STUDY OF RESPIRATION IN HEALTHY AND MOSAIC-INFECTED TOBACCO PLANTS

VIOLETTE F. C.-GLASTONE

(WITH FIVE FIGURES)

Introduction

Comparatively little investigation has been made into the fundamental metabolic processes of plant viruses. BRONFENBRENNER and REICHERT (2, 3, 4) were unable to detect any respiration in either viruses or bacteriophages. The isolation of virus, free from plant cells, and in a condition suitable for such experiments, is obviously very difficult. The effect of virus infections on the metabolism of host plants is also a matter of great interest and may help to elucidate the action of the virus if sufficient data are collected. Since most viruses affect the chlorophyll content of the plant, it is not surprising that the work that has been done so far has been directed primarily on respiration. THUNG (20) observed that the respiration rate of potato leaves infected with leafroll was higher than that of the corresponding healthy leaves. Also working with potatoes infected by leafroll, WHITHEAD (21) studied the course of respiration from the immature tuber up to the development of the plant. In maturing tubers and in leaves, or shoots with expanded leaves, the respiration rate of the diseased plants was higher than that of the healthy plants; but for a period lasting from dormancy of the tuber until the expansion of the leaves, the respiration rate of the healthy material was the greater. This was true both for aerobic and anaerobic conditions, and also for various conditions of light, temperature, and carbon dioxide supply. He concluded that the virus affects the respiration rate not directly, but only by interfering with the translocation of the respirable substrate.

In a number of species of plants naturally infected by mosaic or yellows virus diseases it was found (9) that the respiration rate was increased in young leaves affected by virus but was decreased in older leaves thus affected. This was not apparently connected with the variations in carbohydrate or nitrogen contents of the leaves. On the other hand, DUFRENOY (8) noted that the high rate of respiration in healthy tobacco buds was decreased by tobacco-mosaic virus, whereas the lower respiratory activity of the discolored leaves was increased by the presence of the virus.

Caldwell (6) inoculated tomato plants with aucuba mosaic as seedlings and at the five-leaf stage and measured the respiration of the diseased tops. In all his experiments there was an initial high output of carbon dioxide which fell off to a steady level. Tops of plants inoculated at the five-leaf stage evolved more carbon dioxide than the healthy controls throughout the
experimental period; but those inoculated as seedlings showed a lower output at the beginning, followed by a higher output than the healthy tops once the stable level had been reached. He considered his experiments indicated that the virus affects the enzymes which "activate" the substrate before it is broken down to carbon dioxide in the respiration mechanism.

In a series of experiments on discs of healthy and tobacco-mosaic-infected tobacco leaves carried out by LEMMON (17), the respiration rate of the healthy leaves was always higher than that of the diseased. KEMPNER (15) was unable to find any change in the respiration of tobacco leaves infected by tobacco-mosaic virus, unless necrosis had set in.

There appears therefore to be a certain amount of discrepancy among the published results of the various workers in this field. All the experiments quoted above were made with tissue that was already diseased, and it seemed possible that a study covering the entire development of the disease would be profitable.

During the summer of 1940 the writer made some preliminary observations on the respiration of plants grown in soil. A respiration chamber was sealed over the plant top by means of a wax mixture. This seal was perfectly airtight, and it is usual to assume that the respiration of the top can be measured independently from that of the root by such a device. It was discovered, however, that air can pass easily through plants, especially tobacco, from root to leaf and vice versa (10). Hence it was possible that the air in the soil or roots below the surface of the wax seal was in some way interfering with the measurements.

For this reason it was decided to experiment with entire plants in nutrient solution to avoid effects due to the passage of air from other organs or to cutting, handling, drying out, or partial starvation of isolated material, all of which are known to have some influence on respiration. The present paper therefore is a report of experiments designed to follow the course of the respiration in entire plants from the time of their inoculation up to the appearance of the mosaic mottling, using the same plants throughout the period. The work was done in the spring of 1941.

Material

Ordinary tobacco-mosaic virus was used on Nicotiana tabacum L. var. Samsun as the host plant. This proved to be a very suitable subject for the purpose, as the resulting disease did not distort the plant unduly, merely producing a reduction of chlorophyll in a mottled pattern and causing stunting. There was also the advantage that under favorable conditions of light and temperature it was possible to note the time that the disease became systemic by observing the "clearing of the veins" (13).

The seeds were sown in nutrient sand, and as soon as the plants were
large enough to handle they were transferred to nutrient solution. The solution recommended by Hoagland and Arnon (11) with rather more ferric sulphate was found very satisfactory. The nutrient solution was contained in 4-liter battery jars which were covered with thick black paper to prevent growth of algae on the roots: no algae formed during any experiment. The plants were supported on thick wooden discs, containing holes for the roots. The discs were well waxed with paraffin wax, which was renewed for each set of experiments. Great care was taken to keep separate all the glassware and discs for the healthy and diseased plants, in order to avoid contamination. The only contaminations, which will be noted in the results, were due to an accident, the origin of which was later discovered. The younger plants stood in the wooden discs without further support; the older plants were usually held in place by cotton wads or occasionally by glass rods. The solution was changed twice to four times a week, according to the quantity of plant material. The plants ranged in size from the smallest that could be handled conveniently, of an average individual weight of 0.5 gm., up to the largest that could be put in the respiration chambers, of an average weight of 40 gm. In any case it was considered desirable for this experiment to avoid plants forming flowers, so the range in size was actually the widest that could have been used under the circumstances.

The diseased plants were inoculated in the usual manner by rubbing with a muslin pad dipped in tobacco-mosaic-virus juice (12). The healthy control plants were rubbed with a similar pad dipped in distilled water to equalize the treatment as far as possible. Plants were thus inoculated after being in nutrient solution for periods of 1 day, 1 week, 2 weeks, 3 weeks, and 4 weeks, respectively. The measurements of respiration rate were made on groups of plants from the first day of inoculation up to the time that the mosaic mottling developed. Two weeks were allowed for each group, which was usually long enough. The experiments in nutrient solution were repeated at least once for each time period.

As the respiration measurements were carried out in the daytime, the plants were illuminated artificially at night by means of 1000-watt Mazda bulbs. As a check on the growth and development of the virus, a group of plants in soil grown under normal greenhouse conditions was always used as observational controls. It was found that the clearing of the veins and mottling always appeared on the same days in both the greenhouse plants and in those receiving artificial illumination. The weight measurements were made daily on the same plants by weighing the entire plant. The plants were removed singly from the nutrient solution, placed carefully on a soft tissue, and the roots were gently blotted with another tissue to remove the moisture. The plant was then quickly weighed and replaced in solution. After a little practice, the whole process of weighing could be performed
very rapidly with no apparent injury to the plants. In figure 1 are shown two plants which were weighed in this manner for 50 days. The plant on the left was the healthy control and the other was diseased. It will be seen that the roots as well as the rest of the plants are in good condition.

Method

The apparatus consisted of 6 units, one of which is shown diagrammatically in figure 2. Air from a compressed air manifold was freed from carbon dioxide by passage through a soda-lime tower, A, a sodium hydroxide solution, B, and a dilute barium hydroxide solution tinted with phenolphthalein, C. The latter also acted as an indicator trap. The carbon-dioxide-free air passed through a flowmeter, D, provided with its own overflow trap, and thence via a tube, J, into the respiration chamber, E. This consisted of a large bell jar, 53 cm. high and 26 cm. internal diameter, sealed firmly to a base of ground plate glass, F, by means of a ring of plasticum clay, G, and enclosed during the experiment with a cover of thick black satin to exclude
all light. The air next passed through the tube, K,K, to enter the titration flasks, P (a very slight modification of that used by Mack, 18). The exit tube, Q, from this flask led to a glass tower, R, 65 cm. long, containing glass beads; next, by a glass tube, S, to an indicator trap, T, containing dilute barium hydroxide solution, and thence to the vacuum manifold. With the exception of the brass manifolds, glass tubing, joined by butt to butt joints in thick rubber pressure tubing, was used throughout. The 6 units were connected by the manifolds leading from the compressed air and vacuum mains. Each unit had its own taps to the 2 manifolds, and the vacuum manifold was further protected from sudden changes of pressure by mer-

![Diagram of one unit of the apparatus.](https://example.com/diagram)

The rate of flow of air through the apparatus was kept at 6 liters per hour, which experience proved was sufficient to provide very efficient scrubbing by the barium hydroxide solution in the bead tower. The indicator trap, T, always remained clear, showing that the air was completely freed from carbon dioxide in the tower. The pressure in the respiration chamber was maintained at 1 atmosphere, as indicated by the levels of the n-butyl phthalate in the attached manometer, L. Very slight adjustments of the 2 taps were required for this, and the apparatus was kept under continual observation during all experiments to maintain the required uniform conditions.
The procedure was briefly as follows: Before sealing down the bell jar, the plants, supported on the wooden disc (I) so that their roots dipped into the nutrient solution in the battery jar, H, were arranged so that the air entering by the tube, J, would bubble into the solution. The bell jar was sealed into position and tested for leaks by clamping off K,K and noting the movements of the liquid in the manometer, L. K,K was unclamped and the carbon-dioxide-free air was allowed to flow through the apparatus for one hour. This period had previously been established as more than sufficient to renew the air in the apparatus. Next, a measured amount of standard barium hydroxide and a drop of phenolphthalein were placed in the titration flask through M, which was then shut by a pinch clamp. The two vents protected by soda lime tubes at O and V and the siphon N, previously filled with distilled water, were closed. The vacuum tap, W, was opened enough to draw the liquid in the flask up the glass tower and to maintain atmospheric pressure in the respiration chamber and a uniform rate of flow.

After a definite time interval—usually 4 hours—the vent O was opened, the compressed air and vacuum taps were shut, the vent V was opened, and the tube S was closed, in this order. The barium hydroxide solution was washed down from the tower with carbon-dioxide-free water through the opening made by removing V. The solution was titrated with standard hydrochloric acid solution through the opening M. The burette, connected to an acid reservoir, was permanently attached to the apparatus and was moved to each unit as required. The flask was emptied and rinsed with

![General view of the respiration apparatus. (Photograph by J. A. Carlile.)](image-url)
carbon-dioxide-free water by means of the siphon N, which was left filled with water and clamped off during the experiment.

Results

The respiration rates were all calculated in terms of milligrams of carbon dioxide evolved per gram of fresh weight per hour. The results can best be understood by reference to the curves in figure 4, which are typical examples taken from each age group. It will be seen that the respiration rates of the healthy and diseased plants began at approximately equal levels and remained equal, within experimental error, up to the point at which the respiration rate of the diseased plants rose suddenly above the rate of the healthy plants. In the course of a few days the higher rate of the diseased plants gradually decreased and approached the rate of the healthy plants.

The special significance of these results lies in the observation that the sudden rise in the respiration rate of the inoculated plants coincided with the time at which the virus became systemic, i.e., at the time of the clearing of the veins. Usually when the mosaic mottling appeared on the plant, the respiration rates of the diseased and the healthy plants were either almost or actually equal again. In most cases the experiments were not continued past that point. When the disease became systemic the diseased plants showed a negligible increase of weight whereas the output of carbon dioxide remained the same or was much greater than formerly so that the resulting respiration rate was much higher.

If the conditions were such that the clearing did not show, the rise in respiration rate occurred at such a period before the mottling that it was reasonable to assume that the systemic spread of the virus had coincided with the rise, especially as other indications such as crinkling of the leaves were usually present. On one occasion, when circumstances prevented the observation of the clearing of the veins, the highest rise of respiration rate was missed, but the initial rise and subsequent fall were measured. On another occasion, one unit of the healthy plants showed a sudden rise in respiration; it was found that one of the plants was contaminated with the virus. In the same batch of plants one of the inoculated plants gave a sudden rise on the second day of inoculation. Again one of these plants was seen to be contaminated and, upon removing it, the respiration rate of the unit fell back to normal, rising on the 8th day when the disease became systemic in the remaining plants.

The respiration rate of the healthy plants varied from about 0.30 mg. CO₂/gm./hour for the young plants to about 0.075 mg./gm./hour for the older plants with a tendency to decrease regularly with increasing age and weight. With the inoculated plants the course of the respiration rate was
the same for the plants of all the ages examined, except that the respiration rate was approximately 50 per cent. greater than that of the corresponding healthy plant when the disease became systemic.

![Graph showing respiration rates]

**Fig. 4.** Respiration rates, in milligrams \( \text{CO}_2 \) per gram of fresh weight per hour, of healthy and diseased plants at different ages of inoculation.

The behavior of the plant according to its age is illustrated in the curves of figure 5. The respiration curve of an average healthy plant is shown by a continuous line. The percentage increase in respiration rate of the dis-
eased plants inoculated at the various ages is drawn in a series of dotted curves. It will be seen that the percentage rise, when the disease becomes systemic, is approximately the same irrespective of the ages of the healthy plants at the time of inoculation. When the mosaic develops, the percentage rise apparently decreases with the increasing age of the inoculated plants.

These results indicate that at the time when the virus becomes systemic there is a great increase in the metabolic activity within the plant as indicated by the greater rate of respiration of the inoculated as compared with the healthy plant. A number of investigators have studied the changes and distribution of the virus subsequent to inoculation (5, 7, 14, 16, 19). Their results all indicate that with tobacco-mosaic virus the clearing-of-the-

![Graph showing percentage increase in respiration rate of diseased plants inoculated at various ages.](image)

**Fig. 5.** The percentage increase in respiration rate of the diseased plants inoculated at various ages.

veins period corresponds to the rapid movement and increase of the virus, which would account for a high expenditure of energy whatever actual process is taking place. Further experiments are now being carried out on the respiration problems in order to obtain a fuller interpretation of these results.

**Summary**

The paper describes the apparatus and method for comparing the respiration of entire healthy and mosaic-diseased tobacco plants from the time of inoculation until the appearance of the mottling disease. The same plants were used throughout the period of each experiment.

It was found that the respiration ratio of the diseased plants and healthy plants remained at the same level until the disease became systemic. When the clearing of the veins was apparent, the respiration rate of the diseased plants rose rapidly, followed by a decrease until in the older plants it became approximately equal to that of the healthy plants by the time that the mosaic mottling had developed. The percentage increase in respiration rate
was approximately 50 per cent. higher than the rate of the corresponding healthy plants.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED


