The main environmental conditions that affect plant growth and hence its susceptibility/resistance to viruses, are considered to be temperature and light. It is well known that high temperatures lead to a masking of the symptoms of a tobacco plant systemically infected with tobacco mosaic virus (Bawden, 1950; Kassanis, 1957) and the heat therapy of virus-infected peach trees is well recognised (Hunkel, 1956). Under low temperatures, the leaves of *Nicotiana glutinosa* respond with local necrotic lesions when inoculated with TMV; under high temperatures, such as those occurring in tropical countries, these plants become systemically infected and gradually the plants wilt and die (Bawden, loc.cit.; Kassanis, 1952). These changes in the susceptibility of the host plants perhaps can be attributed to the altered metabolic patterns under the respective changed environments.

Light plays an important role in the predisposition or otherwise of a plant to infection by viruses. It is generally known that darkening, shading or reducing the light intensity prior to inoculation with a mosaic inducing virus like TMV, can increase susceptibility. Conversely, treatment with increased light prior to inoculation by certain viruses is known to confer a slight degree of resistance to the plants and this again depends upon the nature of the virus and the host (Bawden, 1950; Bawden and Roberts, 1947);
1948). These light-induced changes in the susceptibility of the plants probably result from a derangement in the metabolism of the host.

By the term 'susceptibility' is meant that the host is prone to infection by the pathogen and as already stated, is controlled to a large extent by the environmental factors. Particular emphasis has thus to be laid on 'the physiological state' of plants in connection with susceptibility or resistance to viruses. Young tomato plants are more susceptible to TMV than old ones and susceptibility in this case is shown to decrease with age (Samuel, 1934). Even in a single plant, there seems to be variations in susceptibility in the different parts of the plant. Although there is no definite route for the entry of sap-transmissible, mosaic inducing viruses, it is fairly well recognized that the leaf, and particularly the actively growing young leaf, offers a better ground for the virus to enter the tissues of the host. The reasons for this preferential foliar entry may be explained by the fact, that in addition to its being the organ of widest area, it is also the seat of all important metabolic processes.

It is well known that in almost all the sap-transmissible viruses, for the same amount of inoculum, the symptoms in the systemic host appear best in the leaves coming out after inoculation and invariably the inoculated basal leaves remain apparently healthy at least in the initial stages of
the disease. This aspect is very well seen in *Dolichos lablab* inoculated with *Dolichos enation mosaic virus* (see Symptomatology). In this case, the virus is inoculated on to the first pair of leaves six days after germination and the first leaf emerging 6-10 days after inoculation display symptoms of mosaic and vein-banding, while the inoculated leaves remain symptomless almost till the end when they turn yellow and ultimately drop off. In tobacco or tomato infected with TMV, a similar sequence is seen, although when the disease is fully systemic, the inoculated leaves show slight mottling and mosaic in the later stages. It is thus interesting to note that in systemic infections, symptom expression starts almost from the apical region of the shoot, where the plant shows maximum physiological activity of growth and differentiation.

It is the aim of this thesis, to present to the extent possible, some results of the experiments done in the field of host physiology. Particular attention has been paid to the study of metabolic changes of two systemic hosts affected by two different sap-transmissible, mosaic-inducing viruses under controlled conditions of environment. The following aspects of host physiology have been tackled, so as to provide more information to the much discussed problems of virus-host relationships.

**Water in relation to mosaic diseases:**

The changes in water economy of virus infected plants is an almost unexplored field. The fact that many virus
diseases and particularly the mosaics, do not produce any symptoms of wilt, perhaps explains why interest has not been much taken in studying this aspect.

There are, however, a few records of studies on transpiration and on wet and dry weight relationships of virus infected plants. Reuberger and Norton (1933) showed an enhanced transpiration of detached, mosaic infected tomato leaves. In the leaf-roll and other virus diseases of potatoes, an enhanced transpiration rate is reported (Kuprevics, 1934). In tobacco mosaic (Kokin, 1937; Gondo, 1955) and in Phaseolus vulgaris infected with bean mosaic virus (Harrison, 1935a;b), however, a low transpiration is reported.

Howles (1948) showed that the virus causing mosaic of tomato entered a wilting leaf with greater ease than a turgid one. Increased susceptibility induced by an abundance of water supply has been attributed to more succulence, thinner cuticle and less regular palisade tissue (Tinsley, 1953).

Plants infected with viruses and causing changes in transpiration, also reveal changes in the fresh/dry weight ratios. Thus, in mosaic infected tobacco leaves there is a decrease in fresh weight (Bawden, 1950). Increased dry matter has been reported in tomato yellows (Shapovalov and Jones, 1930) and in mosaic of potatoes (Malhotra, 1931; Stone, 1938). In tomato seedlings infected with Nicotiana virus 1, the percentage of water was lower in the early
stages of infection, but increased later (Ainsworth and Selman, 1936). Leaf-roll has been attributed to a reduction in water content (Gibic, 1945). In the tomato mosaic leaves 62% loss in weight by drying as against 39% in healthy has been reported (Neuberger and Norton, 1933).

In the present work, an attempt has been made to study the changes in water economy and fresh weight/dry weight ratios of Dolichos bean mosaic virus-infected Dolichos lablab and tobacco mosaic virus-infected tomato plants, under normal and varied environmental conditions.

Chlorophyll in relation to mosaic disease:

Mosaic is generally known to reduce the chlorophyll content (Dickson, 1931; Cook, 1931). In the mosaic of potato (Rochlin, 1930), in the various virus diseases of potato excepting potato aucuba mosaic (Kuprevics, 1947), in tobacco infected with mild dark green, light green and intense yellow mosaics (Peterson, 1931; Peterson and McKinney 1939), in tomato aucuba mosaic (Sheffield, 1935), in Abutilon infectious chlorosis (Buler, Bertssch, Myrback, Runehjelm and Forsberg, 1930; Buler, 1931), in potato leaf-roll (Kuprevics, 1934), in citrus with tristeza virus (Trippi and Mesias, 1957) and in ash plants with rosette virus (Kuprevics, 1947) the same reductions in chlorophyll content are reported. Carotene and xanthophyll contents similarly show a reduction in tobacco plants showing mild dark green, light green and intense yellow mosaics (Peterson, loc.cit.)
Peterson and McKinney, 1938) and in *Abutilon* infectious chlorosis (Ruler et al., 1930), while an increase in these pigments has been reported for the tristesa disease of citrus (Trippi and Mesias, 1957).

In aubuea yellow mosaic of tomato, the virus is known to inhibit the formation of chloroplasts, while it does not affect those already formed (Sheffield, 1933; Caldwell, 1934). This probably explains why the older leaves on inoculation fails to display the mosaic symptoms, while the younger ones show extreme mosaic mottling. A reduction in protochlorophyll and lipochrome components is seen in the tobacco mosaic and *Abutilon* infectious chlorosis (Ruler, 1931) and the yellow colour of the mosaic infected tobacco plants is explained as due to a preponderance of chlorophyll b (Dunlap, 1928). The reduction in chlorophyll is seen even in older leaves showing no mottling, though leaves just emerging and expanding show the most significant reductions, this condition being seen in all stages of growth (Dunlap, loc. cit.). In the green and yellow strains of TMV, infection causes a marked reduction in the size of chloroplasts from one half to two thirds the normal size (Woods and DuBuy, 1951).

Excessive accumulation of the virus has been noted in the light green areas of the mosaic infected tobacco than in dark green (Grainger, 1933; Matsumoto, 1941). Rod shaped TMV particles have been observed in the isolated
chloroplast granas (Kausebo and Ruska, 1940) and these authors suggested a definite affinity between the protein components of the chloroplast substance and the virus protein. Destruction of the chloroplasts by TMV leads to a reduction of photosynthesis (Dufrénoy, Stamatinés and Sarejanz, 1929) and the chloroplast grains dissolve into fatty globules (Dufrénoy, 1928).

In spite of the great decrease in the chlorophyll content of the infected plants, an increase in the chlorophyllase activity in several types of mosaics is recorded (Peterson, 1931; Peterson and McKinney, 1938). In the yellow mosaic of tobacco affected by TMV, the chlorophyllase activity was double to that of the chlorophyll content (Peterson and McKinney, loc. cit.).

The mechanism of chloroplast destruction and the concomitant mosaic has been explained in several ways: a) fragmentation of chloroplasts due to a hypotonic condition following the transformation of sugars in situ into starch in the leaves of potato leaf-roll (Beauverie, 1926); b) the virus is said to act on the starch producing acids which attack chlorophyll causing mottling in aucuba mosaic of tomato (Bolas and Bewley, 1930); c) suppression of the synthesis of the protein bases of plastids (Kuprevics, 1947); d) in tomato aucuba mosaic the absence of the plastid in some areas is explained as due to the inhibitory effect of the virus on the primordia which are usually destroyed in
the very early stages (Sheffield, 1933); and e) a direct 
anction by the sugar beet yellow virus on the chloroplasts 
(Spikes and Stout, 1955).

The formation of the light green areas in the lamina 
of TNV infected tobacco has been reported to be due to 
the complete vacuolation of palisade cells, thus checking 
cell division, before epidermal tissues are formed. If 
the vacuolation is delayed so that cell division proceeds, 
dark green areas are formed (Grainger and Neafford, 1933).

It is reported (Woods and DuBay, 1941) that the 
synthesis of TMV protein is very much like that of chloro-
phyll protein and probably from the same building units. 
In potato X, the virus multiplication is said to be a 
partial substitute for the synthesis of chlorophyll protein, 
which is stored up beyond the normal minimum for photosynthe-
sis as an inert protein (Bald, 1942).

From the foregoing review, it is clear that there is 
a definite relationship between the mosaic inducing virus 
and the extent of its damage to the chloroplast grana of 
the host, revealed by the absolute losses in chlorophyll 
content. In the present work, experiments have been devised 
to estimate the losses in chlorophyll by DMMV (Dolichos 
enation mosaic virus) and TNV under normal and varied 
environmental conditions.
Manganese is associated with nitrate reductions, enzymes concerned in decarboxylations and hydrolytic reactions and is known to affect photosynthesis (Gerretsen, 1950a; b; Hewitt, Jones and Williams, 1949; McIlroy and Mason, 1954). Chlorosis due to an excess of Mn has been explained as due to the increased photooxidation of the protein protecting the chloroplast grana, thereby inducing the chloroplast to undergo destruction by light (Gerretsen, loc.cit.).

Thus, iron and manganese, separately and in combination are known to be responsible for the various important physiological processes of the plant with particular reference to the chlorophyll formation and maturation. The importance of a definite Fe/Mn ratio, as a key factor in controlling the physiological reactions of respiration and photosynthesis has been stressed by many workers (Somers and Shive, 1942; Somers, Gilbert and Shive, 1942; Gerretsen, loc.cit.).

In the present work, experiments were, therefore, devised to study the iron and manganese contents and the Fe/Mn ratios during pathogenesis of DEMV and TMV under normal and varied environmental conditions.

Carbohydrate metabolism in relation to mosaic diseases:

The photosynthetic apparatus being the main target for the mosaic-inducing virus to display its action, it is
logical to presume that the pattern of photosynthesis in the infected host will be far from the normal. The study of carbohydrate metabolism in this connection becomes essential. While there are many references relating to the accumulation of carbohydrates in potato leaf-roll (Campbell, 1925; Thung, 1928, Cöle, 1945; Martin, 1954), the information regarding the changes in the carbohydrate content consequent on infection by sap-transmissible viruses is scanty and conflicting. Thus, high starch content is recorded in tobacco mosaic (Tollenaar, 1925; Lehrlke, 1930) and increased total and reducing sugar contents in tomato infected with tomato aucuba mosaic virus (Read, 1932). On the other hand, Dimofte (1942) and Rischkov (1943) reported a low carbohydrate content in tobacco mosaic and Dunlap (1930) also recorded a low carbohydrate level for mosaic diseases in general. Kokin (1937) reported a low insoluble carbohydrate content in a tobacco variety infected with the common TMV. In the local lesions of tobacco, the virus is shown to inhibit both the formation and translocation of starch within the infected tissues (Holmes, 1931).

Increased carbohydrate has been recorded in western yellow blight of tomato (Ross, 1927), in Courte-nouvé of vines (Bose and Benesfch, 1941; Ooha, 1937), in cereals affected with wheat mosaic (Rhiakhovsky and Fedulaev, 1941), in sugar beet yellows (Watson and Watson, 1955; van Durren, 1955) and in the yellows of tomato (Shapovalov, 1931).
Reduced carbohydrate content has been reported in tomato plants affected by typical mosaic and necrotic or double streak virus (Brewer, Kendrick and Gardner, 1936) and in tristessa of citrus (Trippi and Mesias, 1957).

Virus infection has also been reported to induce qualitative changes in the nature, synthesis and translocation of carbohydrates. In the healthy potato plants, hexose is the first sugar of photosynthesis, sucrose being the sugar of translocation. In the secondary stage of leaf-roll, the rate of photosynthesis is reduced and starch is converted to hexose, hexose to sucrose and sucrose back again to starch (Barton-Wright and McBain, 1933). Similarly, in the crinkle and paracrinkle of potatoes, there was no difference in the carbohydrate contents between the healthy and infected plants in the early stages of the disease; but, in the later stages sucrose accumulated in the diseased leaf blade formed by the direct hydrolysis of starch whereas, in the comparable healthy plant it was derived by synthesis from hexose, formed by the hydrolysis of starch. In the diseased plants, translocation of sucrose was less readily effected (Barton-Wright and McBain, 1933).

From the foregoing review, it is clear that our present understanding of the carbohydrate metabolism of virus infected plants is far from complete. Though some work has been done in the leaf-roll and yellows diseases, the position with regard to the mosaic diseases needs further
elucidation. With these facts in view, experiments have been devised in the present work to study the changes in the carbohydrate content of healthy and DMV-infected D. lablab and TMV-infected tomato plants, under normal and varied environmental conditions.

**Nitrogen metabolism in relation to mosaic diseases:**

Changes in the nitrogen metabolism of the host can be said to a characteristic feature of virus infections, especially with the mosaic-inducing sap-transmissible viruses. A deranged carbohydrate metabolism in potato mosaic has been explained as the cause for the high nitrogen content of diseased potato leaves (Cookerham, 1939). It is known that even the so called resistant tobacco varieties Ambalaema and T.I. 448A show minor changes in the nitrogen content consequent on inoculation with TMV, while the very highly susceptible Havannah varieties show extreme changes (Martin, Belles and McKinney, 1939; McKinney, 1943). An increase in the total nitrogen content of the host has been reported for tomato yellows (Shapovalov, 1931), potato mosaic (Malhotra, 1931; Cookerham, 1939), tobacco mosaic (Cordingley, Grainger, Pearsall and Wright, 1934; Dimofte, 1942; Hills and McKinney, 1942) and raddish mosaic (Yukawa, 1952). In *Brassica ohirana* infected with turnip yellow mosaic virus, the total as well as the soluble nitrogen levels were higher than the healthy (Borges and Beato, 1955). Tobacco leaves infected with potato virus X (Koslowska, 1958) or TMV (Elbertshagen,
were shown to contain more soluble nitrogen. In the former case there was a higher free amino acid content which reached a peak concentration on the 7th day after inoculation; of these amino acids, aspartic acid was produced in large quantities during protein synthesis by the virus (Koslowska, loc. cit.). A higher soluble nitrogen has also been reported in the potato leaves affected by the leaf-roll virus (Benke, 1956). In *Abutilon* infectious chlorosis, an increase in amino nitrogen has been reported (Euler et al., 1930). Increase in glutamine has been reported in the leaves of sugar beet affected by the sugar beet yellows (Van Durren, 1955) and in potato leaf-roll (Allison, 1953). In the yellow vein mosaic of bhendi (*Abelmoschus esculentus*), leucine, phenylalanine, valine, methionine and asparagine showed an increase (Govindjee, Laloraya and Rajarao, 1956).

A decrease in total nitrogen level is known in sugar beet yellows (Van Durren, loc. cit.), in cereals affected by wheat mosaic virus (Rhiakovsky and Pedulae9, 1941), in western yellow blight of tomato (Rosa, 1927) and in curly top of tomato (Wann and Blood; 1933).

Perhaps the greatest change accompanying virus infections is in protein nitrogen. An increase in protein nitrogen has been reported in TMV-infected tobacco, (Kokin, 1957; Vorobieva, 1939; Dimofte, 1942; Best and Gallus, 1953; Commoner, Schieber and Diets, 1953). In TMV-infected
tobacco (strain 1 of TMV) the lower leaves showed maximum accumulation of virus protein 6 days after inoculation (Martin, Bolls and McKinney, 1939) and a general increase in protein and allied virus protein fractions has also been reported by several other workers (Frampton and Takahashi, 1944; Wildman, Cheo and Bonner, 1949; Commoner, Yamada, Rodenberg, Wang and Baster, 1953; Jeener, Lemoine and Hümme, 1954).

From the foregoing account it is clear, that the changes induced in the nitrogen metabolism of the virus infected host are of varied nature depending upon the nature of the host and the virus. A suggestion has also been made that some symptoms of the virus diseases like foliar distortion, may be due to changes in the free amino acid concentration accompanying virus infections (Commoner and Nehari, 1953).

In the present work an attempt has been made to study the various fractions of nitrogen in the DEMV-infected Dolichos lablab and TMV-infected tobacco and tomato and to correlate these changes with other metabolic derangements and ultimately in the physiology of virus infections. Special attention has been paid to study the distribution of free amino acids in the infected plants.
MATERIALS AND METHODS

Viruses used for study:

*Dolichos lablab* var. *Dolichos lablab* var. DL 231 and *Tobacco mosaic virus* (TMV) were obtained from Dr. S. P. Kapoor, Virologist, Agricultural College and Farm, Poona, South India.

Maintenance of inoculum and inoculation techniques:

DEMV and TMV were maintained on potted *Dolichos lablab* var. DL 231 and White Burley tobacco respectively under insect proof conditions in a glass house.

All inoculations were done using a standard extract of the inoculum i.e., 1 g. of young fully infected leaves ground well in 1 ml of distilled water in a mortar. No abrasive was used for TMV inoculations on tobacco or tomato, since, even without the use of abrasive, very good infection, was attained. For DEMV, celite (BDD) was used as an abrasive.

Mechanical inoculation was carried out in all cases in the usual way by rubbing the infected sap by the forefinger. After rubbing, the excess of inoculum was washed away by a jet of glass-distilled water.

Host plants used for study:

Throughout the course of this investigation, *Dolichos lablab* var. DL 231 for DEMV and *Solanum tuberosum* var. Mar’s Globe for TMV were used.
Environmental conditions:

Plants were grown in garden soil and maintained under glass house conditions at an average temperature of 28-31°C. Tobacco plants were raised in large nursery pans and later transplanted at the 3-4 leaf stage, to individual smaller pots.

Dark and light treatment:

Plants were subjected to dark and light treatments wherever necessary under controlled conditions.

Temperature treatment:

A thermostatically controlled miniature glass house with additional light sources (Sadasivan, Saraswathi-Devi and Suloohana, 1956) was used to vary the temperature and light requirements.

ANALYTICAL METHODS.

Qualitative and quantitative estimation of chlorophyll:

The general method for the extraction and determination of chlorophylls described in the Journal of the Association of Official Agricultural Chemists (A.O.A.C. 1950) has been followed with some modifications. Five hundred milligrams of fresh material, after cleaning and critical sampling are ground in a glass pestle and mortar. About 10 ml of 85% acetone (EDH) are added to the pulp and the contents carefully transferred to a centrifuge tube, the pestle and mortar carefully and repeatedly washed with
small volumes of 85% acetone until the washings are colourless. All washings are added to the centrifuge tube. The material is centrifuged at 3,000 r.p.m. for 5 minutes. The supernatant is poured into a standard flask. About 10 ml of 85% acetone is added to the precipitate in the centrifuge tube and with the aid of a glass rod with a flattened tip, the tissue is ground and mixed. It is again centrifuged and the supernatant added to the first extract and the process of extraction repeated with smaller volumes of acetone until the supernatant is colourless. The filtrates are combined and made up to volume (50 ml) with 85% acetone.

25 ml of the acetone extract are pipetted out into a separatory funnel and about 20 ml of pure ether added. Water is carefully added from a wash bottle along the sides of the funnel, so that all the pigments enter the ether layer, leaving a lower acetone-water layer. The lower layer is discarded and more water is added as before and the ether layer washed about 4 times. The separatory funnel is now placed as high as possible on a tall stand and a second separatory funnel filled with three-fourths full of water is fitted below the first in such a way, that the tail of the upper funnel dips into the water of the lower. The stopper of the lower funnel is kept closed and gently the pigment-ether layer is allowed to trickle through the water, thereby washing itself free of acetone. The lower water layer is discarded and the ether layer again returned to the upper
funnel, the separatory funnel washed with a small volume of ether and the process of washing the ether layer to free the acetone traces repeated 4-5 times. The ether-pigment layer is made up to 25 ml in a standard flask with ether after washing the separatory funnels with small volumes of ether and adding the washings to the main extract. The readings of the purified extract are taken as quickly as possible.

The optical density of the extract is determined in a UV-Spek photoelectric spectrophotometer (Hilger) using an infra-red photocell selector, and two readings are taken one at 6600 Å and the other at 6425 Å, using pure ether as the blank. The chlorophylls are calculated using the formulae given in A.O.A.C. (1950):

**Total chlorophyll (in mg./l.)**

7.72 x O.D. at 6600 Å + 16.8 x O.D. at 6425 Å.

**Chlorophyll a (in mg./l.)**

9.93 x O.D. at 6600 Å - 0.777 x O.D. at 6425 Å.

**Chlorophyll b (in mg./l.)**

Here calculated as the difference between the total chlorophyll and chlorophyll a.

Estimation of free amino acids by paper chromatography:

A known weight of the plant material to be analysed (2-3g. of fresh weight) is ground with about 10 ml of 80% ethanol in a glass mortar using fine, acid-washed sand to aid disintegration of the tissue. The ground tissue is
transferred to a centrifuge tube, and to this are added 2-3 washings of the pestle and mortar to ensure complete extraction. The contents in the centrifuge tube are left overnight in a refrigerator, after which they are centrifuged at 3,000 r.p.m. for about 30 mins. The supernatant is transferred to an evaporating dish and is dried in vacuo over sulphuric acid. The dry precipitate is extracted thoroughly in 1 ml of butanol:phenol mixture (6:4) and cold stored in vials.

Aliquots of these samples are spotted on (about 10 ul. of the sample measured by a calibrated WBC pipette) to Whatman no. 1 filter papers. It has been found that of the two solvents commonly tried, namely, butanol:acetic acid:water (4:1:5) and phenol:water (1:1), the former gave uniformly good separations, without any tailing of the spots. Unidimensional ascending chromatograms were run in butanol:acetic acid:water solvent (4:1:5) and the spots detected using 0.1% ninhydrin in acetone.

The qualitative detection of amino acids is based on the Rf values recorded for pure amino acids by Lakshminarayan, (1955) under our laboratory conditions. A semi-quantitative method of estimation of amino acids has been adopted which consists of representing the concentration of the free amino acids by the area of the spots on the chromatogram in sq.mm. However, this method is not claimed to be wholly satisfactory for the estimation of amino acid concentration.
Estimation of total carbohydrates:

The total carbohydrates are estimated as total reducing sugars by the method of Hagedorn and Jensen (1935). 50 mg. of oven-dried, powdered plant material are transferred to a test tube and 10 ml of 2.5 N HCl added to it. The mixture is digested in a boiling water bath for two hours after which it is filtered, washed with water and the filtrate is neutralised with 5 N sodium carbonate solution. This neutralised solution is then made up to 25 ml (Solution A).

2.5 ml of Solution A are transferred to a test tube and 2 ml of cadmium sulphate mixture (cadmium sulphate, 26.8 and 132 ml of 8 N HgSO₄ - the mixture made up to 1 litre with water) added to it, after which 1 ml of 0.55 N NaOH solution are also added when a bulky precipitate is thrown down. The mixture is then heated over a boiling water bath for 3 mins. It is cooled, filtered and washed. The filtrate is then made up to 20 ml (Solution B).

2 ml of Solution B are taken in a test tube and 2 ml of potassium ferricyanide mixture (0.825 g. of potassium ferricyanide and 5 g. of MgCO₃ dissolved in 500 ml of water) added to it. The mixture is boiled for 15 mins. 3 ml of potassium iodide mixture (2.5 g. KI, 5 g. of zinc sulphate and 25 g. of NaCl in 100 ml water) are pipetted out into a flask to which 2 ml of 3% acetic acid solution and a few drops of freshly prepared starch solution are added. The
sample with potassium ferricyanide solution is added to the flask containing the KI mixture, along with the washings of the tube. The mixture turns blue on shaking and is titrated against N/50 sodium thiosulphate taken in a micro-burette, to attain a colourless end point. The total carbohydrates represented as glucose, is calculated from the equation - 2.86 ml of N/50 sodium thiosulphate = 1 mg. of glucose. The titre value of the sample is taken as the value minus the titre value of the blank. The results are presented as mg/g. glucose of dry weight of the sample.

**Estimation of metallic constituents (Fe and Mn)**

**Sampling and preliminaries:**

The plant part to be analysed is cut with a stainless steel scissors and rapidly washed in Pyrex-glass-distilled water, dried between the folds of a filter paper and dried in an oven at 80°C. for 72 hours. The dried material is ground well in an agate mortar and stored in desiccator.

**Ash ng (General)**

For spectrophotometric estimations of iron and manganese, the nitric acid-perchloric acid digestion technique (Middleton and Stuskey, 1954) was followed in general, except for a few modifications for the individual cases.

**Spectrophotometric estimation of iron and manganese:**

**Ashing:**

100 mg. of dry, powdered plant material are transferred
to a 100 ml Pyrex conical flask, 5 ml of A.R. grade concentrated nitric acid are added and digested over a hot plate. 0.5 ml of 60% perchloric acid are added to aid digestion, and more nitric acid is added to complete the digestion, if necessary. When almost dry, 3-5 drops of A.R. grade concentrated H₂SO₄ are added to the flask and heated to drive away the chlorides present in the sample. When the evolution of white fumes has died down, the precipitate is suspended in about 5 ml of ion-free water (obtained by distilling alkaline potassium permanganate solution of Pyrex-glass-distilled water in an all-glass-Pyrex still) and the contents evaporated to dryness, the process repeated a second time. Finally the precipitate is taken up in about 10 ml of ion-free water and slightly heated to dissolve the salts. A suitable blank is also run.

**Ashing for estimation of iron:**

The digestion procedure for the estimation of iron is similar to that for manganese (see above). After digestion and extraction in ion-free water, the contents of the flask are evaporated to dryness, and resuspended in 2 ml of 6 N A.R. grade HCl. The solution is evaporated to dryness and the process repeated with a second aliquot of the acid. Finally the precipitate is redissolved in 2 N HCl and diluted to about 10 ml with ion-free water. A suitable blank is also run.
Spectrophotometric estimation of manganese:

The samples digested (without the use of HCl) are developed for the permanganate colour in the following manner:

To the contents of the flask, are added 2 ml of 89% syrupy phosphoric acid, (E'Merck) and 5 ml of 0.15% aqueous silver nitrate (Newcomb and Sankaran, 1929). The flasks are shaken and the contents boiled for a minute. 3 ml of a saturated solution of ammonium persulphate in ion-free water are added and the mixture boiled for about 3 mins.

When the full permanganate colour develops a few more drops of persulphate solution are added and after cooling the contents are made up to 50 ml (25 ml if the colour intensity is low) with ion-free water.

The optical density of the solution are read against the blank in a spectrophotometer at 525 μm and the concentration of Mn calculated from the correlation graph prepared by using various concentrations of electrolytically pure manganese metal developed in the same way after digestion with dil. HNO₃.

Spectrophotometric estimation of iron:

The sample digested for the estimation of iron (see above) in 2 N HCl is neutralised with 0.5 N NaOH solution and the pH adjusted to 5.0. 5 ml of a N/10 sodium acetate buffer is added to this, followed by 0.1 ml of thioglycollic acid and 0.1 ml of a 0.1% solution of α-α'-bipyridyl in
absolute alcohol (Hill, 1930). The contents turn red and are made up to volume (usually 10 mg equivalent of the material is enough to give a colour of medium intensity). The concentration of iron is read from the correlation graph prepared in the same way, using ferric ammonium sulphate (reduced to ferrous by digesting with HCl). The colour is read against the blank using a spectrophotometer at the wavelength 540 mp.

**Estimation of sap-soluble iron and manganese:**

Fresh plant material for this estimation is sampled as stated earlier. The material is then ground in a glass mortar using glass-distilled water. A blank is also ground in the same way. The ground material is transferred to a centrifuge tube, after washing the pestle and mortar and the contents of the tube are centrifuged at 3,000 r.p.m. for 20 mins. The supernatant is poured into a 100 ml Pyrex conical flask and the precipitate is re-extracted twice with glass-distilled water. All soluble salts are by now carried into the supernatant. The combined extracts are then dried by evaporation on a water bath and the dried matter digested as stated above, and the individual elements estimated spectrophotometrically as described earlier.

**Estimation of nitrogen:**

**Soluble nitrogen:**

The soluble nitrogen is estimated from the fresh tissue as described by Samborski, Samborski and Clayton (1958).
with some modifications. A weighed quantity of the tissue is homogenised in a mortar with about 10 ml of 80% ethanol and transferred to a Buchner funnel and the soluble nitrogenous fraction extracted exhaustively through Whatman no. 1 filter paper. The residue as well as the pestle and mortar are washed repeatedly with 80% ethanol and the extract evaporated to dryness over sulphuric acid in vacuo. When dry, the residue is extracted in distilled water and made up to volume. A blank is also run. A suitable aliquot of this extract is used for the estimation of nitrogen by the method described by Humphries (1956). The nitrogen is estimated as the amount of ammonia evolved by steam distilling the aliquot to which 30% NaOH has been added (15 ml). The liberated ammonia is collected over 2% boric acid to which 2 drops of the Conway indicator has been added. The boric acid is then titrated against N/28 HCl to attain an end point corresponding to the original colour of boric acid containing the indicator. One ml of N/28 HCl is equivalent to 0.5 mg. nitrogen (Humphries, loc. cit.).

Total nitrogen including nitrate nitrogen:

25 mg. of dried, ground plant material is added to a micro-Kjeldahl flask and 1 ml of sulphuric acid+salicylic acid mixture added to it (5 g. of salicylic acid dissolved in 100 ml of A.R. grade conc. H$_2$SO$_4$). The contents are well shaken and allowed to stand for $\frac{1}{2}$ hour. About 0.3 g. of sodium thiosulphate is added to this and the flask heated
until dense fumes appear. The flask is then removed and about 0.06 g. of digestion mixture (1 g. copper sulphate, 8 g. potassium sulphate and 1 g. selenium dioxide, ground well and mixed) followed by 0.5 ml of conc. H$_2$SO$_4$ are added. The contents are digested for about 2 hours when an apple-green solution is obtained. This is cooled, made up to volume and the nitrogen estimated from an aliquot as described above (Humphries, 1956).

**Total nitrogen without nitrate nitrogen:**

25 mg. of dried, powdered material are transferred into a micro-kjeldahl flask and 0.5 ml of A.R. grade conc. H$_2$SO$_4$ added along with 0.06 g. of digestion mixture (see above). The contents are digested for about 2 hours until an apple-green colour is obtained. The contents are cooled, made up to volume and the nitrogen estimated from an aliquot by the method described above (Humphries, loc. cit.).

**Estimation of water loss and dry weight:**

Potted plants growing under controlled conditions are removed near to a single pan Sartorius Selecta balance and the shoot system of the plant (½" of the stem with the terminal cluster of leaves) is quickly cut and placed on the pan. The fresh weight of the shoot is noted; the loss in weight for every 1 minute is observed using a stop clock and this loss is then calculated per gram fresh weight of the tissue. The water loss is calculated for
the first 5 minutes and the tissue then removed to an air-oven and dried at 80°C for 48 hours and later placed in a desiccator containing anhydrous calcium chloride. The dry weight is then determined. The results of water loss (expressed in mg. water lost per gram fresh weight of tissue) and fresh weight/dry weight ratios represent the mean of 10 samples.
Plate I: Leaf of White Burley tobacco showing mosaic symptoms due to infection by TMV.

Plate II: Young leaves of Mar-Globe tomato showing mild mosaic symptoms (note the youngest leaf) due to infection by TMV.
Tobacco mosaic virus (TMV):

In White Burley tobacco, under the normal greenhouse conditions with a temperature range of 28-31°C, the virus causes systemic infection, the leaves coming just after inoculation (6-8 days) showing early stages of mosaic, distortion and paleness, whereas, the older inoculated leaves seldom show any mosaic pattern except after about a month. The infected plants appear stunted. A fully infected plant shows the typical mosaic as shown in Plate I.

In Mar-Globe tomato, about 10 days after inoculation, the new leaves just emerging show slight downward curling and in about 20 days when fully expanded, display a mild mosaic pattern (Plate II). However, stunting is not very distinct.

In Nicotiana lutosae leaves the virus produces local lesions when the temperature of the greenhouse is below 30°C. and systemic infection, followed at a later stage by the death of plants, occurs when the temperature rises to 32-33°C.

When tobacco plants are grown at a temperature of 36-38°C, after inoculation, the plants do not manifest
Plate III: Two-week old Dolichos lablab plants grown in nutrient solution inoculated with DEMV and showing stunting.

I - Inoculated showing stunting
H - Healthy

Plate IV: Healthy and DEMV-infected leaves of Dolichos lablab.

H - Healthy
I - DEMV-infected leaves showing mosaic symptoms.
any mosaic symptom but are seen to grow normally. When plants fully infected with the virus and manifesting typical symptoms are subjected to high temperatures (36-38°C.), the new leaves emerging after the temperature treatment, appear healthy. But these plants when brought back to the normal greenhouse conditions after temperature treatment redevelop the symptoms.

Tobacco plants display typical symptoms of mosaic when treated with long photoperiods after inoculation (24 hrs. light). When treated with continuous darkness, the inoculated plants dry up much quicker, though they remain greener than the healthy. The inoculated plants survive up to a period of 6 days continuous darkness, while the healthy controls continue to live up to 9 days.

Dolichos enation mosaic virus (DEMV):

This virus has been studied extensively on Dolichos lablab (var.DL 231). Under normal greenhouse conditions, the first visible symptom of infection is a pronounced stunting of the inoculated plants (Plate III). The first trifoliate leaf emerging about 6 days after inoculation display typical mosaic symptoms (Plate IV). By about the 15th day, when the second trifoliate leaf appears, symptoms become much more pronounced, and a one-month old infected plant shows considerable variations in symptoms. These include: tendril outgrowths at the leaflet-tips (Plate V - 1), suppression of one or both or all the
Plate V: DEMV-infected leaves of *Dolichos lablab*
showing various types of foliar abnormalities
(for details see text).

Plate VI: DEMV-infected leaves of *Dolichos lablab*
showing characteristic enations on the lower side of the lamina.
Plate VII: DEMV-infected leaves of *Dolichos lablab* showing enations and reduction in growth in the interveinal areas.

a : Enations in the form of outgrowths

b : Reduction in the interveinal areas
   (the arrow points to a cup-like enation)

Plate VIII and Plate IX : Root and nodule development in DEMV-infected *Dolichos lablab*.

Plate VIII : 15 days after inoculation  
Plate IX : One month after inoculation

H - Healthy
I - Infected (note reduction in root system and number of nodules)
plants, the leaves gradually turn yellow after 5 days of darkness and ultimately drop off, whereas in the inoculated ones, they persist, appear wilted, and are more greenish. By about the 9th day in darkness, all plants begin to collapse. But the inoculated plants retain their green colour as compared to the yellow leaves of the healthy series.

When plants are subjected to continuous illumination after inoculation, the same sequence is seen as under the normal greenhouse conditions excepting that the plants (both healthy and inoculated) were shorter than those under normal conditions. However, in the inoculated plants the mosaic symptoms appear much quicker (4-5 days after inoculation) following the light treatment, than under normal greenhouse conditions.

Summarising symptomatology of DEMV and TMV infected plants, it is evident that they have many features in common. Both induce stunting and both viruses remain apparently unaffected under high temperatures (38-38°C.) and when the temperature effect is withdrawn, both begin to display the normal symptoms. Under darkness, both viruses cause wilting and the inoculated plants are greener than the controls.

In contrast to these similarities, the degree and details of mosaic caused by the two viruses differ; while
Plate X: Variations in the intensity of mosaic due to infection by DEMV and TMV.

a: DEMV-infected leaves of Dolichos lablab.
b: TMV-infected leaves of White Burley tobacco.
c: TMV-infected leaves of Mar-Globe tomato.
TMV causes typical mosaic pattern in White Burley tobacco, and less pronounced mosaic in tomato, DEMV in *Dolichos*

*abláb* causes a more severe mosaic (Plate X), with foliar abnormalities like enations. The acute stunting of the DEMV-infected *Dolichos* plants appears much earlier than the appearance of foliar symptoms and is a characteristic feature of this virus.
EXPERIMENTAL

Chapter I

WATER IN RELATION TO MOSAIC DISEASES

Although the changes in water economy in relation to fungal diseases especially those causing wilts have been much studied, the information regarding the same in plant virus diseases is still incomplete. Perhaps this inadequacy of information is due mainly to the fact that many viruses apparently do not cause any wilt—a disease caused by a physical or physiological deficit of water in plants. If a plant virus is capable of inducing changes in the physiology of its host, especially in a region of greatest metabolic activity, then it is logical to expect changes in water economy consequent on virus infection, although, there are no visible symptoms of wilting or drying up under normal conditions.

In this connection it is interesting to note that DEMV-inoculated Dolichos lablab and to a lesser extent TMV-inoculated tomato plants exhibit a rapid rate of wilting than the controls under increasing periods of dark treatment (vide Symptomatology). In both diseases, the plants show no symptoms of mosaic at the time of wilting. In Dolichos the wilting begins by the 6th day after dark treatment and in tomato by the 4th day. During these periods,
the healthy controls remain turgid and the leaves show varying degrees of yellowing, and defoliation. From these observations, it is evident that the entry of the virus leads to a changed water economy of the plants and, therefore, a study of water relations with reference to viroses seems important.

Experiments have been devised to study the changes in transpiratory rates and fresh weight/dry weight ratios consequent on infection by DEMV and TMV in Dolichos lablab and tomato respectively under normal greenhouse and varied environmental conditions.

1. Effect of DEMV on the rate of water loss and fresh weight/dry weight ratio of Dolichos lablab under normal greenhouse conditions.

Dolichos lablab plants were raised under normal greenhouse conditions in similar-sized pots containing uniform amount of soil, and 6 days after germination, the first pair of opposite leaves were inoculated with DEMV inoculum (vide Materials and Methods). Along with a suitable set of controls, the two sets of plants were maintained under similar moisture and environmental conditions. The rate of water loss and fresh weight/dry weight ratio were determined as described under Materials and Methods. The results of the experiment are presented in Tables 1 and 2 (Text-Fig. 1).
Table 1: The rate of water loss in healthy and DEMV-infected Dolichosc lablab (shoots) under normal greenhouse conditions.

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>5 days</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H I</td>
<td>H I</td>
<td>H I</td>
<td>H I</td>
</tr>
<tr>
<td>(water loss in mg./g. fresh weight of tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.6 2.2</td>
<td>1.1 2.7</td>
<td>2.3 2.4</td>
<td>1.2 1.1</td>
</tr>
<tr>
<td>2</td>
<td>4.2 4.9</td>
<td>2.8 3.8</td>
<td>4.7 5.0</td>
<td>2.5 2.1</td>
</tr>
<tr>
<td>3</td>
<td>5.1 7.2</td>
<td>4.4 5.0</td>
<td>7.1 6.8</td>
<td>3.7 3.1</td>
</tr>
<tr>
<td>4</td>
<td>7.2 10.0</td>
<td>5.4 6.4</td>
<td>8.6 9.1</td>
<td>5.0 4.1</td>
</tr>
<tr>
<td>5</td>
<td>8.8 12.6</td>
<td>7.8 9.1</td>
<td>11.2 11.1</td>
<td>6.2 5.2</td>
</tr>
</tbody>
</table>

( H - Healthy ; I - Infected )

Table 2: The fresh weight/dry weight ratios of healthy and DEMV-infected Dolichosc lablab (shoots) under normal greenhouse conditions.

<table>
<thead>
<tr>
<th>Days</th>
<th>Healthy</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>11.0</td>
<td>8.5</td>
</tr>
<tr>
<td>10</td>
<td>11.2</td>
<td>10.9</td>
</tr>
<tr>
<td>20</td>
<td>9.0</td>
<td>8.5</td>
</tr>
<tr>
<td>30</td>
<td>7.7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Results:

From Table 1, it is clear that the rate of water loss in the infected shoots is considerably higher by the 5th day after inoculation. By the 10th day the rate continues to be high in the infected plants. By the 20th day the difference between the healthy and infected plants
becomes negligible, while by the 30th day, the infected plants show a lower rate of water loss than the healthy. Thus, when the plants show intense mosaic symptoms between the 20-30th days after inoculation, the rate of water loss is almost the same if not lesser than the healthy.

Table 2 reveals that the infected plants uniformly show a lower fresh weight/dry weight ratio than the healthy and the difference in the ratio between the healthy and infected plants is greatest by the 5th day. In both series, there is an increase in the ratio by the 10th day, followed by a gradual decrease during the subsequent stages.

2. The rate of water loss and fresh weight/dry weight ratio of healthy and TMV-infected tomato (shoots) under normal greenhouse conditions.

Tomato plants were raised under normal greenhouse conditions and 15 days after germination they were inoculated with TMV. The inoculated and control plants were maintained under identical conditions of environment and moisture requirements. Periodical samplings were made and the rate of water loss and fresh weight/dry weight ratios determined as described in Expt. 1. The results are presented in Tables 3 and 4 (Text-Fig. 2).
Table 3: The rate of water loss in healthy and TMV-infected tomato shoots under normal greenhouse conditions.

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>5 days</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>I</td>
<td>H</td>
<td>I</td>
</tr>
<tr>
<td>(water loss in mg./g. fresh weight of tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.3</td>
<td>3.6</td>
<td>3.2</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>7.0</td>
<td>5.2</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>8.7</td>
<td>11.5</td>
<td>7.7</td>
<td>8.8</td>
</tr>
<tr>
<td>4</td>
<td>11.8</td>
<td>13.8</td>
<td>9.6</td>
<td>10.3</td>
</tr>
<tr>
<td>5</td>
<td>14.2</td>
<td>17.0</td>
<td>11.3</td>
<td>12.5</td>
</tr>
</tbody>
</table>

(H - Healthy; I - Infected)

Table 4: The fresh weight/dry weight ratios of healthy and TMV-infected tomato shoots under normal greenhouse conditions.

<table>
<thead>
<tr>
<th>Days</th>
<th>Healthy</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>16.4</td>
<td>12.5</td>
</tr>
<tr>
<td>10</td>
<td>18.7</td>
<td>13.5</td>
</tr>
<tr>
<td>20</td>
<td>18.1</td>
<td>15.3</td>
</tr>
<tr>
<td>30</td>
<td>14.8</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Results:

Table 3 reveals that the rate of water loss in the TMV-infected tomato shoots of the 5th day series is considerably higher than the healthy. By the 10th day the infected plants continue to lose more water than the healthy but not as much as the 5th day series. By the 20th day the rate of water loss in the infected plants is lower.
than the healthy and by the 30th day, there is not much
difference between the healthy and infected plants. In
both, there is a decrease in the water loss with increasing
age.

The data on fresh weight/dry weight ratios from
Table 4 shows that it is lower in the infected in the
age levels tried, the maximum difference being in the
first 20 days after inoculation. By the 30th day, the
difference in the ratio between the healthy and infected
plants is negligible.

5. Effect of darkness on the ratio of water loss in healthy
and DMV-Inoculated B. napus (leaves, shoots).

Do six sets of plants were raised and inoculated as
described in the earlier experiments. After inoculation,
the plants were removed to a dark chamber (vide Materials
and Methods) along with a suitable set of control plants
and samplings were done on the 2nd, 4th, 6th and 9th days
after the dark treatment. The plants were maintained
under identical moisture conditions. The rate of water
loss and the fresh weight/dry weight ratio were calculated
as described in experiment 1. The results are presented
in Tables 5 and 6 (Text-Fig. 3).

(Tables on the following page).
Table 5: Effect of darkness on the rate of water loss in healthy and DEMV-inoculated Dolichos lablab (shoots).

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>2 days H</th>
<th>I</th>
<th>4 days H</th>
<th>I</th>
<th>6 days H</th>
<th>I</th>
<th>9 days H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.2</td>
<td>2.5</td>
<td>0.9</td>
<td>1.1</td>
<td>0.7</td>
<td>0.7</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>4.8</td>
<td>1.7</td>
<td>2.2</td>
<td>1.5</td>
<td>1.4</td>
<td>2.5</td>
<td>1.8</td>
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<tr>
<td>3</td>
<td>3.8</td>
<td>7.5</td>
<td>2.5</td>
<td>3.4</td>
<td>2.1</td>
<td>2.0</td>
<td>3.8</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>9.6</td>
<td>3.2</td>
<td>4.5</td>
<td>2.8</td>
<td>2.5</td>
<td>5.1</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>6.2</td>
<td>11.9</td>
<td>4.3</td>
<td>5.6</td>
<td>3.5</td>
<td>3.1</td>
<td>6.6</td>
<td>3.2</td>
</tr>
</tbody>
</table>

(water loss in mg/g. fresh weight of tissue)

(H = Healthy; I = Inoculated)

Table 6: Effect of darkness on the fresh weight/dry weight ratios of healthy and DEMV-inoculated Dolichos lablab (shoots).

<table>
<thead>
<tr>
<th>Days</th>
<th>Healthy</th>
<th>Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.2</td>
<td>8.8</td>
</tr>
<tr>
<td>4</td>
<td>11.1</td>
<td>8.6</td>
</tr>
<tr>
<td>6</td>
<td>13.1</td>
<td>6.1</td>
</tr>
<tr>
<td>9</td>
<td>7.8</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Results:

From Table 5 it is evident that the rate of water loss in the DEMV-inoculated Dolichos is nearly twice as much as the healthy by the 2nd day after inoculation and treatment with darkness. By the 4th day, the rate of water loss continues to be higher than the healthy but not as much as in the 2nd day series. By the 6th and
9th days, the loss is lower than the healthy and by the 9th day, the inoculated plants lose only half as much water as the healthy. It must be mentioned here that the inoculated plants manifest symptoms of wilting by the 6th day onwards and by the 9th day the plants become almost completely wilted. No symptoms of wilting are seen in the healthy plants even at the 9th day level.

The fresh weight/dry weight ratios (Table 6) are interesting in that the ratio goes on gradually decreasing in the inoculated plants with increasing dark periods. In the healthy series there is no decrease till the 5th day but by the 9th day the ratio is much reduced. The ratio in the inoculated plants at all age levels is lower than the healthy, the greatest difference being seen on the 6th day.

4. Effect of darkness on the rate of water loss and fresh weight/dry weight ratios of TMV-inoculated tomato.

Tomato plants were raised in the usual manner and 16 days after germination, they were inoculated with TMV inoculum. After inoculation, the plants were subjected to continuous darkness as described in experiment 3 for six days. A suitable control was similarly maintained. The inoculated plants began showing symptoms of wilting by the 6th day onwards. The rate of water loss and fresh weight/dry weight ratio were determined as described in the earlier experiments. The results are presented in Tables 7 and 8 (Text-Fig. 4).
Table 7: Effect of darkness on the rate of water loss in healthy and TMV-inoculated tomato shoots.

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>2 days (H)</th>
<th>I</th>
<th>4 days (H)</th>
<th>I</th>
<th>6 days (H)</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>(water loss in mg./g. fresh wt. of tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.2</td>
<td>1.6</td>
<td>2.0</td>
<td>1.8</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>2.3</td>
<td>2.0</td>
<td>3.7</td>
<td>3.5</td>
<td>2.8</td>
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<tr>
<td>3</td>
<td>3.6</td>
<td>3.0</td>
<td>5.7</td>
<td>5.3</td>
<td>4.2</td>
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<td>7.1</td>
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</tr>
<tr>
<td>5</td>
<td>6.1</td>
<td>5.2</td>
<td>9.6</td>
<td>9.0</td>
<td>6.8</td>
<td>8.5</td>
</tr>
</tbody>
</table>

(H - Healthy; I - Inoculated)

Table 8: Effect of darkness on the fresh weight/dry weight ratios of healthy and TMV-inoculated tomato shoots.

<table>
<thead>
<tr>
<th>Days</th>
<th>Healthy</th>
<th>Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>18.3</td>
<td>16.3</td>
</tr>
<tr>
<td>4</td>
<td>18.5</td>
<td>8.9</td>
</tr>
<tr>
<td>6</td>
<td>11.7</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Results:

From the results presented in Table 7 it is evident that the rate of water loss in the TMV-inoculated tomato shoots remains lower than the healthy between the 2nd and 4th days after the dark treatment, but increases by the 6th day, when it is higher than the healthy. It is interesting to observe here, that this trend is contrary to what
is seen in the DHMV-inoculated *Dolichos* subjected to
darkness.

Table 8 shows that the fresh weight/dry weight
ratios of the inoculated plants is lower than the healthy
at all age levels. The difference is very great on the
4th and 6th days after inoculation, when the ratio is
nearly half as much as the healthy.

5. **Effect of continuous illumination on the rate of
water loss and fresh weight/dry weight ratio of DHMV-
inoculated *Dolichos* lablab shoots.**

*Dolichos* lablab plants were raised and inoculated
with DHMV as described in the previous experiments. After
inoculation, the plants were removed to a light chamber
(vide Materials and Methods). A set of controls was also
maintained under similar conditions. The plants were
treated with 2, 4, 6 and 9 days of continuous illumination
and sampled periodically. The rate of water loss and
fresh weight/dry weight ratios were determined as described
in Expt.1. The results are presented in Tables 9 and 10
(Text-Fig. 5).

Table 9: Effect of continuous illumination on the rate of
water loss in healthy and DHMV-inoculated
*Dolichos* lablab shoots.

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>9 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>I</td>
<td>H</td>
<td>I</td>
</tr>
<tr>
<td>(water loss in mg./g. fresh wt. of tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.2</td>
<td>3.3</td>
<td>3.3</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>4.4</td>
<td>6.3</td>
<td>6.7</td>
<td>6.2</td>
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<td>6.4</td>
<td>9.4</td>
<td>9.4</td>
<td>9.3</td>
</tr>
<tr>
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<td>8.4</td>
<td>12.7</td>
<td>12.2</td>
<td>12.0</td>
</tr>
<tr>
<td>5</td>
<td>10.1</td>
<td>15.7</td>
<td>15.1</td>
<td>14.8</td>
</tr>
</tbody>
</table>

(H - Healthy; I - Inoculated)
Table 10: Effect of continuous illumination on the fresh weight/dry weight ratios of healthy and DEMV-inoculated Dolichos lablab shoots.

<table>
<thead>
<tr>
<th>Days</th>
<th>Healthy</th>
<th>Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.2</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>10.1</td>
<td>9.7</td>
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<td>9.9</td>
</tr>
<tr>
<td>9</td>
<td>10.2</td>
<td>9.4</td>
</tr>
</tbody>
</table>

**Results:**

Table 9 reveals that the rate of water loss in the DEMV-inoculated Dolichos plants is considerably higher than the healthy on the 2nd and 6th days after the light treatment. By the 4th and 9th days, however, the rate equals almost that of the healthy.

The fresh weight/dry weight ratios of the inoculated plants are always lower than the healthy at all age levels (Table 10). The maximum difference is seen on the 9th day after inoculation.

6. **Effect of continuous illumination on the rate of water loss and fresh weight/dry weight ratios of TMV-inoculated tomato shoots.**

Tomato plants were raised under normal greenhouse conditions and inoculated with TMV, 15 days after germination. After inoculation, the plants were transferred to a light chamber along with a set of control plants. The two sets of plants were subjected to identical conditions and were sampled periodically. The rate of water loss and fresh weight/dry weight ratios were determined
as in Expt. 1. The results are presented in Tables 11 and 12 (Text-Fig. 6).

Table 11: Effect of continuous illumination on the rate of water loss in healthy and TMV-inoculated tomato shoots.

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>9 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>I</td>
<td>H</td>
<td>I</td>
</tr>
<tr>
<td>(water loss in mg./g. fresh wt. of tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>2.6</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
<td>5.1</td>
<td>4.4</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>4.3</td>
<td>7.4</td>
<td>6.1</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>5.6</td>
<td>9.4</td>
<td>7.8</td>
<td>9.1</td>
</tr>
<tr>
<td>5</td>
<td>6.4</td>
<td>11.4</td>
<td>9.4</td>
<td>11.3</td>
</tr>
</tbody>
</table>

( H - Healthy; I - Inoculated)

Table 12: Effect of continuous illumination on the fresh weight/dry weight ratios of healthy and TMV-inoculated tomato shoots.

<table>
<thead>
<tr>
<th>Days</th>
<th>Healthy</th>
<th>Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>18.6</td>
<td>17.3</td>
</tr>
<tr>
<td>4</td>
<td>17.2</td>
<td>15.6</td>
</tr>
<tr>
<td>6</td>
<td>16.6</td>
<td>14.3</td>
</tr>
<tr>
<td>9</td>
<td>17.8</td>
<td>16.1</td>
</tr>
</tbody>
</table>

From the results presented in Table 11 it is clear that the rate of water loss is uniformly higher in the inoculated plants at all age levels. By the 2nd day, the inoculated plants lose nearly twice as much water as the healthy, but the difference gradually narrows down till
the 6th day. By the 9th day again, the inoculated plants show a greater loss of water than the healthy.

Table 12 reveals a uniformly lower fresh weight/dry weight ratio of the inoculated plants at the age levels tried, the greatest difference being seen by the 6th day after inoculation.