PHYSIOLOGY OF VIRUS-INFECTED PLANTS

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Abstract

Little information is available on the sequence of physiological changes from virus inoculation to full development of disease symptoms. In this paper, we discuss (1) activity of chlorophyllase, (2) ferrous and ferric iron changes, (3) inorganic and organic phosphorus, and (4) respiration in pigeon pea sterility mosaic infected pigeon pea plants and cassava mosaic infected cassava plants.

In both healthy and diseased plants, chlorophyll *a* and *b* increased with age. However, these were significantly lower in diseased plants than in healthy plants, from early stages. Chlorophyllase activity increased with age, such increase being much steeper in diseased plants. There appeared to be a progressive conversion of ferrous Fe to ferric Fe in diseased leaves. Diseased leaves at all ages had higher levels of total P. There was a greater conversion of inorganic P to the organic form as the disease progressed. Respiration increased with progress of disease up to the production of full symptoms, and dropped thereafter reaching levels lower than those in healthy leaves of corresponding age. In PSMV-affected pigeon pea leaves activity of catalase, peroxidase, ascorbic acid oxidase and cytochrome oxidase increased while polyphenol oxidase activity decreased. Mitochondrial nitrogen was much higher in diseased leaves than in healthy leaves.

Introduction

A Study of imbalance in the major metabolic processes in virus-infected plants would help to elucidate the complex host-virus interactions and has, therefore, received considerable attention from many workers (Wynd, 1943; Grieve, 1951; Bawden and Pirie, 1952; Bawden, 1959; Porter, 1959). Recently, the subject has also been ably reviewed by Diener (1963) and Sadasivan (1963). Much of the information gathered so far relates to mosaic type diseases. Derangements in all major metabolic activities

such as respiration, photosynthesis and carbohydrate metabolism, organic acid and nitrogen metabolism have been noticed. Much of the information has, however, been gathered from studies on plants usually employing the fully developed leaves showing optimum symptoms. A study of physiological changes in the infected plant at a particular stage will not give a full picture of the sequence of physiological derangements in the host plant. In this paper, we discuss the sequence of changes in pigeon pea plants affected by the sterility mosaic disease, and cassava plants affected by the cassava mosaic disease. We present here only results obtained in (1) chlorophyllase activity, (2) status of ferrous and ferric iron, (3) organic and inorganic phosphorus, and (4) respiration.

Pigeon pea sterility mosaic (PSMV) produces severe mosaic mottling of the *Cajanus cajan* leaves, reduction of leaf size, stunting of the plant and abortion of flowers without pod-set. The natural vector of the virus is not definitely known, but is suspected to be a soil-borne agency. As artificial transmission of the disease is difficult and uncertain, naturally infected material was used in the present studies. Leaves from the first to the sixth node were analysed for the various constituents. Symptoms of the disease become progressively severe with increase in age of the leaves, the sixth leaf showing the most severe symptoms.

Cassava mosaic disease (CsMV) is very common on *Manihot esculenta* (cassava, tapioca) in the Kanyakumari District of Madras State. The disease produces conspicuous mosaic mottling and distortion of leaves and severe stunting of the plants. Root tuber production is severely reduced. The virus is easily transmitted by grafting and by the whitefly *Bemisia tabaci*. Optimum symptoms usually develop in about 15 days from inoculation. In the studies reported here, the first leaf produced after graft inoculation was analysed 5, 10, 15, 20 and 25 days after graft inoculation for the various constituents.

Standard methods of analysis were followed.

Chlorophyll and Chlorophyllase

In both the diseases, *viz.*, pigeon pea sterility mosaic disease and cassava mosaic disease, the most conspicuous symptom is the mosaic mottling of leaves. Figure 1 indicates the chlorophyll content and chlorophyllase activity as influenced by age and disease in both hosts. In healthy pegeon pea leaves slight increase in chlorophyllase activity was observed with increasing age. In the diseased leaves of both hosts, however, striking

increase in chlorophyllase activity was observed with the progress of disease. This increase was much more than can be accounted for by age alone. It was observed that the yellow patches in diseased pigeon pea leaves had much higher chlorophyllase activity than the green areas (Nambiar, 1966). Chlorophyll *a* and *b* increased with age in healthy and diseased leaves; but diseased leaves had generally lower contents of these pigments at all stages. The difference was more striking in chlorophyll *b*.

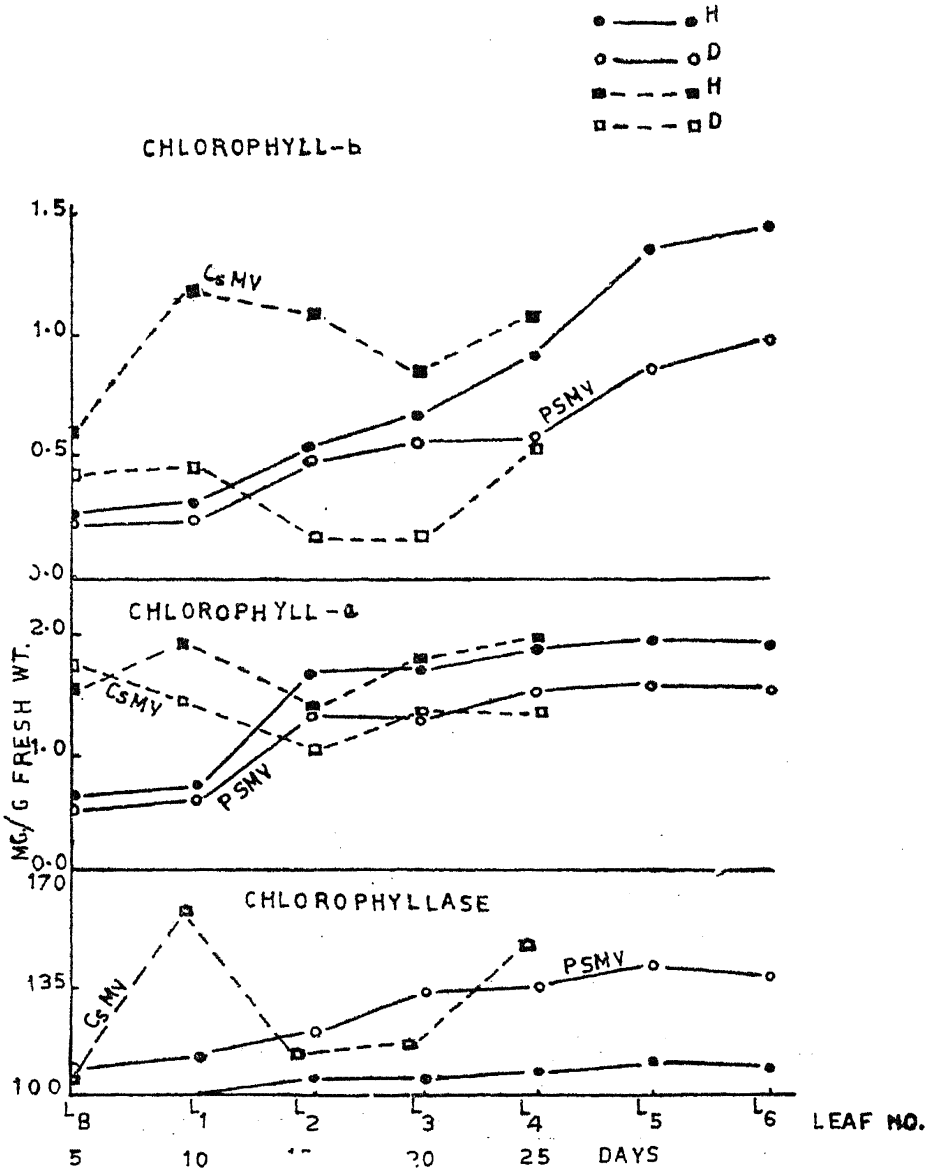

FIG. 1. Chlorophyll *a*, chlorophyll *b* and chlorophyllase activity in PSMV-affected pigeon pea and CsMV-infected cassava.

Chlorosis produced in virus-affected leaves has been attributed to two causes: inhibition of chloroplast development (Esau, 1956) and stimulation of cell enzymes, which attack chlorophyll (Peterson and McKinney, 1938). In PSMV-infected pigeon pea leaves, Narayanasamy and Rama-

krishnan (1965) could not find any alteration in chloroplast size. It would appear, therefore, that the mosaic effect in pigeon pea is largely due to stimulation of chlorophyllase activity. In diseased cassava plants, in addition to a stimulation of chlorophyllase, a reduction in chloroplast size has also been observed (Alagianagalingam, 1967). The mosaic effect appears to be largely due to stimulation of chlorophyllase, though other factors which inhibit chloroplast development are also active in diseased plants (*see* below).

Iron status.—Iron is indispensable for chlorophyll synthesis though it does not directly enter into the chlorophyll molecule. Thus, it is of interest to study the status of iron *vis-a-vis* a reduced level of chlorophyll in virus-infected leaves. Though the reduction in total and ferric iron in

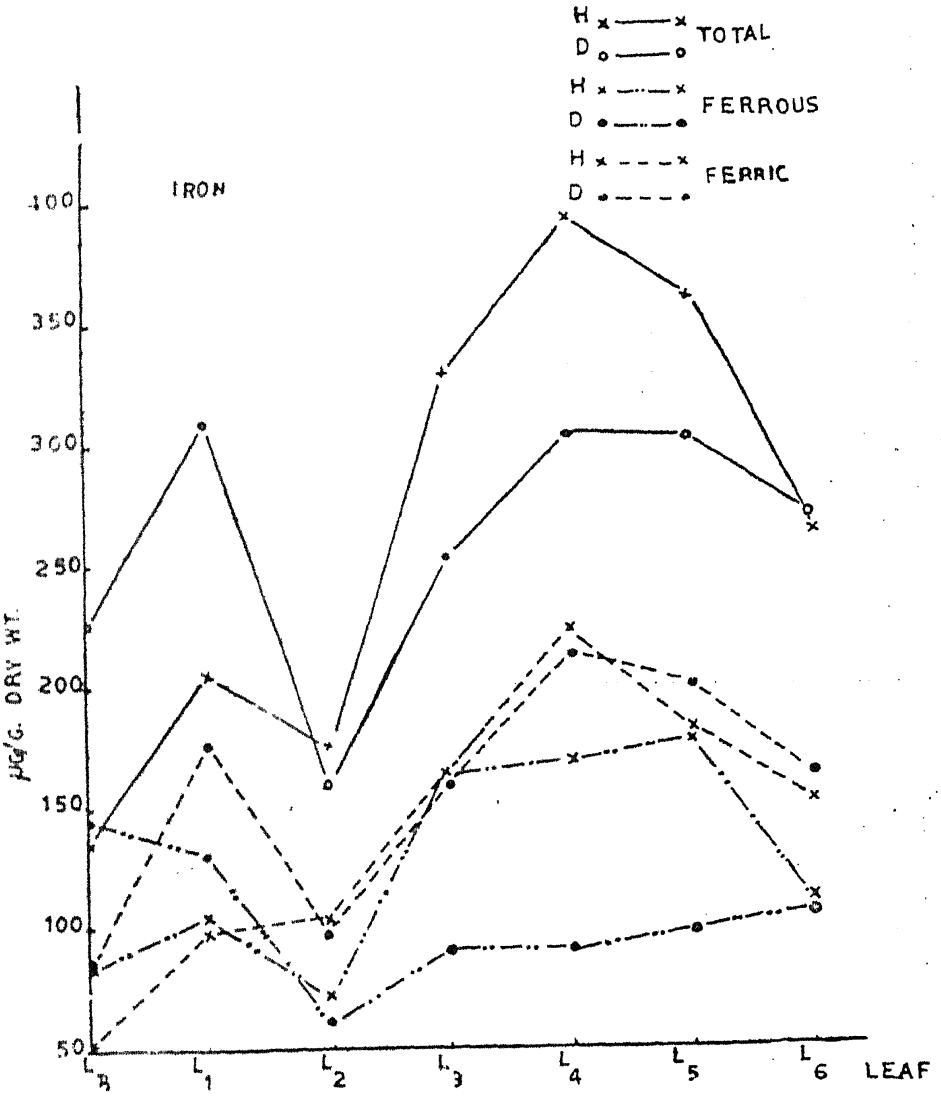

FIG. 2. Effect of PSMV infection on total, ferrous and ferric iron contents in PSMV-affected pigeon pea leaves.

diseased when compared to healthy leaves was not significant, the level of ferrous iron showed a significant decrease in diseased leaves over healthy leaves (Figs. 2 and 3). It is interesting to note that while in leaf-bud and first leaf of the diseased pigeon pea plant the ferrous iron content was higher than ferric iron, in leaves below first node in diseased pigeon pea plant the ferric form constituted the major part. This finding is in agreement with that of John (1963) who found that the major part of total iron in *Dolichos lab-lab* infected by *Dolichos* enation mosaic virus (DEMV) was in the insoluble state, perhaps in the ferric form. A similar trend was observed in diseased cassava leaves also. Infection probably leads to a greater conversion of ferrous to the ferric form of iron.

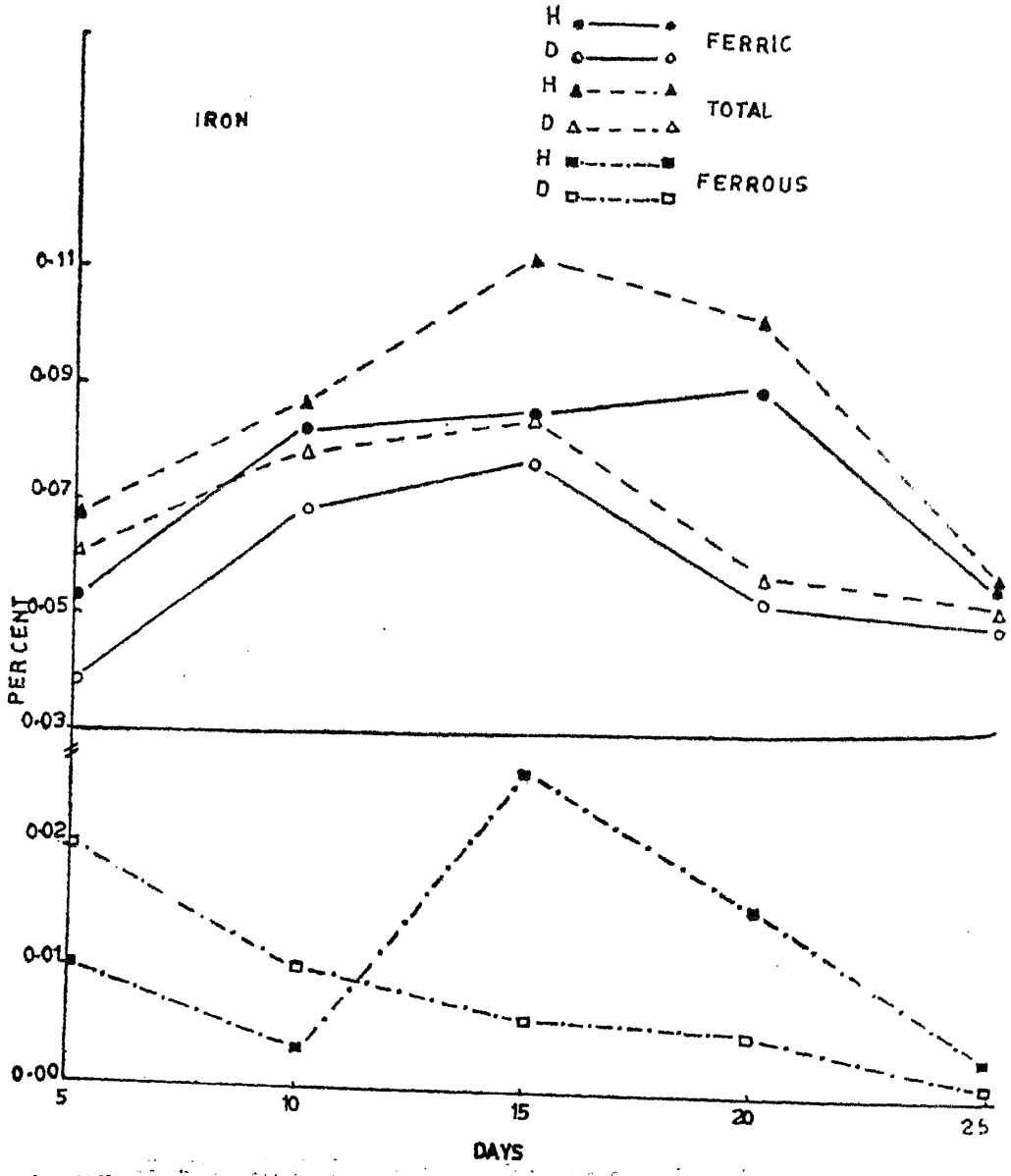

FIG. 3. Effect of CsMV infection on total, ferrous and ferric iron contents in CsMV infected cassava.

Phosphorus status.—Both the PSMV-affected pigeon pea and CsMV-affected cassava leaves recorded higher level of total phosphorus at all ages when compared to healthy leaves. Inorganic phosphorus level was significantly less in diseased tissues except in older leaves of Cassava (Fig. 4). The differences in phosphorus level between healthy and

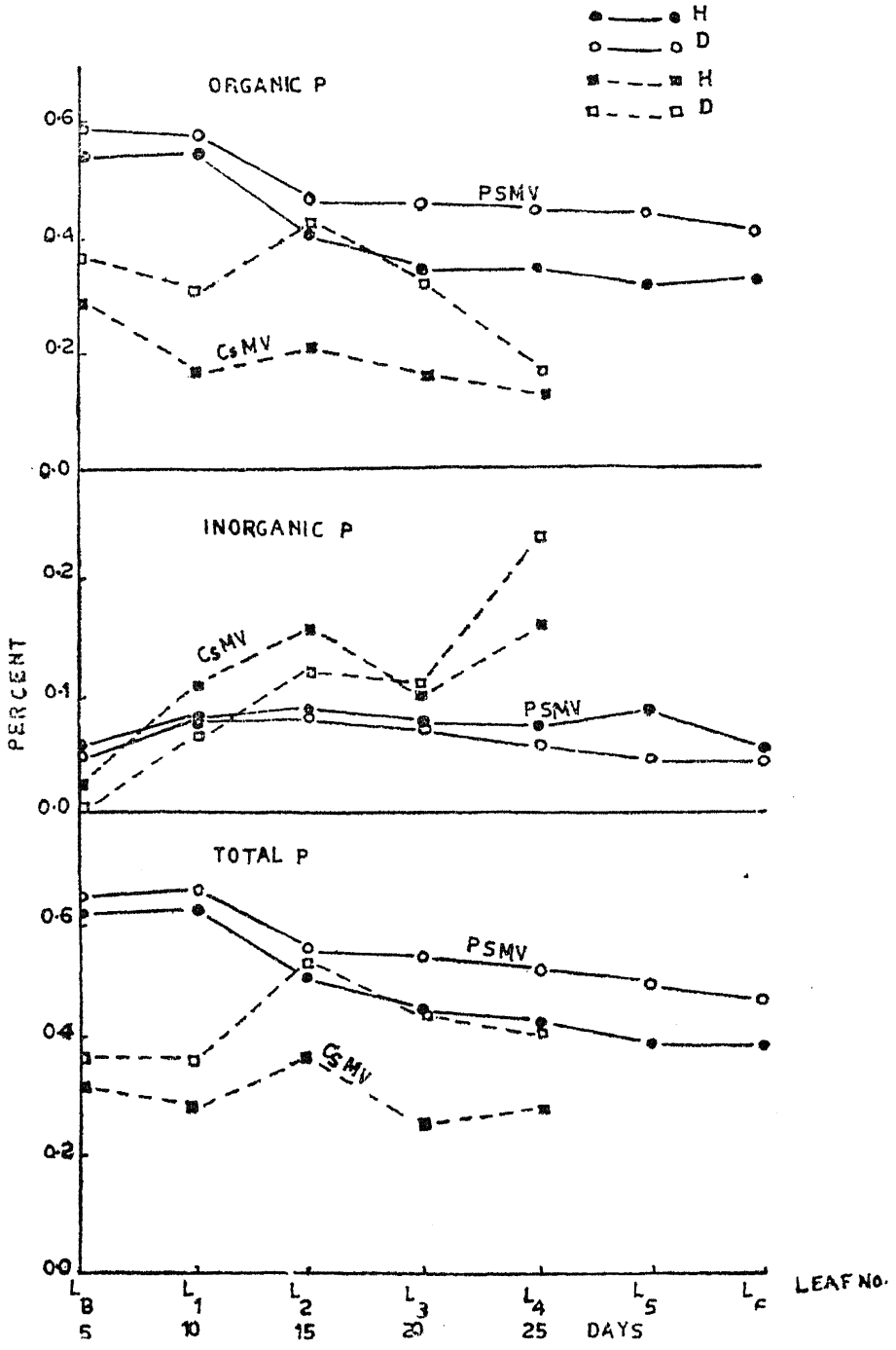

FIG. 4. Total, inorganic and organic phosphorus in PSMV-affected pigeon pea and CsMV-infected cassava.

diseased leaves steadily became wider with the age of the leaf and the progress of the disease. The inorganic phosphorus content was significantly less in the fourth and fifth leaves in diseased pigeon pea. The organic phosphorus levels in diseased pigeon pea and cassava leaves were much higher than in healthy leaves and this difference became wider as disease progressed. It appears that not only did diseased leaves contain higher levels of phosphorus but with the progress of the disease a larger fraction of inorganic phosphorus was converted to the organic form. It is known that phosphorus enters into the composition of nucleic acids. A higher level of nucleic acid observed in diseased pigeon pea leaves (Nambiar, 1966) is of interest in this connection. Jeyarajan (1965) found higher levels of total phosphorus in chilli leaves affected by potato virus Y. However, Ramadasan (1962) reported a decrease in phosphorus in leaves of DEMV-affected *D. lab-lab* plants while Porter and Weinstein (1960) could not observe any significant difference in the levels of total, inorganic and organic phosphorus between healthy and cucumber mosaic virus (CMV)-infected tobacco.

Respiration.—Respiratory changes during pathogenesis has been investigated for a number of years with different hosts and diseases. In general, virus-infected tissues respire at an accelerated rate (*see* Diener, 1963). The data on alteration in respiration in pigeon pea and cassava leaves as brought about by disease and age are presented in Fig. 5. It may be seen that the respiration rate in diseased leaves was higher than the control. In the case of PSMV disease, the respiratory rate in diseased leaves tended to fall in older leaves reaching even levels lower than in healthy leaves. In cassava at all ages diseased leaves respired at a higher rate than healthy leaves. The maximum difference, however, was on the 15th day after inoculation and the difference narrowed down thereafter.

The changes in activity of some enzymes in pigeon pea plants affected by PSMV was investigated (Fig. 5 and Table 1). The activity of catalase, peroxidase, ascorbic acid oxidase and cytochrome oxidase increased with progress of the disease while polyphenol oxidase activity was less in diseased leaves at all stages. The significant increase in catalase activity in second, third and fourth leaves on diseased plant over their healthy counterparts is important in that these leaves showed the full disease symptoms and higher percentage of stimulation in respiration. Wynd (1942) studying catalase activity in tobacco mosaic virus (TMV)-infected tobacco leaves at different ages could not attach any physiological significance to changes that occurred in the catalase activity of these leaves.

The increased peroxidase activity has, however, been interpreted to be a factor causing enhanced respiration by Pantanelli (1912). Loebenstein

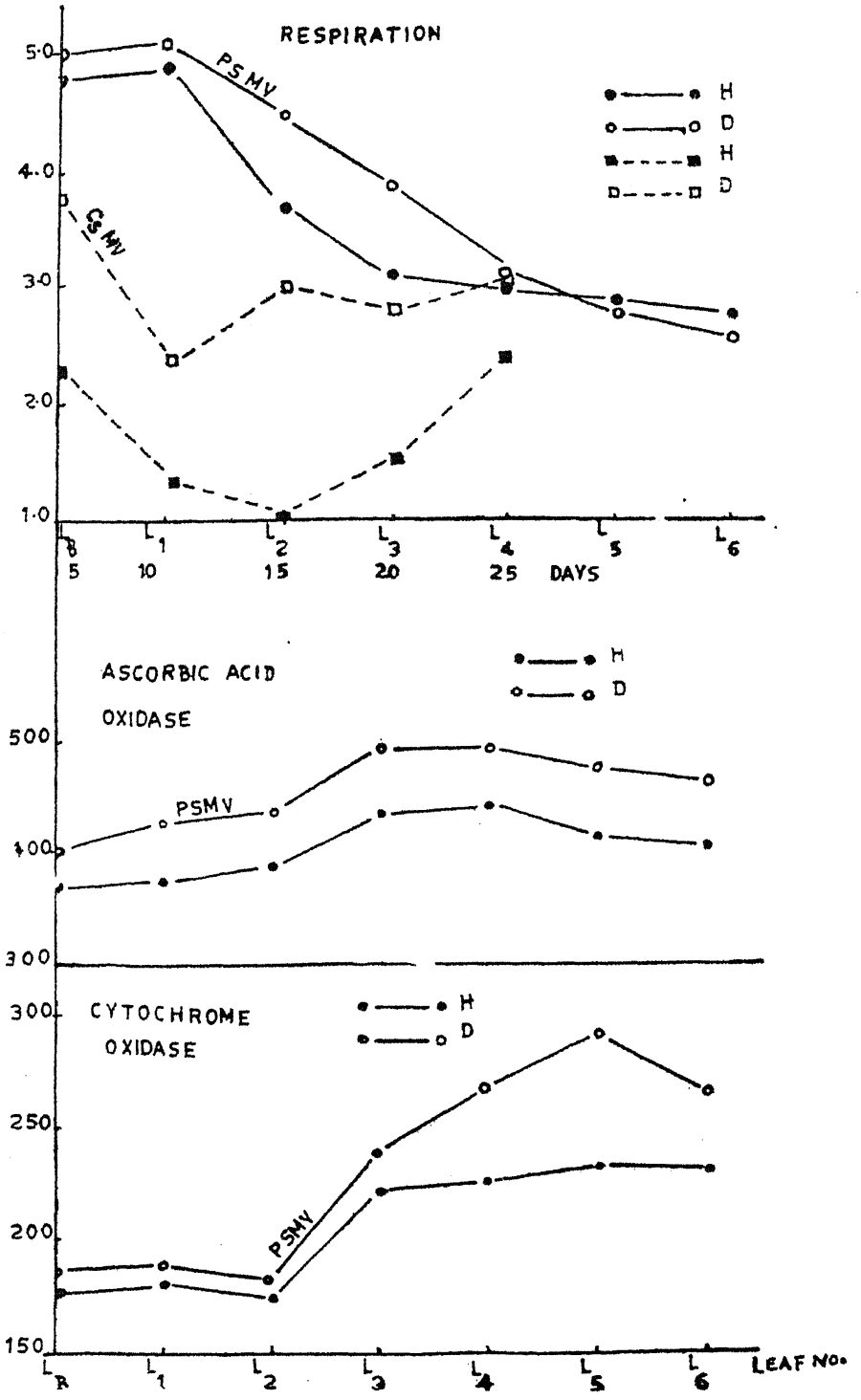

FIG. 5. Top: Respiration rate in PSMV-affected pigeon pea and CsMV-affected cassava.

Middle and bottom: Ascorbic acid oxidase and cytochrome oxidase activity in PSMV-affected pigeon pea.

TABLE I

Activities of catalase, peroxidase and polyphenol oxidase in leaves at different ages of healthy and PSMV-affected pigeon pea plants

Leaf position	CATALASE (Expressed as unit enzyme activity)			PEROXIDASE (Expressed as difference in optical density in 10 min.)			POLYPHENOL OXIDASE (Expressed as μl O_2 per hour per g. over endogenous oxidation)		
	Healthy	Diseased	% increase (+) or decrease (−) over healthy	Healthy	Diseased	% increase (+) or decrease (−) over healthy	Healthy	Diseased	% increase (+) or decrease (−) over healthy
Leaf-bud	0·719	0·724	+ 0·72	0·013	0·016	+23·30	1133·40	953·85	−15·90
Leaf No. 1	0·788	0·910	+13·48	0·014	0·018	+28·60	1228·48	693·94	−43·52
„ 2	0·979	1·152	+17·76	0·015	0·019	+26·60	1533·98	636·36	−58·65
„ 3	1·474	1·545	+ 4·82	0·018	0·022	+22·20	1116·74	712·27	−36·22
„ 4	1·651	1·711	+ 3·67	0·021	0·025	+19·05	1201·88	814·22	−32·25
„ 5	1·568	1·711	+ 9·12	0·025	0·029	+16·00	1105·23	1063·16	− 3·81
„ 6	1·457	1·488	+ 2·16	0·021	0·026	+23·80	1226·65	1202·30	− 1·99

and Linsey (1963) found that increased peroxidase activity was related to severity of symptoms in tobacco plants infected by PVY, TMV and PVX + TMV. Peroxidase activity has been correlated with virus content and symptom development in CMV-infected plants (Menke and Walker, 1963). In the present investigation, the gradual increase of peroxidase activity from leaf-bud downwards in diseased leaves indicates perhaps its relation not only to the symptom development but also to respiration. Merrett (1962) studying TMV-infected tomato plants found an initial decrease in polyphenol oxidase activity followed by an increase reaching a maximum in 8 to 10 days depending upon the leaf position. Thereafter, it fell gradually till it was below the level observed in healthy plants. The present finding of an increased cytochrome oxidase activity in diseased pigeon pea leaves compared to their healthy counterparts is in agreement with those of Merrett (1962) and Rubin and Zeleneva (1964 *a*, 1964 *b*).

In view of the increased level of most of the enzymes studied in diseased tissues it is not clearly known whether the increased respiration of the PSMV-infected plant can be attributed to an increased activity of one or all of these enzymes. Mitochondria are known to be the seat of cytochrome oxidase activity. In this connection, the observation

of an increased number of mitochondria in TMV-infected *N. glutinosa* (Weintraub *et al.*, 1964) is of interest. In diseased leaves of pigeon pea an increase in mitochondria nitrogen has been observed probably indicating a similar increase in mitochondrial numbers (Nambiar, 1966).

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