.7 | 3.0 | 3.0 | 7.2 | 2.8 | 3.1 |
| EARLY BLOOM | Upper leaves | 1.6 | 1.8 | 1.5 | 1.8 | 1.3 | 5.6 | 2.5 | 2.6 |
| | Middle " | 2.3 | 2.6 | 1.7 | 2.3 | 2.0 | 6.8 | 2.7 | 2.9 |
| | Lower " | 2.5 | 4.7 | 1.8 | 2.7 | 3.1 | 7.2 | 2.9 | 3.2 |
| HARVEST-ING. | Upper leaves | 2.0 | 3.3. | 1.6 | 1.7 | 1.6 | 5.9 | 2.7 | 3.3 |
| | Middle " | 2.3 | 4.7 | 2.0 | 2.4 | 2.1 | 6.8 | 3.0 | 3.5 |
| | Lower " | 2.6 | 5.2 | 2.1 | 2.9 | 3.4 | 7.3 | 3.1 | 3.6 |

## TABLE V

Measurements of leaf cells of mosaic tobacco plants topped once and suckered three times.

| Stages | | Epidermal Cells | | | | Palisade | | Stomata | |
|---|---|---|---|---|---|---|---|---|---|
| | | Upper | | Lower | | | | | |
| | | W | L | W | L | W | L | W | L |
| BUD | (Upper leaves | 1.5 | 1.9 | 1.4 | 2.0 | 1.5 | 4.7 | 2.5 | 2.6 |
| | (Middle " | 2.4 | 3.0 | 1.5 | 2.4 | 1.9 | 6.3 | 2.6 | 2.8 |
| | (Lower " | 2.6 | 5.0 | 1.7 | 3.0 | 3.0 | 7.2 | 2.8 | 3.1 |
| EARLY BLOOM | (Upper leaves | 1.7 | 1.9 | 1.5 | 2.0 | 1.5 | 5.2 | 2.5 | 2.8 |
| | (Middle " | 2.3 | 2.9 | 1.7 | 2.4 | 2.0 | 6.5 | 2.6 | 3.0 |
| | (Lower " | 2.6 | 4.9 | 1.8 | 2.9 | 3.1 | 7.0 | 2.9 | 3.3 |
| HARVEST-ING | (Upper leaves | 2.8 | 4.4 | 2.1 | 2.0 | 2.0 | 6.1 | 2.6 | 3.4 |
| | (Middle " | 2.8 | 4.5 | 2.0 | 2.5 | 2.5 | 7.3 | 2.6 | 3.3 |
| | (Lower " | 2.9 | 6.1 | 2.1 | 3.0 | 2.7 | 9.0 | 3.2 | 3.6 |

## TABLE VI

### Measurements of leaf cells of mosiac tobacco plants not topped or suckered.

| Stages | | Epidermal cells | | | | Palisade | | Stomata. | |
|---|---|---|---|---|---|---|---|---|---|
| | | Upper | | Lower | | | | | |
| | | W | L | W | L | W | L | W | L |
| BUD | (Upper leaves | 1.5 | 1.9 | 1.4 | 2.0 | 1.5 | 4.7 | 2.5 | 2.6 |
| | (Middle " | 2.4 | 3.0 | 1.5 | 2.4 | 1.9 | 6.3 | 2.6 | 2.8 |
| | (Lower " | 2.6 | 5.0 | 1.7 | 3.0 | 2.6 | 7.2 | 2.8 | 3.1 |
| EARLY BLOOM | (Upper leaves | 1.7 | 1.9 | 1.5 | 2.0 | 1.5 | 5.2 | 2.4 | 2.6 |
| | (Middle " | 2.3 | 2.9 | 1.7 | 2.4 | 2.0 | 6.5 | 2.6 | 2.8 |
| | (Lower " | 2.7 | 4.8 | 1.8 | 2.9 | 2.8 | 7.0 | 2.7 | 3.0 |
| HARVESTING | (Upper leaves | 1.9 | 3.3 | 1.7 | 1.9 | 1.5 | 6.0 | 2.5 | 3.0 |
| | (Middle " | 2.7 | 3.7 | 1.8 | 2.5 | 1.9 | 8.1 | 2.9 | 3.2 |
| | (Lower " | 2.9 | 5.0 | 2.2 | 2.6 | 2.8 | 9.2 | 3.0 | 3.5 |

Considering these tables in a similar manner to those for healthy tobacco leaves we find much the same conditions. One notable feature is the apparent increase in size of the stomata in both treatments as compared with the normal. This can probably best be accounted for as a result of the slight enlargement of the leaf due to topping and to a lesser extent suckering and also to an increase in the number of cells due to hypoplasia.

Comparing the results for healthy with those of the mosaic samples, a few general deductions can be made.

Generally speaking there is a slight increase in the width of the epidermal cells, but a decrease in their length in the mosaic leaves.

The palisade cells of mosaic material are somewhat shorter than normal. This is of course particularly true where hypoplasia has resulted from the division of the palisade cells into two, but even in undivided types there is a slight reduction in length. (Plate I).

In both, the effect of many suckerings has led to a general enlargement of the cell components.

Additional factors:

Apart from the many cell measurements which were made, a number of other factors were noticed when examining the various samples of material.

In many cases the lower leaf tissue showed partial disintegration. In the field these older leaves are always the first to show wilting symptoms under dry conditions. Maximov (51) states that as a result of severe wilting the guard cells of the stomata become super-saturated with water. This results in an abnormally wide opening with a loss of power to reclose the stomata, and partial or complete desiccation of the leaf may take place due to distortion. The measurement of the cells of these leaves are therefore not as accurate as those from the middle and upper portions of the plant.

In the normal healthy tobacco plant there occur many crystalline bodies located mainly in the upper and lower epidermal cells. These are thought to be for the most part calcium oxalate crystals, and probably aggregate to form the "grain" of the cured tobacco leaf. The term grain refers to the crystalline bodies which are held in the leaf throughout curing and play an important role in the general grade and burning quality of the leaf. In all the healthy material examined an abundance of these crystal deposits was observed, but they occurred even more commonly in the mosaic tissues, (Plate II). Assuming these crystalline materials to be a product of the metabolism of the plant, a difference is at once observed between the respective metabolic activities of healthy and mosaic tobacco. There is an apparent speeding up

in the metabolic rate of mosaic plants, a deduction which is further borne out by testing for the catalase content of various healthy and mosaic leaves. This question is dealt with in a later experiment.

In the heavily diseased portions of the leaf, there is a distinct reduction in the intercellular spaces of the spongy tissue accompanied by hypoplasia of the palisade and spongy parenchyma cells. In healthy tissue the intercellular spaces are quite definite, but in nearly all the mosaic tissue studied there was a reduction in these spaces with a tendency for the cells to adopt somewhat cuboidal shapes, (Plate III).

No evidence of the disintegration of the chloroplasts, as reported by Dickson was found. The staining methods employed, however, may not have been satisfactory for studying this possible modification.

Thicknesses of leaves:

In addition to the various measurements taken of cell sizes in the leaf samples from the topping and suckering treatments, the thicknesses of the leaves were also measured at bud, early bloom and harvesting stages.

A Randall and Stickney cover glass standardizer, consisting of a small metal plate and plunger between which the leaf was inserted and which registered the distance between plate and plunger on a dial attached to the instrument, was used

for making the observations. The dial was divided into degrees corresponding to thousands of an inch, and the values in the following tables are in these terms.

Thirty measurements were taken in the centre of the leaf near the midrib, care being taken to avoid large veins, and with mosaic leaves to avoid all but chlorotic areas. The averages of these thirty measurements are given in the tables.

## Table V11

### Leaf thicknesses of healthy tobacco plants subjected to various cultural treatments.

| Treatment | Stage | Leaves (in $\frac{1}{1000}$ inch.) Lower | Middle | Upper |
|---|---|---|---|---|
| Topped once, suckered once. | Bud | 14.97 | 9.43 | 9.00 |
| | Early Bloom | 15.43 | 10.80 | 9.03 |
| | Harvesting | 18.16 | 13.50 | 11.83 |
| Topped once, suckered three times | Bud | 14.97 | 9.43 | 9.00 |
| | Early Bloom | 16.06 | 11.10 | 9.50 |
| | Harvesting | 20.16 | 14.06 | 13.50 |
| Not topped or suckered. | Bud | 14.97 | 9.43 | 9.00 |
| | Early Bloom | 15.00 | 10.60 | 9.06 |
| | Harvesting | 16.00 | 11.24 | 12.80 |

## Table VIII

### Leaf thicknesses of mosaic tobacco plants subjected to various cultural treatments.

| Treatment | Stage | Leaves (in $\frac{1}{1000}$ inch.) Lower | Middle | Upper |
|---|---|---|---|---|
| Topped once, suckered once | Bud | 10.64 | 9.20 | 8,79 |
| | Early Bloom | 12.00 | 10.11 | 9.50 |
| | Harvesting | 16.36 | 13.27 | 10.88 |
| Topped once, suckered three times | Bud | 10.83 | 9.53 | 9.30 |
| | Early Bloom | 12.48 | 10.30 | 9.86 |
| | Harvesting | 16.99 | 14.00 | 11.55 |
| Not topped or suckered | Bud | 10.52 | 9.20 | 8.60 |
| | Early Bloom | 12.00 | 9.94 | 9.16 |
| | Harvesting | 12.81 | 11.62 | 10.87 |

It will be observed from the foregoing tables that the leaves of both healthy and mosaic plants are thicker than the normal where those plants were subjected to topping and suckering. Three suckerings have produced the heaviest type of leaf, while the unsuckered untopped plants gave rise to comparatively thin ones. Mosaic leaves are consistently thinner than healthy ones, irrespective of any particular treatment.

In concluding this experiment, it is well to recall that, so far as can be deduced, the spread of the virus through the leaf is very uneven. The reaction of the leaf may depend on its age at the time of infection or on the virulence or amount of the active principle, but the variations in both thickness and cell structure in a single leaf, and sometimes within a single area, indicate that the virus may be unequally distributed throughout the plant or one of its organs. There is no reason to believe that the cell structure is modified by the virus as such. The effects are apparently only limited to the inhibition or retardation of development.

## Experiment No. II

### The catalase content of healthy and mosaic tobacco leaves.

The enzyme catalase is present in all living tissues, including various animal organs, blood, secretions and excreta, plants and microorganisms. It is defined by Miller (57) as "a substance which is capable of decomposing peroxide into water and molecular oxygen without being able apparently to activate the oxygen thus liberated toward oxidizable substances." The equation for the reaction is as follows:

$$2H-O-O-H = 2H_2O + O_2.$$

Catalase, which was first isolated by Loew (46) in 1901 from tobacco, is apparently the most widely distributed of any of the enzymes.

In many cases it has been found that catalase activity and the intensity of respiration have paralleled each other. There are, however, certain variations from this. Rhine (73) noted that in the germination of the seeds of wheat, feterita, clover, mustard, radish and buckwheat there was always a drop in the catalase activity at the onset of germination, the low point being reached in from thirty minutes to several days, depending upon the type of seed. This decrease was nevertheless immediately followed by a sharp rise in the catalase activity. With regard to the association of respiration and catalase activity Rhine believes that the most plausible theory of catalase

formation in the plant seems to be that it is formed as an enzyme according to the theory of need - the presence of the substance which it attacks acting as a stimulus for the production of the enzyme. The decrease of catalase with germination could thus be explained by supposing the catalase reserve to be used up in attacking the respiratory products faster than it is produced. The high stimulation of the suddenly increased quantity of by-products would thus lead in a short time to the increased production of catalase observed. If this is the case, the catalase curve would follow, but never precede the respiration curve.

Harvey (36) finds that while they hydrogen ion concentrations of both leaf types were approximately the same, catalase activity is increased in mosaic tobacco as compared with healthy tobacco.

From the standpoint of studying the metabolic activity of a plant, while the amount of catalase cannot always be used as an index, yet for comparative purposes it has been adopted by many investigators. Thus Crocker and Harrington (20) find the amount of catalase in germinating grain to be correlated with their rate of metabolism.

Heinicke (37) concludes that the catalase activity of apple-leaf tissue is influenced by the factors which affect its nutritive or physiological conditions. He believes that the activity of catalase is a more sensitive measure of the metabolic status of the tissues than the usual chemical analysis,

and that it may serve, along with other measures as an indicator of the physiological responses of the plants to various cultural conditions or treatments.

Neller (60) studied the catalase content of Jonathan apples at various times after picking. He describes the apparatus used in these studies and with slight modifications his methods and apparatus were used in the present experiment.

Experimental procedure:

Apparatus:

As stated above, the apparatus used in these catalase studies was a slightly modified form of that employed by Neller. It consisted essentially of a glass container in which the macerated leaf was placed and into which a known quantity (10 c.c.) of hydrogen peroxide could be admitted. Through the rubber stopper placed in the neck of this container passed a glass stirrer and a delivery tube through which the oxygen evolved passed to a gas burette. In this burette the amount of water displaced registered the amount of oxygen discharged from the leaf tissue. A small electric motor rotated the stirring rod at a fairly rapid but constant speed. (Plate IV).

After placing the macerated leaf section in the container the rubber stopper was sealed in place to prevent any loss of oxygen. A slight modification was introduced at the point when the glass stirring rod passed through the cork. The bearing

described by Neller was not found to be entirely satisfactory, and eventually one in which the rod passed through a bearing composed of a short piece of rubber tubing contained in a slightly larger piece of glass tubing was perfected. This bearing was lubricated frequently with a mixture of graphite and glycerin to ensure of an air-tight connection.

To lessen further possibilities of leakage, as the water was displaced in the gas burettes both liquid levels were maintained at the same place. This counteracted the possibilities of excess strain through the stirrer bearing due to back pressure.

The material in the glass container was held at a temperature of 20°C in a water bath during the reaction.

Preparation of material.

In all cases tested, 10 sq. cms. of leaf tissue cut out of the centre of the leaf immediately to the right of the midrib, were macerated carefully in a mortar with a small amount of sand. To this macerated tissue were then added 30 c.c. of distilled water, the mixture being next placed in the container equipped with stirrer and delivery tubes.

Owing to the fact the activity of catalase deteriorates on ageing the material was tested immediately after maceration.

After sealing the stopper and testing for leakages by raising one column of the burette, 10 c.c. of fresh hydrogen

peroxide were run in through the side delivery arm. The stirrer was started immediately and the temperature of the water bath carefully checked at 20°C.

With all the samples tested the oxygen evolved was greatest in the first two minutes.

The stirrer, however, was allowed to rotate for twelve minutes and when no more bubbles were observed in the mixture, denoting that all the oxygen had been evolved, the reading on the burette was taken. The amount of water displaced represented the amount of oxygen evolved. The time for the completion of the reaction varied from 85 to 30 minutes, depending on the sample tested.

All fresh leaf material was taken from the middle leaves of the plants starting on September 24, 1931. Leaves in process of curing were tested between September 18th and 23rd. They were cut and transported to the curing barn on September 2nd.

In the following table "fresh" denotes that the leaves were taken from living plants. Curing leaves are described on the basis of their colour at the time they were tested. All results are for five different samples.

## Table IX

Time and amount of $O_2$ evolved from healthy and mosaic tobacco leaves.

| Leaf sample | | Time $O_2$ evolved | Amount $O_2$ evolved |
|---|---|---|---|
| Fresh healthy (young) | | 70 minutes | 10.7 c.c. |
| " " | (mature) | 60 minutes | 9.1 c.c. |
| Curing leaves | Green | 55 minutes | 8.5 c.c. |
| | Yellow | 40 minutes | 5.7 c.c. |
| | Brown | 40 minutes | 2.3 c.c. |
| Fresh mosaic (young) | | 85 minutes | 12.3 c.c. |
| " " | (mature) | 70 minutes | 10.0 c.c. |
| Curing leaves | Green | 45 minutes | 8.8 c.c. |
| | Yellow | 30 minutes | 2.9 c.c. |
| | Brown | 30 minutes | 2.5 c.c. |

From the foregoing tables it is clearly shown that the catalase content of fresh mosaic tobacco leaves is greater than that of healthy leaves, and with the sole exception of the yellow coloured leaves, this is true of leaves in three stages of curing.

In the cases of both healthy and mosaic samples, the catalase activity is reduced where the plants have been cut for some time.

Young leaves in both cases showed greater catalase content than mature ones. On the basis of metabolic status, this was to be expected.

Using the amount of oxygen evolved on the addition of hydrogen peroxide to the tissue as a comparative index of metabolism, diseased leaves show greater metabolic activity than healthy ones, this increase being presumably a direct result of the virus infection.

## Experiment No. III

## Studies to determine the external and internal effects of nitrogen and potassium deficiencies on the leaves of healthy and mosaic tobacco plants.

The tobacco plant reflects to a considerable degree in its outward appearance the nutritive condition of a soil. As an indicator plant in studying nutritional deficiencies of soil tobacco has of late years been used quite extensively in Germany and the United States. Lack of potassium shows up in the appearance of dark brown patches on the leaf, stunting of the plant and drying out of the lower leaves; lack of magnesium is indicated by the loss of the green colour at the tip and margins of the lower leaves; calcium deficiency is indicated by wrinkling and abnormalities of shape in the young leaves and nitrogen deficiency is indicated by the marked yellowing of the leaves successively from the base to about one-third the distance to the top.

Morgan (58) found tobacco to be more satisfactory than alfalfa, corn, lettuce, carrots, beets, onions, buckwheat and barley as an indicator plant of soil nutritive deficiencies under greenhouse conditions. In a large number of tests the specific mineral deficiencies showed up more quickly and more consistently on tobacco than any of the plants mentioned above.

McMurtrey (55) finds tobacco an excellent plant for indicating lack of calcium, potassium and magnesium in both the greenhouse and field. He attributes this to the fact that the large leaf registers growth responses very delicately.

Experimental methods:

Since the response of the tobacco plant to such mineral deficiencies is so definite, it was considered worthwhile to study the effect of very reduced amounts of nitrogen and potassium on tobacco both macro- and microscopically. Accordingly, thirty-six healthy Resistant Havana tobacco seedlings of uniform size and age were planted in six-inch pots coated on the inside with a thin layer of paraffin wax. Of this number, eighteen had been inoculated two days previously with tobacco mosaic virus. The pots contained a mixture of 90 parts of fine sand and 10 parts of surface soil very low in both nitrogen and potassium. The soil was included in order to improve slightly the general consistency of the sand which, in its pure form, is not very satisfactory from the point of view of aeration. Distilled water was added daily to the pots for four days, at which time it was considered that the seedlings had become established. The thirty-six pots were then divided up into six lots of six. These were treated as follows:

6 check healthy plants, fed a normal solution.

6 healthy plants, fed a solution deficient in nitrogen.

6 healthy plants, fed a solution deficient in potassium.

6 check mosaic plants, fed a normal solution.

6 mosaic plants, fed a solution deficient in nitrogen.

6 mosaic plants, fed a solution deficient in potassium.

The solution used for nutritional purposes was that of Crone. The normal and modified forms are given below.

Crone's solution.

| | Normal. | -N | -K |
|---|---|---|---|
| Potassium nitrate | 1.0 gm. | 0.0 gm. | 0.0 gm. |
| Iron phosphate | 0.5 " | 0.5 " | 0.5 " |
| Calcium sulphate | 0.25" | 0.25" | 0.25" |
| Magnesium sulphate | 0.25" | 0.25" | 0.25" |
| Potassium chloride | 0.0 " | 1.0 " | 0.0 " |
| Calcium nitrate | 0.0 " | 0.0 " | 1.0 " |
| Distilled water | 2000 c.c. | 2000 cc. | 2000 c.c. |

These solutions were given to the plants at the rate of 150 c.c. every two days.

Results:

(a) Macroscopic.

Growth and development of the plants was slow in all cases, and the treatments were conducted over a period of eighteen weeks. At the end of that time the outward appearances of the plants were as follows:

Check, healthy, normal: Plants normal in colour, and a tendency for a spreading type of growth. Height to growing point 3.5 inches.

Healthy, -N: Plants very yellow, small leaved and stunted. Height to growing point 1.6 inches.

Healthy, -K: Plants showing yellow blotches in some of the lower leaves. Somewhat stunted. Height to growing point 2.8 inches. (Plate V).

Check, mosaic, normal: Plants show typical mosaic symptoms, but quite stunted. Height to growing point 2.3. inches.

Mosaic, -N: Plants very small. Leaves pale yellow in colour. Hyperplastic areas more or less obscured. Height to growing point 1.1 inches.

Mosaic, -K: Plants somewhat smaller than those on the normal solution. Yellow areas on leaves less common than on healthy plants. Mosaic symptoms clearly defined. Height to growing point 2.0 inches.(Plate VI).

(b) Microscopic.

After these notes were made, typical leaf samples were taken for sectioning in precisely the same manner as outlined in Experiment I. Different stains however were used, the tissue being first stained for 3 hours in Delafield's haematoxylin and counterstained in alcoholic safranin.

Sections of material from four leaves taken from different plants were made and studied for abnormalities in upper and lower epidermis, palisade cells, parenchyma cells, intercellular spaces, crystalline bodies, cuticle and chloroplasts.

Cross sections were made of leaf tissue and also of the midrib about the centre of the leaf. Other details as to methods etcetera apply as for Experiment I.

The following table gives in a concentrated form the results obtained from the measurements of various cells comprising the leaf tissue samples examined. The figures given are averages of fifty measurements made in each case, with the exception of the cuticle, where only twenty were made.

## Table X

Comparison of leaf tissues of healthy and mosaic tobacco grown in sand cultures deficient in Nitrogen and Potassium.

| Material | Epidermal cells | | | | Palisade | | Cuticle | Width of entire leaf. |
|---|---|---|---|---|---|---|---|---|
| | Lower | | Upper | | | | | |
| | W | L | W | L | W | L | W | |
| Healthy, Normal | 1.8 | 2.4 | 2.1 | 3.4 | 2.8 | 7.9 | 0.36 | 20.6 |
| Healthy, - N | 2.3 | 2.4 | 2.4 | 3.6 | 2.6 | 8.0 | 0.5 | 24.3 |
| Healthy, -K | 2.0 | 2.2 | 2.2 | 3.5 | 2.4 | 7.0 | 0.4 | 21.1 |
| Mosaic, Normal | 2.0 | 2.4 | 2.7 | 3.1 | 2.6 | 6.5 | 0.36 | 19.2 |
| Mosaic, - N | 1.9 | 2.1 | 2.8 | 3.0 | 2.5 | 6.9 | 0.48 | 22.4 |
| Mosaic, - K | 2.0 | 2.2 | 2.5 | 3.0 | 2.4 | 6.5 | 0.40 | 19.8 |

Measurements in U.

It will be seen from this table that despite the very outstanding external appearances of plants deficient in nitrogen and potassium, internally changes of cell size etcetera are comparatively slight. The palisade cells of the mosaic tissue are generally shorter, but this applies where the plants were fed normal solutions equally as much as where they were fed solutions deficient in nitrogen and potassium.

One interesting change is that which has apparently taken place in the thickness of the leaves. Both with healthy and mosaic types, the result of deficiencies in nitrogen and potassium has been to produce a slightly thicker leaf. In studying fertilizer requirements where tobacco is being grown commercially, and where leaf "body" is of major importance, this factor must be taken into consideration.

Amongst the other features noticed during the study of the sections was the reduction in the number of chloroplasts in both healthy and mosaic leaves grown in deficiencies of N and K. This was particularly true where there was a lack of nitrogen, this tissue also showing greater intercellular spaces than the other types. The leaf material from mosaic plants grown in a deficiency of potassium showed very strikingly the hypoplasia and tendency towards cuboidal cell shapes described by Dickson (21). The palisade cells were quite rectangular in

outline, and spaces between cells were very limited.

Crystalline bodies appeared more profusely in the leaves of mosaic plants fedon normal Crone's solution than in the leaves of healthy plants fed on a normal solution or on solutions deficient in potassium and nitrogen. Hardly any traces of crystalline bodies of any sort could be found in the mosaic leaves grown under conditions of potassium and nitrogen starvation. No reason can be given for this phenomenon, unless it be that the reduction in the chlorophyll content of the leaf as a result of the mineral deficiencies, coupled with the effects of the virus, cause a great reduction in the metabolic activities of the plants concerned.

Cross sections of the leaf midribs showed no abnormalities existing in these structures. In every case, the arrangement of the vascular elements was apparently quite normal.

## Experiment No. IV

## Animal nutrition studies with potato and clover mosaic diseases.

One of the interesting possibilities which suggests itself to the worker on plant virus diseases is whether or not chemical changes take place within the plant as a result of the infection, and along what lines these changes might develope. Apart from chemical analysis, in the case of a few mosaic diseases, it should be theoretically possible to determine if there has taken place any change in vitamin content and general nutritive value, by studying the growth and development of experimental animals fed on infected plant material. Since many plants which are hosts to one or more mosaic diseases are normally unpalatable to suitable animals, the possibilities of research along these lines is limited. Potatoes and clover, however, the two hosts used in these experiments, are generally speaking quite suitable foods for most herbivorous animals, and it was considered an interesting problem to study the changes in growth, and development, if any, when potatoes and clover infected with mosaic disease were fed to experimental animals.

The writer has been unable to find any reports of previous work on this particular problem, and the results presented herewith are purely of a preliminary nature. From the results obtained, it would appear that a much closer analysis

of the various factors involved is necessary before any conclusive deductions can be drawn.

Experimental procedure:

Preliminary trials were made in which young rabbits, both male and female, were fed on mosaic potato tubers augmented with a small daily portion of whole oats. A number of deaths occurred, however, all animals losing weight and quite obviously failing to thrive on the ration.

The guinea pig was therefore substituted as the experimental animal, since large numbers were available and this animal has been used extensively in many types of research work. In short preliminary trials it appeared to take to the potato diet more satisfactorily than the rabbit.

Experiment A.

Blood counts (red cells, white cells and percentage haemoglobin) and weights were taken of nine guinea pigs, five of which were males and four females. All the animals were of fairly uniform size and weight, ranged in ages from ten to fourteen weeks, and had been previously fed on a diet of roots, oats and cabbage.

After carefully noting the weights of all animals, including an additional nine guinea pigs which were used as checks, they were gradually placed on a new diet composed of

50 parts by weight of mosaic or healthy potato tuber, and 50 parts by weight of whole oats, over a period of approximately twelve days. The experimental animals were given 20 grams of mosaic tuber and oat mixture each day, and the checks 20 grams of healthy tuber and oat mixture. The potato variety was Early Rose, and the healthy tubers were from certified stock. Weights of all animals were taken every second day after their introduction to the new diet.

To ensure against the possibility of faecal contamination, the guinea pigs were kept in separate wire cages with a ½ inch mesh floor. The room temperature was always cool and never exceeded 68°F. Distilled drinking water was before the animals at all times.

Results:

At various times up to 24 days from the time they were started on the potato diet, eleven of the animals died. Of these, three males and three females were check animals, and two males and three females had been feeding on the mosaic potato diet. In all cases post mortem examinations were made, but none of the pathological conditions discovered could in any way be assumed to have resulted from the effect of the virus in the diseased tubers. Eight of the eleven dead guinea pigs showed an abnormal amount of gas in the intestinal tract, notably

the stomach, but this condition occurred more in the check animals than in the experimental ones. Other complications, common to both check and mosaic-potato fed animals, were in some cases definite signs of haemorrhage in the lung and large and small intestines, enlargement of the gall bladder, occasional spotting on the liver and enlargement of the adrenal glands.

The semi-digested food in the stomach was in a very liquid acidified condition, and there were no symptoms of constipation which might at first be considered to result from the heavy carbohydrate ration.

Blood counts taken of the remaining seven living animals showed no signifiant changes apart from a slight increase in the percentage haemoglobin exhibited by those fed on mosaic potato. Since only four animals were tested, however, and the increases were slight, nothing definite can be assumed from this observation.

After reading the blood counts, the remaining living animals were etherized and examined twenty-seven days after starting on the new diet. Abnormal gas formation was exhibited in all but one case, and other symptoms were found which had been observed in the dead animals previously examined. No symptoms which could in any way be associated with the virus were discovered. This also applied to the weights of the animals which had been taken every two days. Very erratic changes

both gains and losses in weight, were exhibited quite irrespective of diet.

It was assumed that either the guinea pig was not a suitable type of animal for this type of investigation or else the ration was not sufficient to support normal life. One interesting observation was made, however, in connection with the palatability of the healthy and mosaic tubers. Ten days after the experimental animals had been feeding on the mosaic potato diet, pieces of healthy and mosaic tuber were placed in their cages at feeding time. In all cases the healthy tuber was consumed first, the diseased one often remaining untouched until the following day. It was thus demonstrated that in the cases of seven animals tested, healthy Early Rose potato tuber was more palatable to guinea pigs than mosaic tuber.

Experiment B.

As a result of previous complications, it was decided that the ration was unsatisfactory from the standpoint of supporting life. A balanced ration for guinea pigs was therefore sought, in which the carbohydrate portion could be made up as far as possible of healthy or mosaic potato tubers.

Dr. E.B. Hart, of the University of Wisconsin, suggested the following ration:

69 parts of rolled oats.

25 " " alfalfa meal heated in autoclave for 30 minutes.

5 " " casein.

1 " " salt.

This was modified to make up a ration including potato as follows:

69 parts of potato dry weight (350 parts wet weight)

25 " " alfalfa meal heated in autoclave for 30 minutes.

5 " " casein

1 " " salt

The method of mixing the ration was to pass the potato tuber through a meat chopper and then mix all the constituents together in a bowl in their proper proportions. Approximately 50 grams of the mixture was given to the animals each day, and distilled drinking water was before them at all times.

Eight young guinea pigs, weights ranging between 150-200 grams, were used in this experiment. Of these, two males and two females were fed on a mixture containing mosaic tuber, and two males and two females on a mixture containing healthy tuber. As before, blood counts were taken and weights recorded every two days. The new ration was introduced to the animals over a period of ten days.

Results:

Here also the mortality rate was high, only one animal remaining alive after 21 days from the time of starting on the new diet. Post mortem examinations showed very similar conditions to those found in Experiment A, though the formation of gas was less common. No single cause could be given to account for the many deaths, and it was perforce concluded that either the diet or the animals were unsatisfactory.

Experiment C:

This experiment was conducted in precisely the same manner as Experiment B, above, with the exception that dried healthy and mosaic clover plants were substituted for healthy and mosaic potato tuber in the ration. The proportion of clover used was calculated on a dry weight basis. It was then broken up by hand into small pieces and mixed in with the alfalfa meal, casein and salt.

Results:

Despite the fact that clover is normally very palatable to the guinea pig, and that the ration given would thus be assumed to be almost ideal, all the eight animals died within seventeen days.

Examination showed many of the symptoms found in potato-

fed animals, there being evidence of lung and intestinal haemorrhages, enlarged gall bladders and some gas formation.

From the above results, only two conclusions can be made. In the first place the guinea pig would not appear to be a suitable animal for studies of this nature, reacting very unfavourably, as it presumably does, to changes of diet. And in the second place, mosaic infected Early Rose potato tubers were shown to be less palatable to guinea pigs than healthy tubers of the same variety. During the course of the work, however, many interesting problems were uncovered, and on this account it should be of value at least to the animal pathologist. Such features as gas formation and its relation to the particular diet fed to the animal, the occurrence of haemorrhages and their relationship with other pathological symptoms and the precise cause for complications in the adrenal glands are all problems worthy of investigation.

The blood counts of guinea pigs at this period of the year, based on counts made on 42 animals, are as follows:

| | Red cells. | White cells. | Haemoglobin |
|---|---|---|---|
| Males | 5,700,000 | 9,500 | 97% |
| Females | 5,650,000 | 9,400 | 98% |

It is the writer's opinion that if a suitable animal could be found for this study, the results would be of considerable importance and interest. In this regard, it may be that

white rats would prove satisfactory, since the rat is normally of a scavenging nature and appears to subsist on a very assorted and often meagre supply of food.

## Experiment No. V

### Blood and serum reactions.

Gruber and Durham (35) in 1896 observed that when a suspension of bacteria was mixed in vitro with serum from an animal that had been injected previously with cultures of the same microorganism, the individual bacteria were brought together in clumps, i.e. agglutinated. These investigators also recognized the specificity of the reaction. The following year, Kraus (41) demonstrated that cell-free culture filtrates of bacteria precipitated the serum drawn from animals that had received injections of the filtrate. While the early work of Kraus dealt chiefly with bacterial proteins, it was soon discovered that many different kinds of foreign proteins may cause a similar flocculation of the serum of animals into which they have previously been injected.

Practical application of these phenomena of specific agglutination and precipitation and of other antigen/antibody reactions to the diagnosis of disease and the identification of proteins rapidly followed their discovery. Among the various uses to which these reactions have been put may be mentioned: the diagnosis of typhoid fever by the Widal agglutination test, the diagnosis and typing of Pneumococcus, blood groupings necessary in blood transfusions, detection of food adulteration and delicate tests for proteins present in so minute a quantity

as to render their identification by the application of chemical methods exceedingly difficult.

In the field of plant studies it is only within recent times that use has been made of these serological relations. Purdy (67, 68), Matsumoto (49) and Matsumoto and Somazawa (50) between the years 1928 and 1930 produced precipitins in rabbits for virus extracts of tobacco affected with the mosaic disease.

In studies with plant pathogenic bacteria work has been done by Sharp (79), Link and Hull (44), Link and Taliaferro (45) and Goldsworthy (31), as a result of which it has been shown that serological methods are of value in recognizing and separating the various species. The possibility of the existence of distinct serological strains within any single species, however, has not as yet been the subject of any extensive amount of work. Williams and Glass (89) produced a high-titred agglutinating serum for Phytomonas malvacearum, the causal organism of leaf spot of cotton, but absorption tests failed to establish any fundamental serological differences between the strains used.

It seems natural that serological technique should be applied as an aid in determining the systematic relations of economic plants in breeding work, and that geneticists have given heed to it as a possible means for determining invisible characters. Zade (92) applied the precipitin test in an effort

to determine the systematic relationships of oats and wheats. He found that a close agreement obtained between serological relationships and the relationships determined by morphological and breeding criteria.

Nelson and Birkeland (62) used immunological technique to differentiate varieties within species, and demonstrated that the precipitin test, when conducted with the purified globulin fraction of wheat proteins, is of value to the geneticist in selecting hybrids for desired invisible characters, such as resistance of wheats to Puccinia graminis tritici. When any one variety is used as a standard, others can be classified serologically, the wheats showing the greatest number of genetic factors in common exhibiting the closest relationship serologically.

Immunological technique has also been used to determine compatability of individuals for grafting - Dontcho (23) and Green (34).

In recent work, Helen Purdy Beale (68b) found that no precipitate occurred when leaf extracts of Sudan grass, Hippeastrum, lily and abutilon, each affected with its respective mosaic disease, were treated for their ability to precipitate antiserum for virus extract of tobacco mosaic disease. Beale concludes that the specific antigenic substance in mosaic tobacco extract is foreign antigenic material, possibly virus itself,

rather than altered host protein.

Experimental procedure:

Method of hyperimmunization.

Preparation of antigens:

Extracts of healthy and mosaic tobacco plants, healthy and mosaic potato tubers and healthy and mosaic potato plants were used for injection purposes in these experiments. All were prepared by first grinding the tissue in a meat chopper, squeezing out the juice through cheesecloth and passing it through a Berkefeld N filter under aseptic conditions.

Injection of animals:

Since most investigators have found rabbits to be satisfactory for studies of this nature, these animals were used exclusively. Injections were made in all cases into the marginal ear-vein of the rabbit. Five injections of 1 c.c. of the antigen were given at two day intervals, the last being followed with a rest period of 20 days. This rest period was included in order to ensure of a high precipitin titer. Only 0.5 c.c. of antigen was administered at the first injection following a rest period in order to avoid possible anaphylactic shock. The remaining 0.5 c.c. was injected about an hour later.

Collection of serum:

From 6 to 8 days after the last injection, the animals

were etherized and bled to death from the heart. The blood was collected in sterile vials, the antiserum being allowed to separate from the clot overnight. The following morning it was inactivated at 56°C (water bath) for one half-hour, and stored in sterile tubes in an ice chest until used.

Precipitin experiments.

The following antigens were used for injection purposes during the course of these studies:

1. Aged mosaic infective tobacco juice.
2. Freshly extracted mosaic infective tobacco juice.
3. " " healthy tobacco juice.
4. " " mosaic infective potato plant juice.
5. " " healthy potato plant juice.
6. " " mosaic infective potato tuber juice.
7. " " healthy potato tuber juice.

One-half c.c. each of undiluted antigen and antiserum thoroughly mixed in sterile tubes were incubated for one and one-half hours at 37°C in an incubator room and examined for the presence of precipitate. The tubes were then placed in in the ice chest, a final record being made the following morning of the presence or absence of precipitate. A large number of antigen/antiserum combinations were studied, each test being repeated at least three times and control tubes containing saline solution being included.

**Results:**

In the following tables are presented the results of the antigen/antiserum combinations using tobacco and potato, with the amounts of precipitate obtained in each case. The results from test control tubes are not included, but in all instances whether saline solution replaced antigen or antiserum, no sign of a precipitate was observed. This applies also in control tests using serum from non-inoculated rabbits.

## Table XI

Precipitin tests with antisera for tobacco mosaic virus and extracts of tobacco plants.

| Antigen | Antiserum | Precipitate ++ |
|---|---|---|
| Aged infective tobacco juice. | For aged infective tobacco juice | X X X X |
| Fresh infective tobacco juice. | For fresh infective tobacco juice. | X X X X |
| Fresh healthy tobacco juice. | For fresh healthy tobacco juice. | X X |
| Fresh infective tobacco juice. | For fresh healthy tobacco juice. | X X |
| Fresh healthy tobacco juice | For fresh infective tobacco juice. | X X |

++

X X = moderate precipitate.

XXX X = very heavy precipitate.

## Table XII

Precipitin tests with antisera for potato mild mosaic virus and extracts of potato plants and tubers.

| Antigen | Antiserum | Precipitate ++ |
|---|---|---|
| Healthy tuber juice | For healthy tuber juice | XXX |
| " " " | " mosaic tuber juice | XX |
| " " " | " healthy plant juice | X |
| " " " | " mosaic plant juice | X |
| Mosaic tuber juice | " mosaic tuber juice | XXXX |
| " " " | " healthy tuber juice | XX |
| " " " | " healthy plant juice | XX |
| " " " | " mosaic plant juice | XXX |
| Healthy plant juice | " healthy plant juice | XX |
| " " " | " healthy tuber juice | X |
| " " " | " mosaic tuber juice | X |
| " " " | " mosaic plant juice | XX |
| Mosaic plant juice | " mosaic plant juice | XXXX |
| " " " | " healthy tuber juice | X |
| " " " | " mosaic tuber juice | XX |
| " " " | " healthy plant juice | XX |

++
X = slight precipitate.
XX = moderate precipitate.
XXX = heavy precipitate.
XXXX = very heavy precipitate.

Heaviest precipitates occur in the mixtures of virus tobacco and potato extracts and the homologous antisera. This is clearly shown to be the case when using aged mosaic tobacco juice, fresh tobacco juice, fresh potato tuber juice and fresh potato plant juice as the antigens.

It will be observed that precipitation also occurs between mixtures of undiluted antigen and all the antisera prepared. In most cases the amounts are small, and are attributable to the presence of precipitins to the normal proteins in the juice extracts. The precipitate obtained from the mixture of healthy potato tuber juice and its homologous antiserum, however, and the equivalent where healthy plant juice is used is significantly greater in amount. It is assumed that the protein materials present differ in their antigenic properties, resulting in different amounts of precipitate.

The results indicate clearly the presence of antigenic material in virus plant extracts which does not occur in healthy ones. This may be foreign protein material (virus), or host protein altered by the action of the virus. It is impossible to say which from the results, though Purdy (68) concludes from similar studies that the antigenic material present is foreign protein.

Inoculations.

Series of Resistant Havana tobacco plants were inoculat-

ed with samples of the top liquid and precipitate of all the homologous virus antigen/antiserum combinations. No symptoms of disease showed where the potato mosaic virus was involved, although preliminary tests with juice extract showed that the mosaic developed well on tobacco as its host.

With the tobacco mosaic virus, no symptoms developed from inoculations with the top liquid, but is presence was demonstrated in the precipitate. Of ten plants inoculated in each case, one showed symptoms after twelve days where aged infective juice was used as the antigen, and two where freshly extracted infective juice was used as the antigen. It was assumed that the 50/50 mixture of antigen/antiserum was incorrectly balanced to allow of complete inactivation or neutralization of the virus.

Inoculations with mosaic antisera and blood clots to which had been added 5 c.c. of saline solution showed no evidence of the viruses of either tobacco or potato mosaic. Even supposing the virus to be present, however, the dilution factor would probably be sufficiently great to offset the possibility of reinfection phenomena.

Absorption tests.

The observation that virus extract reacts more readily with the homologous antiserum, as indicated by the production of a very heavy precipitate when using both tobacco and potato, led

to the assumption that there might be some substance present in virus extract not found in healthy leaf extract that is responsible for the reaction. Consequently, precipitin absorption experiments were undertaken in which the precipitins to normal tobacco extract were absorbed from the antiserum for virus extract by adding small amounts of juice from healthy tobacco leaves, healthy potato tubers and healthy potato plants and incubating the mixtures for one and one-half hours at 37°C. After standing in the ice chest overnight the mixtures were centrifugalized to throw down the precipitate. The supernatant liquid was then drawn off and more juice added to the partially absorbed serum. This process was continued until no precipitate was formed upon the addition of normal juice, whereupon the absorption was regarded as complete. The process required five days in the case of freshly extracted tobacco juice, seven days in the case of freshly extracted potato plant juice, and ten days in the case of potato tuber. The amounts of juice added amounted to 1.1 c.c. for tobacco, 1.4 c.c. for potato plant and 1.9 c.c. for potato tuber.

Subsequent addition of the corresponding virus extracts to the antisera from which all the precipitins to normal juice had been absorbed resulted in the formation of heavy flocculent precipitates, only slightly smaller than where the absorption had not been attempted. The precipitate in the mosaic potato

tuber mixture was not increased as much as in the other two, and no explanation for this can be given unless it is supposed that the tubers from which the juice was extracted were themselves slightly infected. Since the Early Rose potato is one of the red-centre types, which are notoriously difficult to produce perfectly free from mosaic, this explanation may suffice.

The results are interpreted however as additional evidence of the presence of material in virus extract not found in normal juice and capable of stimulating the production of antibodies, in this instance precipitins, upon injection into an animal.

Hypersensitivity.

Using guinea pigs as experimental animals, a few preliminary tests were carried out with mosaic-infected and healthy tobacco plant extracts in an effort to determine whether or not the reactions of the animals were correlated with the protein differences between the two juices. One-fifth c.c. of aged mosaic, fresh mosaic and fresh healthy juice was injected intraperitoneally followed by a second injection of one-half c.c. after ten days. Reactions, very similar to anaphylactic shock, were demonstrated in all cases, particularly where the aged mosaic juice was used. With this extract, a one c.c. injection into a medium sized guinea pig not sensitized by a previous injection, was sufficient to cause death in five minutes.

The conclusion was drawn that the symptoms were those of acute nicotine poisoning, and not anaphylactic shock as a result of the introduction of the protein into the blood stream. This was further substantiated by the fact that animals injected with fresh healthy juice showed almost the same reactions as those injected with fresh mosaic juice.

The results obtained show the necessity of first precipitating the nicotine in these juices if further studies on hypersensitivity are to be attempted. If this is not done, anaphylactic shock may be confused with nicotine poisoning, particularly where the amounts of juice injected are small.

## Experiment No. VI

## Filtration studies with tobacco mosaic virus.

In 1982, Iwanowski (38) first demonstrated that the causal agent of the mosaic disease of tobacco was filterable through a filter which would not allow the passage of Bacteria.

Much work in connection with filtration of viruses has been carried out since that time, the majority of it in an effort to determine the approximate size of the virus particles. Using porcelain filters, Duggar and Armstrong (27) calculated the size of the particles of tobacco mosaic virus to be 30 μμ. Olitsky (64) employing similar methods estimated the size of the virus particles of foot and mouth disease to be somewhere between 20 and 100 μμ. Pransnitz (66) estimated the size of Flexner bacteriophage as approximately 20 μμ.

In very recent work, Storey (86) has tried the effect of filtration through a number of different filters on the virus causing streak disease of maize. At a hydrogen-ion concentration of 6.0, juice from infected maize plants, to which had been added an equal quantity of 20% sucrose solution, was shown to retain its virulence after passage through Chamberland LI and Berkefeld V and N filters. The juice was less virulent after passage through a Chamberland L3 filter, and the virus failed to penetrate througha Seitz E.K. filter disc. Storey used leaf hoppers in determining whether or not the virus had passed through the filter. These insects, which are commonly

the vectors of streak disease of maize, were allowed to ingest the juice - made more palatable by the addition of the sucrase- and transferred to healthy maize plants. If, after a period of time, symptoms of the disease failed to develope, it was concluded that the particular filter used with that sample of juice had not permitted passage of the virus principle.

Experimental methods:

In our experiments the Seitz E.K. Germproofing filter only was used. The model was the "Salle," and it consisted of a metal chamber and delivery tube clamped tightly together on each side of one or two standard Seitz asbestos filter pads. The filter was held by means of a closely-fitting rubber stopper in the neck of a 1000 c.c. vacuum flask, and the apparatus was sterilized in the autoclave for 20 minutes before use.

Two lots of mosaic infected tobacco juice, one freshly extracted and the other which had aged under a layer of thymol for eighteen months, were used in the experiment.

Both extracts were filtered in 150 c.c. lots under suction pressure of 70 mm. of mercury, samples of the filtrate being collected in sterile test tubes after 30 seconds, 2 minutes, 5 minutes, 10 minutes, 30 minutes and when all the volume of 150 c.c. had been drawn through. The filtrates were in all cases very clear, and there was an approximate correlation between their colour due to the presence of pigments and the

time taken for their passage through the filter. This colour ranged from a very light brown after thirty seconds to a dark brown at the completion of the filtration.

In parallel ranges of tests either one or two asbestos pads were used.

Results:

These are given in the tables which follow.

## Table XIII

Virulence of 150 c.c. of eighteen month old tobacco mosaic virus juice at various times during passage through a Seitz E.K. Filter.

| Filtrate collected after: | No. of pads. | Plants inoculated. | Plants infected. |
|---|---|---|---|
| 30 seconds. | 1 | 5 | 0 |
| 2 minutes | 1 | 5 | 0 |
| 5 minutes | 1 | 5 | 0 |
| 10 minutes | 1 | 5 | 0 |
| 30 minutes | 1 | 5 | 0 |
| 10 hours | 1 | 5 | 0 |
| 30 seconds | 2 | 5 | 0 |
| 2 minutes | 2 | 5 | 0 |
| 5 minutes | 2 | 5 | 0 |
| 10 minutes | 2 | 5 | 0 |
| 30 minutes | 2 | 5 | 0 |
| 12½ hours | 2 | 5 | 0 |

## Table XlV

Virulence of 150 c.c. of freshly extracted tobacco mosaic virus juice at various times during passage through a Seitz E.K.Filter.

| Filtrate collected after: | No. of pads. | Plants inoculated. | Plants infected. |
|---|---|---|---|
| 30 seconds | 1 | 10 | 0 |
| 2 minutes | 1 | 10 | 0 |
| 5 minutes | 1 | 10 | 1 |
| 10 minutes | 1 | 10 | 10 |
| 30 minutes | 1 | 10 | 10 |
| 12 hours | 1 | 10 | 10 |
| 30 seconds | 2 | 10 | 0 |
| 2 minutes | 2 | 10 | 0 |
| 5 minutes | 2 | 10 | 0 |
| 10 minutes | 2 | 10 | 7 |
| 30 minutes | 2 | 10 | 9 |
| 11 hours | 2 | 10 | 10 |

The foregoing tables are self-explanatory. It would seem that the virus is prohibited from passing through the filter pad from the stored juice on account of the protein substances present being drawn down and forming a thick shining layer over the surface. The fermented juice took much longer to pass through the filter than did the freshly extracted material, doubltess on account of these colloidal substances and protein material, and examination of the pads after filtration showed a heavy, shining, dark-brown layer coated over the surface. The spaces through this coating were apparently too small to allow the passage of the virus particles, although the juice was quite virulent before filtration.

With the freshly extracted juice an electrical phenomenon is apparently involved in which the charge held by the pad must be neutralized before the virus can pass through. Between pH 4.0 and pH 9.0 the virus in freshly extracted juice carries a negative charge, according to electrophoretic tests made by Willstatter (90) and others. Assuming this to be the case, therefore, the charge held by the filter pad is presumably a positive one, which, when neutralized by the negative charges held by the virus will allow it to pass through. The time taken for the neutralization is between five and ten minutes when using one pad, and a somewhat extended period when the juice is passed through two pads.

It is curious that Storey found the Seitz filter immune to the passage of the virus causing streak disease of maize. No explanation of this phenomenon can be given, unless colloidal materials were present in the juice in sufficient quantities to form a layer over the pad surface impermeable to the virus particles. Additional work on a number of different viruses and their virulence after passage through a Seitz Germproofing filter should prove of considerable interest.

## Experiment No. VII

## Studies to determine the method of spread of the tobacco mosaic virus within the leaf.

It is, at the present time, not known in exactly what manner the infectious agent spreads within the tobacco leaf from the time of inoculation with the mosaic virus until the appearance of the disease symptoms.

Cook (19) concludes from recent studies with a number of mosaic diseases that the spread is very uneven, and that the virus may be unequally distributed throughout the plant. He considers that the active agent inhibits the differentiation of the cell structure from the time it reaches the meristomatic tissue, which remains in the stage of development it had attained at the moment of infection.

Caldwell (12) in studies on the physiology of the virus diseases of plants, has demonstrated that in the case of tomato mosaic the spread of the virus up the leaf, from inoculations made at the base, is not through the xylem vessels. He treated tomato leaves with chloroform in such a manner as to leave only the upper and lower portions attached by the xylem strands of the midrib. Inoculations with infective juice in the lower leaf portion resulted in their becoming infected, but the virus failed to travel in the xylem stream and infect the upper portion. Caldwell concludes that virus

movement takes place through the living ground structure of the leaf.

In testing mosaic and healthy tobacco leaves for starch content, the writer observed that in leaves affected with the virus the amount of starch in the chlorotic areas was considerably reduced. It was decided, therefore to make use of this modification in an effort to trace the method of spread of the infective agent within inoculated leaves.

Experimental procedure:

Inoculations:

During this investigation a large number of Resistant Havana tobacco leaves, of different sizes and ages, were inoculated with either freshly extracted or aged infective juice. All the plants used were reared in the greenhouse.

Inoculations were made at various places on the leaf surface, and were accomplished either by gently rubbing a blunt ended glass rod faced with linen and soaked in the inoculum on the leaf surface, or by making small punctures in the leaf with a piece of finely drawn out glass tubing dipped in the inoculum. In the former method, care was taken to avoid injuring the leaf, it being desired only to rupture the trichomes in the small area touched by the rod end, thus allowing the infective agent to gain access into the leaf.

A number of different areas on the leaf surface were inoculated. Using the glass rod, inoculations were made at the tip, middle and base of the midrib, and on the leaf tissue at tip, middle and base on either side of the midrib. In some cases small leaf veins were included, in others excluded from the areas inoculated. Similar points were chosen when the finely drawn out glass tubing was used in making the inoculations, and also punctures were made about $\frac{1}{2}$ cm. apart over the entire leaf surface.

Check plants, using sterile distilled water in place of the infective juice, were inoculated in a parallel series to the above. In all cases, any excess of inoculum was washed off the leaves with water.

Testing for starch content:

At various times after inoculation, usually two day intervals, check and treated leaves were cut off the plants close to the stalk. This was done only on bright days, at about 3.30 p.m. when it was considered that the amount of starch would be high. Check and inoculated leaves to be tested were boiled for five minutes in water, immersed for about thirty minutes in 95% methyl alcohol held at 80°C until all the chlorophyll was extracted and finally placed in a solution of iodine and potassium iodide. This solution consisted of 1 gm. of iodine and 0.25 gms. of

potassium iodide crystals dissolved in 100 c.cs of distilled water.

After immersion in the iodine solution for twenty minutes, the leaves were removed, placed in cold water for twelve hours and finally pressed and dried on photographic ferrotyping plates. When held against a source of bright light, a deep blue colour denoted the presence of starch, which was reduced in amount or absent in areas showing a brown or light yellow colo ration.

Results:

Leaves tested at two day intervals showed a progressive reduction in the amount of starch, correlated with the spread of the virus. (Plates VII and VIII). It was not possible to follow the exact path of migration, which would appear to be in a very irregular manner, since in some cases the amounts of starch were reduced in areas farthest away from the point of inoculation, and in others quite close. Generally speaking, however, the most significant reduction occurred at the edges of the leaf usually towards the tip. Points inoculated by means of the glass rod showed as black circular areas delimited by a thin blue line radiating irregularly from which were blotchy areas showing little or no starch. These areas were so indefinite that it was impossible to trace their exact path. After about twelve

days in the case of medium sized plants (10 - 12 leaves), the blotches seemed to merge, the whole leaf showing mixed starch-containing and non-starch-containing areas. (Plates VII and VIII). Where definite punctures were made in the leaf tissue, black circular spots developed surrounded by an irregular white halo which increased slightly in size up to about ten days after inoculation. After this period the fringes of the halo could not be traced, a general merging process apparently taking place as before. (Plate IX).

No evidence that the virus migrated along the midrib and leaf veins was obtained from midrib inoculations. The starch-testing method was apparently not sufficiently delicate for registering deficiencies in these areas.

It was concluded that the spread of the mosaic virus within the tobacco leaf was very irregular, but was correlated with a reduction in the amount of starch. It was apparent also that there was an unequal distribution of the infective agent, the first areas affected being in most cases the edges of the leaf. After the first ten days starch reduction tests were insufficiently delicate to trace the path of virus migration.

## Experiment No. VIII

## The effect of the mosaic disease on yield and quality of tobacco.

That mosaic disease of tobacco is a cause of loss to the tobacco grower is generally accepted, but just how much loss results under given conditions has been reported in only instance. McMurtrey (56), working with the variety Maryland Broadleaf in the southern Maryland tobacco district of the United States, found that three recognizable forms of damage were exhibited by green mosaic tobacco leaves. These were the ordinary mottling or mosaic form, the form in which the mottling is accompanied by necrotic spotting and the blister form which occurs on leaves showing no mottling. All forms were accompanied by reduction in total yields, and the cured leaf was as a rule badly spotted and discoloured. McMurtrey found that inoculation of the plants shortly after they were set out in the field resulted in 30-35 per cent reduction in yield and 55 per cent reduction in quality. When the infection took place one month after transplanting, the damage was almost as severe. Inoculation at topping time did not produce a significant reduction in yield, but even at this late date the quality of the crop was lowered by the development of the disease following late inoculation.

According to the Plant Disease Reporter (Supplement 80, 1931) of the U.S. Department of Agriculture, a survey of

the diseases affecting tobacco revealed the fact that in eleven of the sixteen states studied mosaic was reported as being the most common and severe of any of the diseases. Severe necrosis or "rusting" of the leaves, with its resultant deleterious effect on yield and quality, was reported as occurring commonly in Wisconsin, Massachusetts, South Carolina, Maryland, Connecticut and to a lesser extent California. The spread of tobacco mosaic in the field was observed to be associated with the topping and suckering processes, although such infection appeared late in the season and in most instances did not produce as severe damage as outbreaks which appeared earlier. In some instances mosaic infections appeared to be attributable to tobacco refuse around plant beds, and also to the use of natural leaf by persons working in the beds. In other instances infection was thought to have been carried to the beds during weeding on the hands and clothes of workmen who were engaged in handling the crop of the preceding year, or the virus may have been spread from plant to plant in weeding the beds or in transplanting.

The importance of tobacco mosaic disease cannot be too heavily stressed, since it is of world-wide distribution, and has been reported of epidemic proportions in some years from the tobacco growing districts of the United States, Canada,

South America, Africa, Italy, Turkey, Russia, India, the West Indies, Java and Sumatra. With this in view, it is surprising that more work has not been done on the actual losses sustained as a result of mosaic. On this account, the following results should be of value.

Experimental methods:

During the summer months of 1930 and 1931, crops of Resistant Havana tobacco were grown at Macdonald College, Quebec, in which plants were inoculated with the mosaic virus at various times during growth. In both years the tobacco plots consisted of 80 rows, 3 feet apart, with plants 1½ feet apart in the rows. The soil was a heavy clay loam, with a southern slope. In the spring of 1931, an 8-1-1 nitrogen-phosphoric acid-potash fertilizer was applied to the field at the rate of 1400 lbs per acre after the addition of manure.

Seedlings were raised either in the greenhouse (1930) or in hot beds (1931) and they were set out in the field when about 6 inches in height. In 1931 the seedlings were obtained from the Tobacco Division, Central Experimental Farm, Ottawa. They possessed somewhat longer stalks than those raised in the greenhouses at Macdonald College, and exhibited a more upright type of growth. Due to excessive heat at the time of planting, many renewals were necessary in the 1931 crop due to planted

seedlings dying off. The crop, taken as a whole, was therefore somewhat less uniform in growth and development than that for 1930, when only greenhouse seedlings were used and very few renewals made.

In both years, the weather was extremely hot at the time of planting, which necessitated watering the young plants in the field one or two days after setting.

The plots were divided up into four parts or replications of twenty rows each. Each replication was further divided into five plots of four rows each, with a general average of 27 plants per row. The plots were inoculated at various times with the mosaic virus, the method of inoculation being to rub infective juice on the under surface of the leaves with the aid of a piece of cheesecloth. Each replication was made up of 4-row plots inoculated at various times, with one 4-row plot not inoculated and used as a check. Times of inoculation were as follows:

| Inoculation. | 1930 crop. | 1931 crop. |
|---|---|---|
| Seedling. Seedlings inoculated in greenhouse before planting. | May 22nd | May 23rd |
| Planting. Seedlings inoculated at time of planting in field. | June 16th | June 14th |
| Hoeing. Plants inoculated at time of hoeing or cultivating in field. | July 26th. | August 8th. |
| Topping. Plants inoculated at time of topping. | August 2nd | August 12th |

Check. No inoculations.

Each of the four replications contained four check rows, four seedling inoculation rows, four planting inoculation rows, four hoeing inoculation rows and four topping inoculation rows, making a total of twenty rows in all.

In 1930, on September 10th and 11th, and in 1931 on August 25th and 26th, all but the end plants in each row were cut down, speared on wooden laths and taken to the curing shed. Here the laths, with seven plants on each, were suspended about four inches apart on wooden beams, and allowed to remain over winter until the plants had dried out and cured. When fully cured, the plants were removed from the curing shed, the leaves being later stripped off and graded into top leaves, middle leaves, bottom leaves and trash. Weights were taken of all grades in all plots, representative samples made up of 10% of the yield for each plot being later sent to the Tobacco Division, Ottawa, for quality grading and yield calculations.

Results:

In the following tables are presented the data as to yield and quality of the crops grown in 1930 and 1931. The results were calculated from 10% samples by weight from all plots replicated four times. Plot numbers are given collectively according to treatment, and on the basis of four replications.

## TABLE XV.

Acre yields of tobacco inoculated at different periods of growth with mosaic virus. Macdonald College, 1930.

| Plot No. | Treatment | No. of Plants | Plot Yields | Acre Factor | | Yield per Acre |
|---|---|---|---|---|---|---|
| | | | Lb. | | | Lb. |
| 1B | Seeding | 111 | 13.4063 | 81.1 | | 1087 |
| 2B | " | 92 | 11.5625 | 97.8 | | 1131 |
| 3B | " | 92 | 11.0625 | 97.8 | | 1082 |
| 4B | " | 66 | 6.9688 | 136. | | 948 |
| | | | | | Ave. | 1064 |
| 1C | Planting | 108 | 12.5625 | 83.3 | | 1047 |
| 2C | " | 84 | 10.3125 | 107 | | 1103 |
| 3C | " | 85 | 10.8281 | 106 | | 1148 |
| 4C | " | 84 | 10.3281 | 107 | | 1105 |
| | | | | | Ave. | 1101 |
| 1D | Hoeing | 100 | 13.3406 | 90 | | 1201 |
| 2D | " | 84 | 11.9062 | 107 | | 1274 |
| 3D | " | 100 | 15.0313 | 90 | | 1353 |
| 4D | " | 101 | 14.5547 | 89.1 | | 1297 |
| | | | | | Ave. | 1281 |
| 1E | Topping | 76 | 9.9168 | 118 | | 1170 |
| 2E | " | 100 | 13.8856 | 90 | | 1250 |
| 3E | " | 85 | 12.1093 | 106 | | 1284 |
| 4E | " | 99 | 15.1640 | 90.9 | | 1378 |
| | | | | | Ave. | 1270 |
| 1A | Check | 84 | 12.1718 | 107 | | 1302 |
| 2A | " | 101 | 12.2813 | 89.1 | | 1094 |
| 3A | " | 92 | 14.4431 | 97.8 | | 1413 |
| 4A | " | 100 | 14.2026 | 90 | Ave. | 1272 |
| | TOTALS | 1844 | 246.0386 | | | |

## Table XVI

Grade percentages and indices of tobacco inoculated at different periods of growth with mosaic virus.

Macdonald College 1930

| Time of Inoculation | Plot No. | Percentage of Assorted Grades | | | | Grade Index. |
|---|---|---|---|---|---|---|
| | | Trash | Lights | Darks | Totals | |
| Seeding | 1 | 100 | - | - | 100 | 7.0 |
| | 2 | 100 | - | - | 100 | 7.0 |
| | 3 | 100 | - | - | 100 | 7.0 |
| | 4 | 100 | - | - | 100 | 7.0 |
| | | | | | Av. | 7.0 |
| Planting | 1 | 100 | - | - | 100 | 7.0 |
| | 2 | 100 | - | - | 100 | 7.0 |
| | 3 | 100 | - | - | 100 | 7.0 |
| | 4 | 100 | - | - | 100 | 7.0 |
| | | | | | Av. | 7.0 |
| Hoeing | 1 | 93.8 | 6.2 | - | 100 | 8.4 |
| | 2 | 93.8 | 6.2 | - | 100 | 8.4 |
| | 3 | 95.2 | 4.8 | - | 100 | 8.1 |
| | 4 | 95.7 | 4.3 | - | 100 | 8.0 |
| | | | | | Av. | 8.2 |
| Topping | 1 | 56.9 | 25.0 | 18.1 | 100 | 15.5 |
| | 2 | 62.6 | 24.6 | 12.8 | 100 | 14.6 |
| | 3 | 62.1 | 28.7 | 9.2 | 100 | 15.0 |
| | 4 | 54.7 | 26.3 | 19.0 | 100 | 15.9 |
| | | | | | Av. | 15.3 |
| Check | 1 | 52.3 | 28.6 | 19.1 | 100 | 17.4 |
| | 2 | 54.5 | 25.8 | 19.7 | 100 | 16.9 |
| | 3 | 59.4 | 28.0 | 12.6 | 100 | 16.3 |
| | | | | | Av. | 17.0 |

## Table XVll

Acre yields of tobacco inoculated at different periods of growth with mosaic virus.

Macdonald College, 1931.

| Plot No. | Treatment | No. of Plants. | Plot Yields Lb. | Acre Factor | Yield per Acre. Lb. |
|---|---|---|---|---|---|
| 1B | Seeding | 88 | 16.656 | 102.2 | 1702 |
| 2B | " | 98 | 16.719 | 91.8 | 1535 |
| 3B | " | 99 | 17.125 | 90.9 | 1557 |
| 4B | " | 102 | 16.969 | 88.2 | 1497 |
| | | | | | Ave.1572 |
| 1C | Planting | 64 | 11.063 | 140.6 | 1555 |
| 2C | " | 57 | 10.563 | 157.8 | 1667 |
| 3C | " | 108 | 16.750 | 83.3 | 1395 |
| 4C | " | 100 | 15.875 | 90.0 | 1429 |
| | | | | | Ave.1512 |
| 1D | Hoeing | 102 | 19.938 | 88.2 | 1759 |
| 2D | " | 95 | 20.563 | 94.7 | 1947 |
| 3D | " | 97 | 17.250 | 92.7 | 1599 |
| 4D | " | 86 | 15.75 | 104.6 | 1647 |
| | | | | | Ave.1738 |
| 1E | Topping | 89 | 20.563 | 101.1 | 2079 |
| 2E | " | 94 | 20.625 | 95.7 | 1974 |
| 3E | " | 74 | 11.625 | 121.6 | 1414 |
| 4D | " | 69 | 13.032 | 130.4 | 1699 |
| | | | | | Ave.1791 |
| 1A | Check | 84 | 20.125 | 107.1 | 2155 |
| 2A | " | 83 | 15.750 | 108.4 | 1707 |
| 3A | " | 87 | 17.25 | 103.4 | 1784 |
| 4A | " | 110 | 22.500 | 81.8 | 1841 |
| | TOTALS | 1286 | 336.691 | | Ave.1872 |

## Table XVlll

Grade percentages and indeces of tobacco inoculated at various periods of growth with mosaic virus. Macdonald College, 1931.

| Time of Inoculation. | Plot No. | Percentage of Assorted Grades | | | | Grade Index |
|---|---|---|---|---|---|---|
| | | Trash | Lights | Darks | Total | |
| Seeding | 1 | 84.2 | - | 15.8 | 100 | 8.4 |
| | 2 | 100.0 | - | - | 100 | 7.0 |
| | 3 | 91.7 | 1.4 | 6.9 | 100 | 8.4 |
| | 4 | 100.0 | - | - | 100 | 7.0 |
| | | 94.1 | 0.3 | 5.6 | 100 | Ave.7.7 |
| Planting | 1 | 86.4 | - | 13.6 | 100 | 9.0 |
| | 2 | 68.5 | - | 31.5 | 100 | 11.7 |
| | 3 | 97.5 | - | 2.5 | 100 | 7.4 |
| | 4 | 100.0 | - | - | 100 | 7.0 |
| | | 88.2 | - | 11.8 | 100 | Ave.8.8 |
| Hoeing | 1 | 67.7 | 11.2 | 21.1 | 100 | 12.7 |
| | 2 | 65.9 | 6.3 | 27.8 | 100 | 12.6 |
| | 3 | 78.9 | 11.3 | 9.8 | 100 | 11.1 |
| | 4 | 61.8 | 22.0 | 16.2 | 100 | 14.5 |
| | | 68.6 | 12.6 | 18.8 | 100 | Ave.12.7 |
| Topping | 1 | 48.1 | 9.5 | 42.4 | 100 | 15.5 |
| | 2 | 57.3 | 8.5 | 34.2 | 100 | 14.1 |
| | 3 | 41.7 | 28.5 | 29.8 | 100 | 18.0 |
| | 4 | 52.4 | 15.3 | 32.3 | 100 | 15.4 |
| | | 49.8 | 15.6 | 34.6 | 100 | Ave.15.8 |
| Check | 1 | 45.3 | 20.2 | 34.5 | 100 | 18.9 |
| | 2 | 49.5 | 13.5 | 37.0 | 100 | 16.7 |
| | 3 | 48.5 | 7.8 | 43.7 | 100 | 17.4 |
| | 4 | 60.5 | 6.9 | 32.6 | 100 | 14.3 |
| | | 51.0 | 12.1 | 36.9 | 100 | Ave.16.8 |

From a study of Tables XV, XVI, XVII and XVIII, it will be clearly seen that mosaic infection has an adverse effect on both the yield and quality of Resistant Havana tobacco. With the exception of the average yields obtained from seedling and planting inoculations in 1931, due principally to the uneven growth and development of the plants as a result of resetting a large number at the time of transplanting, the severity of the damage is in direct relation to earliness of inoculation. Inoculations which were made up to almost six weeks after transplanting caused the most marked reductions in yield and quality of the cured leaves. Infection at the time of topping did not produce a significant reduction in yield, particularly in the 1930 crop, but on the basis of grade indeces the quality was adversely affected. Climatic conditions are of importance at this period, since where growth is retarded, the virus will not spread rapidly throughout the plant, and the leaves may not become sufficiently infected to cause the malformation and spotting which reduced their value when cured.

The figures presented in the tables show as much as 20% reduction in yield in 1930 where the plants were inoculated in the seedling stage, and almost the same reduction in 1931. For the two year period, the average yield of inoculated plants compared with the checks was reduced 12 to 15%, the reduction in the quality being as high as 40%. Plants inoculated in the seedling stage or at the time of transplanting show yield re-

ductions of about 20%, and quality 55%. The percentage reductions in yield and quality decrease quite consistently with the lateness of inoculation, the quality of the cured product being more adversely affected than the yield.

On the basis of total number of plants harvested and total yields, the 1931 crop yielded more than that of 1930. This was undoubtedly due to the fertilizer application made in the spring of 1931.

The effect of the disease was expressed in three major ways on the appearance and quality of the cured leaf. In the first place, mosaic patterning gave rise to a wrinkled, dwarfed type of leaf which was much discoloured; secondly, the patterning was often accompanied by necrotic spotting which necessarily persisted on the cured leaf; and lastly, compared with uninfected types, a general discolouration varying from dark red to dirty brown occurred commonly on mosaic leaves. Singly, or in combination, these symptoms accounted for a very significant lowering of the grade index.

While the results obtained are fairly consistent, a two year period is not sufficiently long to allow of definite conclusions being drawn. Many variable factors must be taken into consideration with studies of this type, notably climatic conditions, soil fertility and the general growth and development of the plants. If, for instance, growth is rapid just prior to the hoeing inoculation, and is retarded at the time of the topping inoculation, differences in yield and quality

between these two types may not be very great. The results do indicate, however, that every possible precaution should be taken by the commercial tobacco grower to avoid spreading the disease, or to apply active eradication methods where it has already occurred. Care and judgment exercised in preventing infection in the seed bed, the destroying of weed hosts occurring in the vicinity and the rogueing of diseased plants occurring in the field will do much to prevent the tobacco mosaic disease reaching proportions sufficient to materially effect the yield and quality of the crop.

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## Summary and Conclusions.

This paper reports the results of a number of experiments with the mosaic diseases of tobacco, potato and clover, principally the first mentioned. A general review of the more pertinent literature is also presented.

Studies to determine the effect of two degrees of topping and suckering, namely topped once and suckered once, and topped once and suckered three times, on the leaf structure of healthy and mosaic tobacco plants, indicate that the principal effect, compared with untopped unsuckered plants is to initiate a general enlargement of the leaf cells as a result of the treatments. Stained and sectioned leaf samples taken from the lower, middle and upper leaves of healthy and diseased plants at three stages of growth show that several suckerings tend towards the production of a thicker leaf. Mosaic leaves are consistently thinner than healthy ones irrespective of treatment. They also show increased crystalline material content, decreased intercellular spaces in the sprongy parenchyma tissue, increased width of epidermal cells and decreased length of palisade cells.

On the basis of cell measurements, there is a tendency for a dimensional decrease in cell size from the lower to the upper leaves. Lower leaves show signs of

desiccation due to wilting.

There is a slight increase in the size of the stomata in mosaic leaves taken from plants subjected to the cultural treatments.

The effects of the virus are apparently only limited to the inhibition or retardation of development, and its spread throughout the leaf is very uneven.

The catalase content of living and partially cured tobacco leaves infected with the mosaic disease, is greater than in the corresponding non-infected leaves. Using the amount of oxygen evolved on the addition of hydrogen peroxide to the macerated leaf tissue as an index of metabolic activity, the effect of the virus has been to cause increased activity.

Nitrogen deficiency in the food supply of healthy and mosaic tobacco plants is indicated in both cases by stunted growth and extreme yellowing of the plant. Lack of potassium causes stunting and the development of irregular yellow areas on the lower leaves. The thickness of the leaf is in both cases increased over the corresponding normal.

Microscopically, lack of nitrogen produces increased intercellular space areas of both healthy and mosaic leaves, and a reduction in the number of chloroplasts. There

is a reduction below normal in the crystalline content of healthy and mosaic leaves taken from plants grown under conditions of nitrogen and potassium starvation.

Preliminary nutrition studies designed to show whether the mosaic disease has any effect on the nutritive value of potato tubers infected with mild mosaic, and clover plants infected with mosaic, indicate that guinea pigs are not satisfactory experimental animals for use in studies of this nature. A number of digestive disturbances, and attendant complications of interest to the animal pathologist are introduced. Early Rose potato tubers, infected with the mild mosaic disease, are definitely less palatable to guinea pigs than healthy ones.

The sera from rabbits given a series of injections of virus extracts from mosaic tobacco plants, mild mosaic potato tubers and mild mosaic potato plants form heavy precipitates when mixed with these extracts and incubated for a suitable period. Smaller precipitates occur where the antisera for mosaic extracts are mixed with healthy tobacco, potato tuber and potato plant juices, and none at all where mosaic extracts are added to serum drawn from non-inoculated rabbits.

Where the precipitins to normal tobacco, potato tuber and plant extracts are absorbed from the corresponding

antisera for mosaic extracts, addition of the virus extracts causes the formation of heavy flocculent precipitates slightly reduced in amount from mixtures where no absorption was carried out.

Inoculations with the precipitate and supernatant liquid from a mixture of tobacco mosaic antigen and antiserum indicate that in a ratio of 1:1 the virus is not entirely inactivated in the precipitate.

Preliminary tests on the hypersensitivity of guinea pigs to mosaic and healthy tobacco plant extracts, show the necessity for first precipitating the nicotine, since the symptoms of anaphylaxis and nicotine poisoning may be confused.

Filtration studies with aged and freshly extracted mosaic tobacco juice using the Seitz Germproofing filter show that the electrical charge held by the filter pad must be neutralized before it is able to pass through. The neutralization takes from five to ten minutes when using one pad, and a somewhat longer period when using two. The protein materials in aged juice collect in a thick slimy layer over the pad surface and completely prevent the passage of the virus.

Applying the phenomen of a reduction in the amount of starch in tobacco leaves as a result of mosaic infection

in attempts to trace the path of migration of the virus from various points of inoculation, iodine tests are insufficiently delicate after about ten days. During that period there is a gradual reduction in the amount of starch in inoculated leaves, but the area of spread cannot be followed in later stages. The distribution of the virus within the leaf is apparently very uneven. Needle inoculations show as definite circular areas of reduced starch, increasing slightly in size up to about twelve days. These areas seem to merge into each other in later stages.

The yield and quality of tobacco are shown to be adversely affected by the mosaic disease, a correlation existing between the earlines of infection and the intensity of its effects. Averages computed for the two years covered by this test indicate that inoculation at the seedling stage and at transplanting time reduce the yield 20 - 25 per cent, and the quality 50 per cent. Inoculation at topping time did not produce a significant reduction in yield, but even at this late date the quality was lowered. Three main types of damage are found on the cured leaf; dwarfing, necrotic spotting and discolo ration. Any or all of these are responsible for the significant reductions in yield and quality found. The importance of controlling the disease where tobacco is being grown on a commercial scale cannot be over emphasized.

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## Explanation of plates.

Photomicrographs were made with the aid of a Zeiss microscope, fitted with oil immersion 1/12 and 4 mm. apochromatic (N.A. 0.95) objectives and a Leitz "Makam" camera. Kodak verichrome filmpacks were used in all but the contact photographs.

### Plate I.

Fig. A. Cross-section of part of middle leaf from a healthy tobacco plant not topped or suckered, showing general configuration. (X 270)

Fig. B. Cross-section of part of middle leaf from a mosaic tobacco plant not topped or suckered, showing general configuration. (X 270)

### Plate II.

Fig. A. Longitudinal section of lower epidermal cells of middle leaf takan from a mosaic tobacco plant topped and suckered once. To show abundance of crystalline bodies. (X 800)

Fig. B. Cross-section of upper epidermal cell from upper leaf of mosaic tobacco plant topped and suckered once. To show presence of crystals. (X 900)

### Plate III.

Fig. A. Longitudinal section of spongy parenchyma tissue of upper leaf of healthy tobacco plant topped once and suckered three times. To show intercellular spaces. (X 270)

Fig. B. As above, but from mosaic tobacco plant (X 270)

Plate IV.

Photograph showing the apparatus used in determining the catalase content of healthy and mosaic tobacco leaves.

Plate V.

To show the effect of nitrogen and potassium deficiencies on the growth and development of healthy tobacco plants twelve weeks old.

Plate VI.

Same as Plate V, but plants infected with the mosaic disease.

Plates VII and VIII.

Figs. A, B, C, D, E, and F. Contact prints showing the decrease in starch content in tobacco leaves inoculated with the mosaic virus, and tested every third day. The point of inoculation is clearly shown as an irregular black circle. Abundance of starch indicated in white areas, reduction or absence in dark areas. (Natural size).

Plate IX.

Fig. A. Contact print showing effect of glass needle inoculation with mosaic virus on tobacco leaf, after four days.

Fig. B. As above, but after fifteen days. The irregular halo due to the absence of starch is clearly shown round the ruptured areas.

Fig. C. Contact print showing tobacco leaf inoculated with sterile water and glass needles after ten days. All natural size.

PLATE I.

Fig. A.

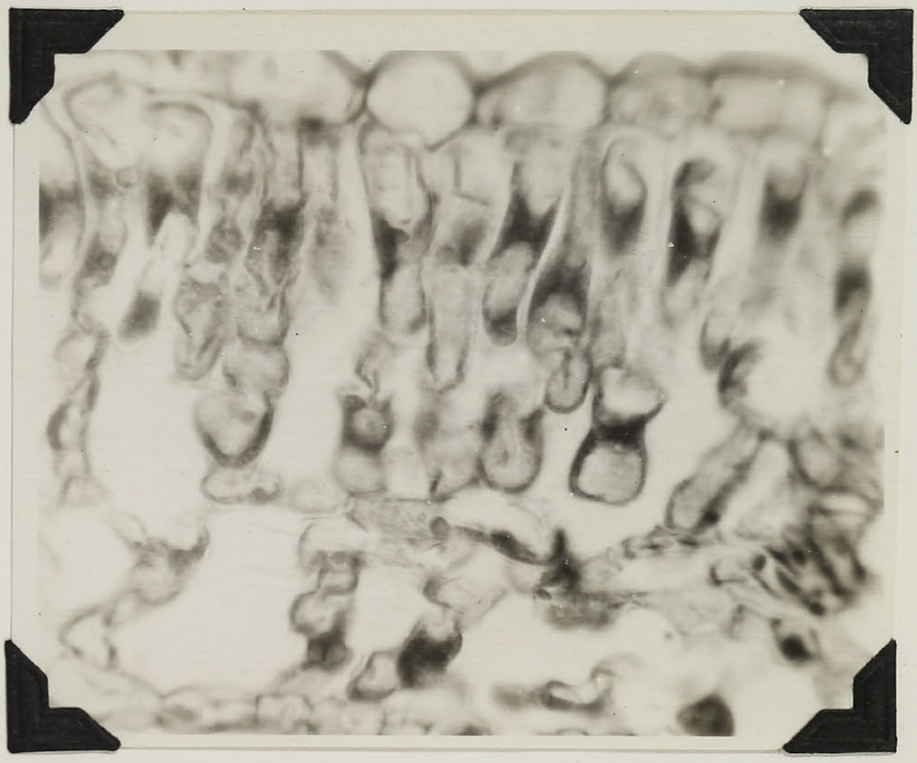

Fig. B.

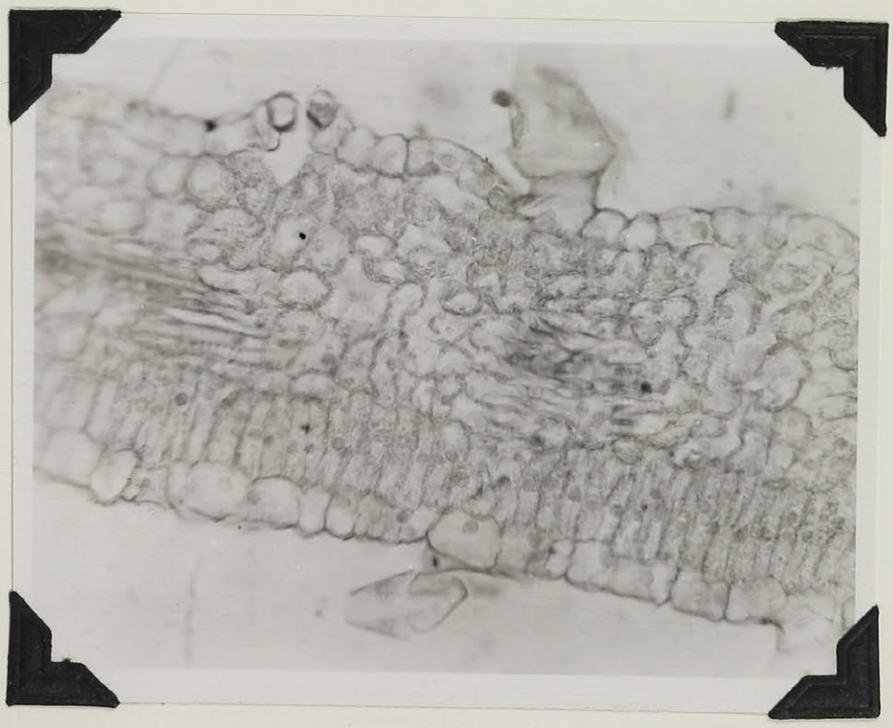

PLATE II.

Fig. A.

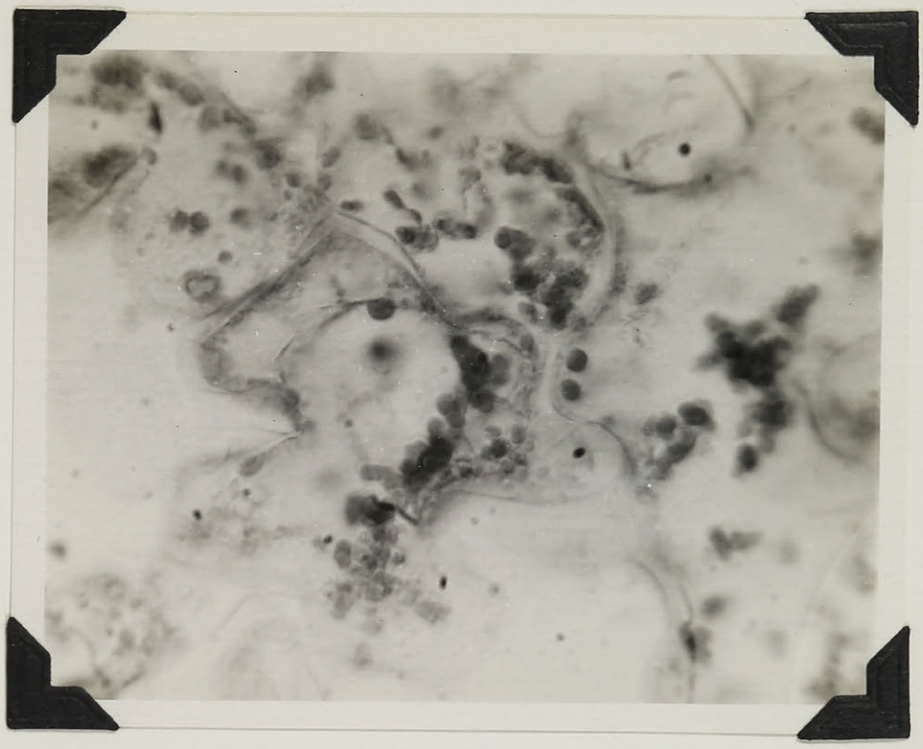

Fig. B.

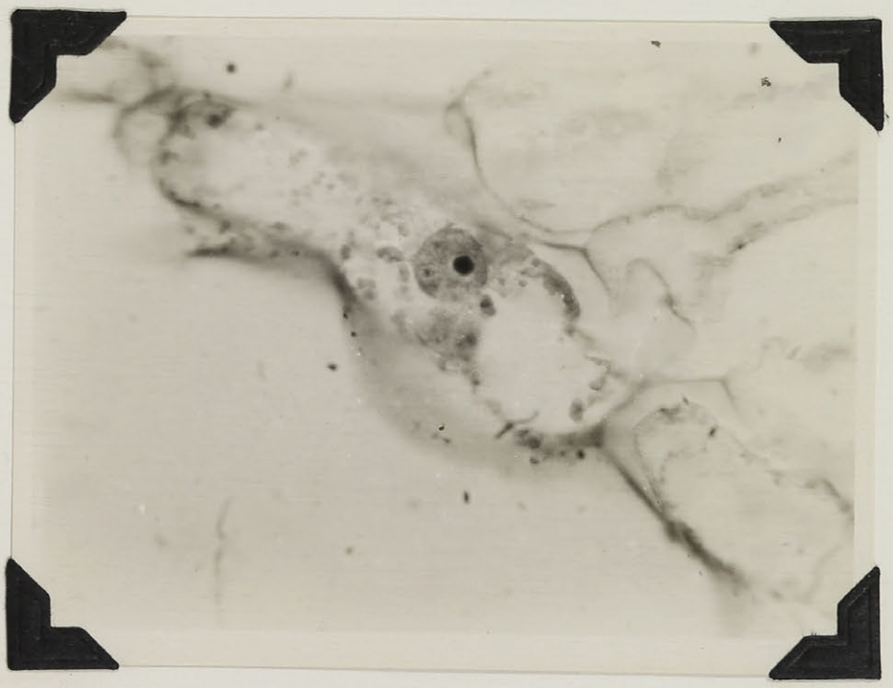

PLATE III.

Fig. A.

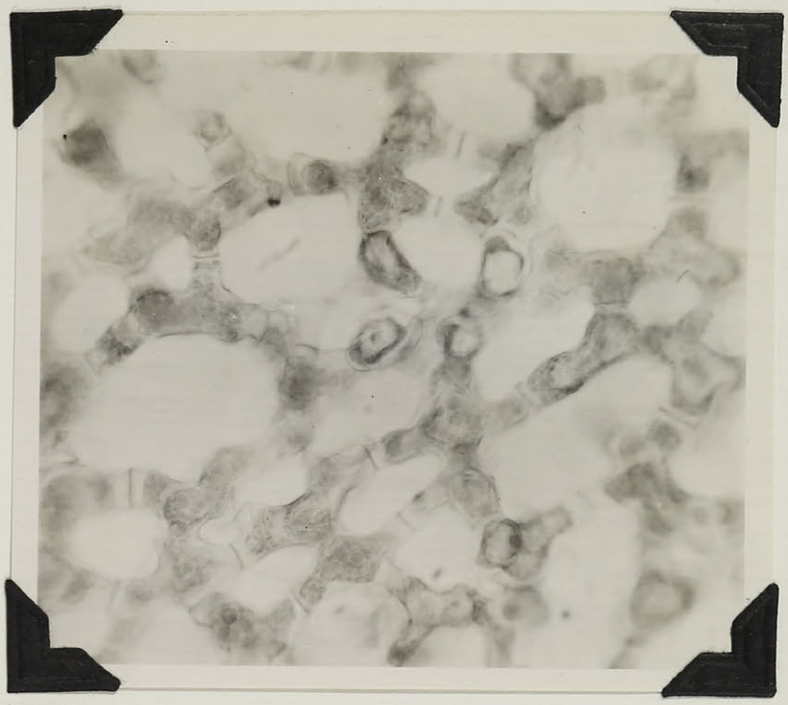

Fig. B.

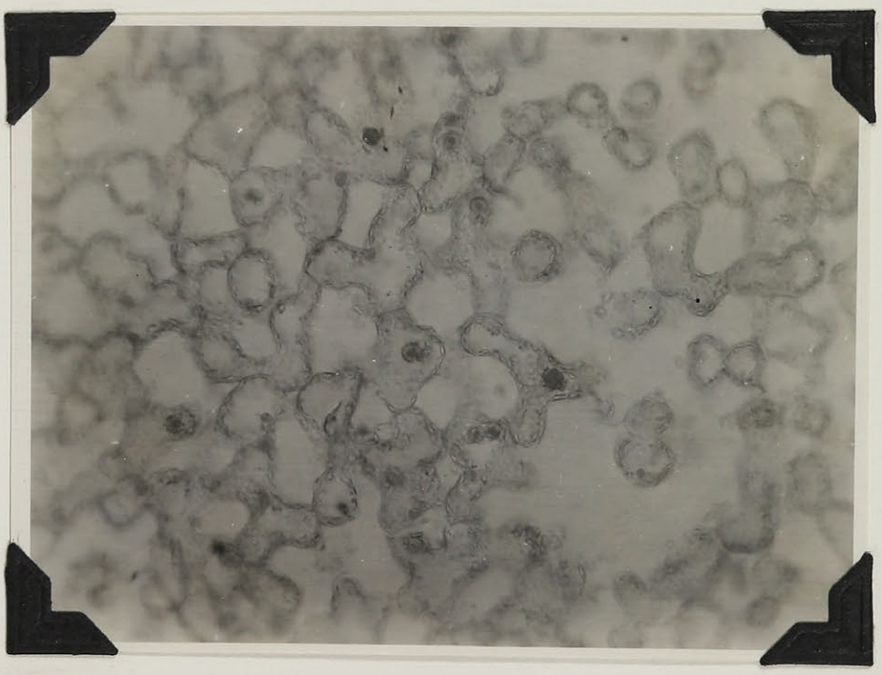

PLATE IV.

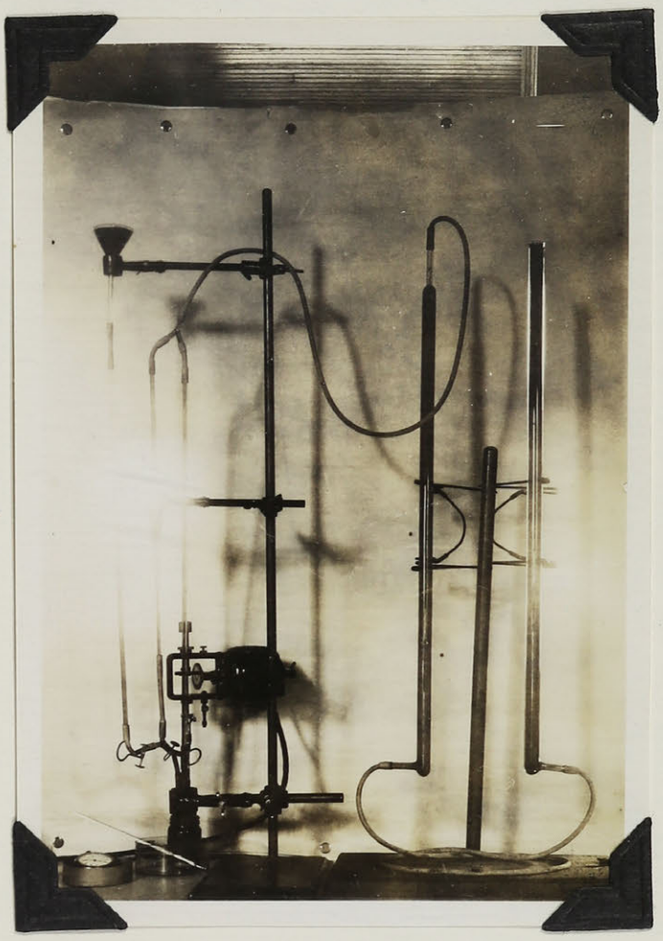

PLATE V.

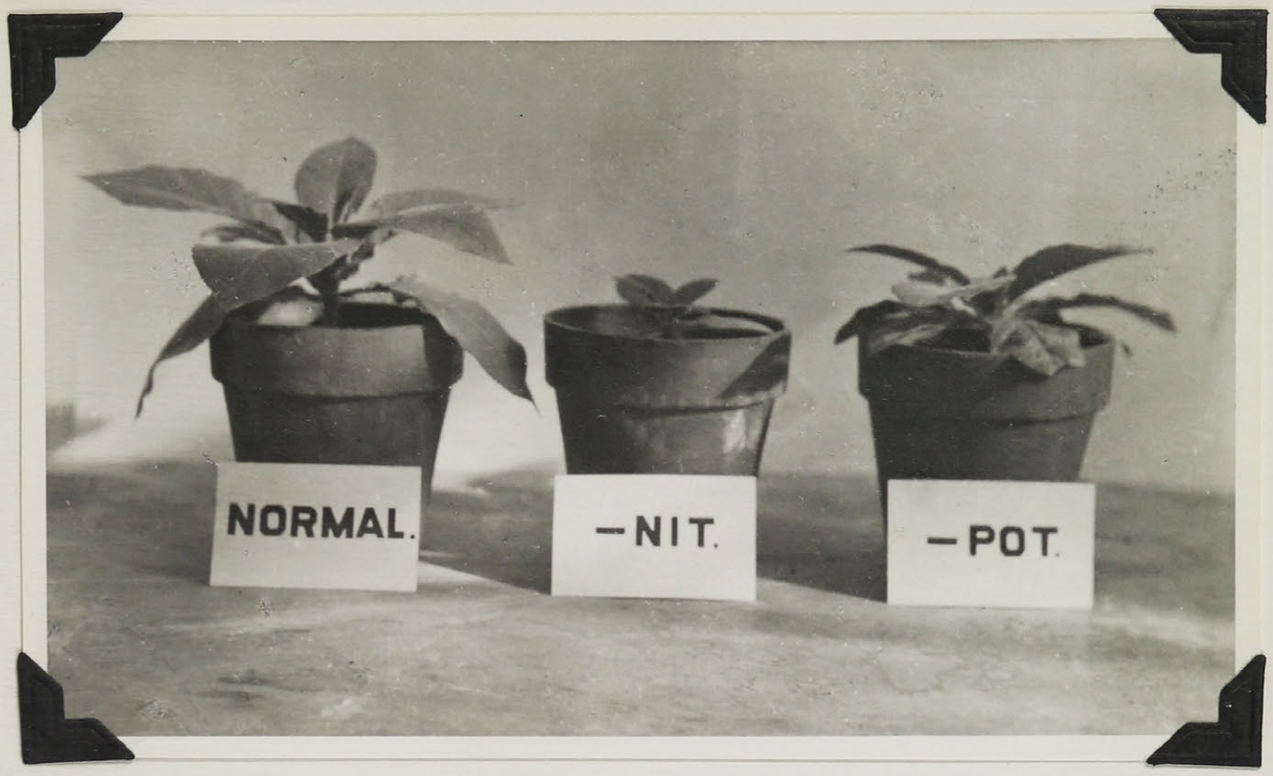

PLATE VI.

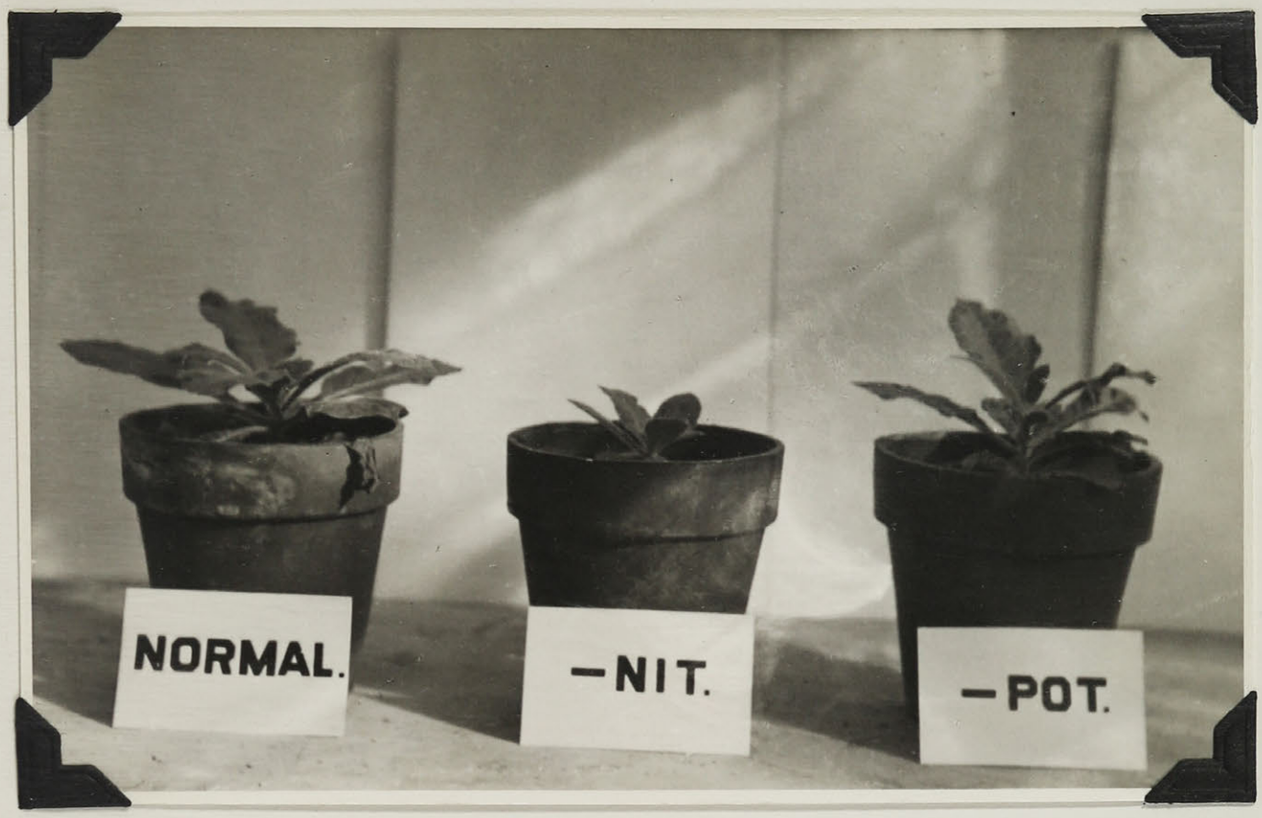

PLATE VII.

Fig. A.

Fig. B.

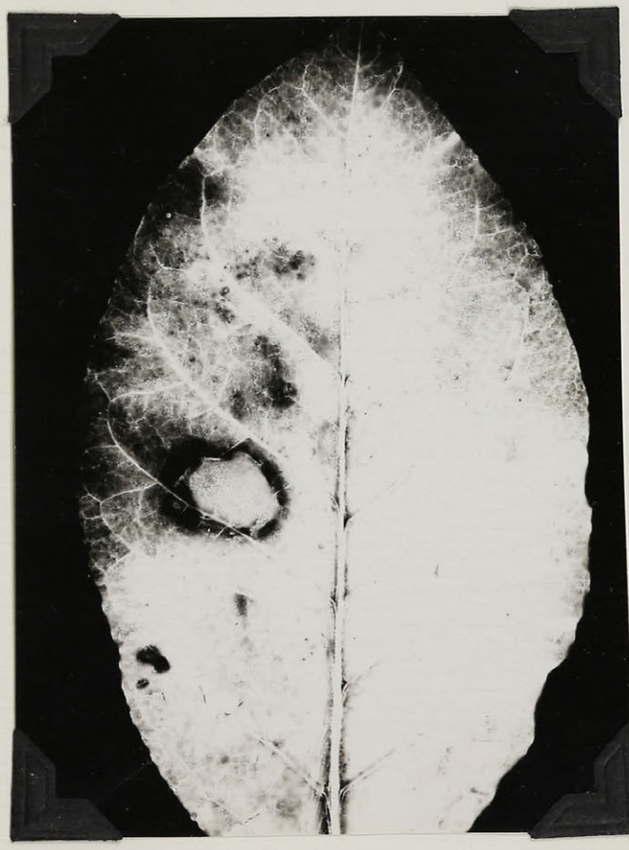

Fig. C.

PLATE VIII.

Fig. A.

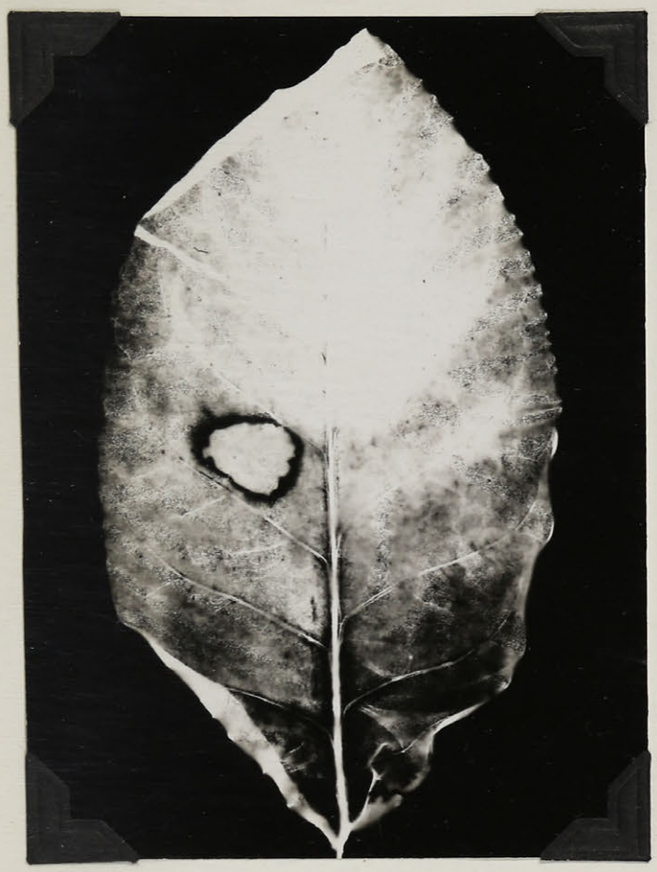

Fig. B.

Fig. C.