Respiration Rates and Peroxidase Activity in Virus-Infected *Phaseolus vulgaris*

The respiration rate of virus-infected leaves usually increases during the period of virus multiplication and the increase in intensity of disease symptoms. Such changes have been observed where viruses cause local lesions, e.g. tobacco mosaic virus (TMV) in *Nicotiana glutinosa* L.[1] or in *N. tabacum* L. var 'Xanthi'[2] and in systemic infections e.g. vein clearing in sweet potato[3]. These increases may reflect changes in the activity of specific oxidative enzyme systems[4,5] and the following is an account of the effect of the local lesion producing tobacco necrosis virus (TNV) on respiration rate and peroxidase activity in French beans (*Phaseolus vulgaris* L. var. 'Prince').

The upper surface of one of the pair of fully expanded primary leaves of *P. vulgaris* was inoculated by rubbing with the forefinger wet with a partially purified preparation of the D strain of TNV[6] and the other with distilled water to constitute a control.

For respiration studies, discs 12 mm in diameter were punched from the leaves and the oxygen uptake of these was determined by conventional Warburg manometry. For measuring peroxidase activity 0.6 g of leaf discs was ground in 10 ml ice cold 0.1 *M* sodium acetate in a M.S.E. homogeniser for 5 min, and the resulting macerate centrifuged for 1 h at 15,000 *g* at 4 °C. The activity of the supernatan was determined by a colourometric method[5]. The reaction time of 0.3 ml of supernatant was measured on a hydrogen peroxide and pyrogallol substrate from O.D. 1.0–3.0 in an 'Eel' colourimeter with a 622 filter. Absolute peroxidase values for each sample were expressed as (sec · mg fresh weight)$^{-1}$ or (sec · cm^2 leaf area)$^{-1}$ and relative activity was taken as the ratio of

$$\frac{\text{absolute activity infected}}{\text{absolute activity healthy}}$$

with the relative activity of the healthy control as 1.0.

Increase in respiration rate following infection. In virus infected leaves respiration rate increased 48 h after inoculation although local lesions were not observable until some 12 h later (Figure 1). The respiration rate increased with time and was higher in those discs with the greatest number of lesions. In other experiments no respiratory increase was found in discs cut from between the lesions of infected leaves.

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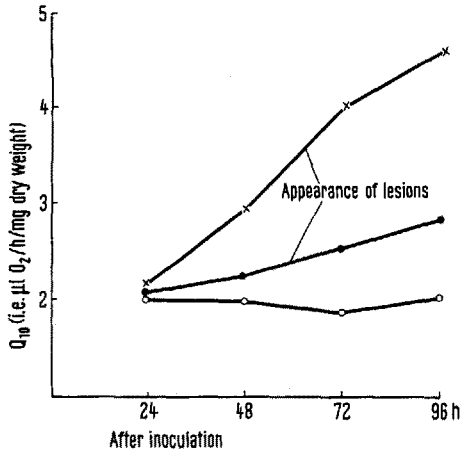

Fig. 1. The respiration rate of TNV infected and healthy leaf discs of *Phaseolus vulgaris*. x——x TNV infected tissue (20 lesions/disc). •——• TNV infected tissue (5 lesions/disc). o——o Healthy tissue.

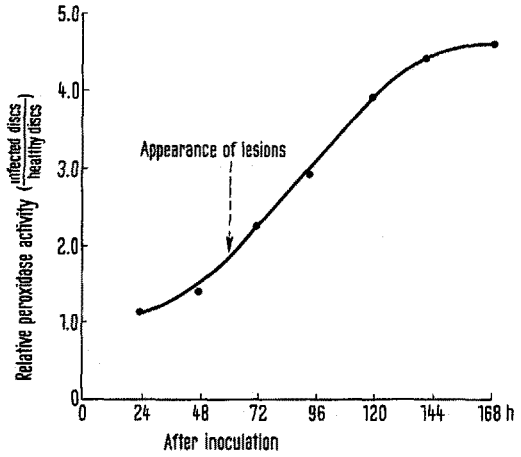

Fig. 2. Increase in relative peroxidase activity of *Phaseolus vulgaris* leaves after inoculation with TNV.

Peroxidase activity in leaf discs of *Phaseolus vulgaris* showing different numbers of local lesions 4 days after inoculation with TNV

Mean No. of lesions per disc	Reaction time (sec) O.D. 1.0–3.0	Fresh weight basis Absolute activity (sec · mg)$^{-1}$	Relative activity	Leaf area basis Absolute activity (sec · cm^2)$^{-1}$	Relative activity
0 (healthy leaves)	58[a]	$0.86 \cdot 10^{-3}$	1.0	$0.52 \cdot 10^{-3}$	1.0
0 (infected leaves)	58	$0.86 \cdot 10^{-3}$	1.0	$0.52 \cdot 10^{-3}$	1.0
1	41	$1.22 \cdot 10^{-3}$	1.42	$0.73 \cdot 10^{-3}$	1.40
5	30	$1.61 \cdot 10^{-3}$	1.87	$0.92 \cdot 10^{-3}$	1.79
10	24	$2.08 \cdot 10^{-3}$	2.42	$1.08 \cdot 10^{-3}$	2.08
20	14	$3.57 \cdot 10^{-3}$	4.15	$2.01 \cdot 10^{-3}$	3.87
LSD ($P = 1\%$)		$0.34 \cdot 10^{-3}$		$0.14 \cdot 10^{-3}$	

[a] 0.3 ml enzyme extract mixed with 5 ml 0.05 *M* pyrogallol in phosphate buffer (pH 6.0) and 0.5 ml 1% H_2O_2.

Effect of degree of infection on peroxidase activity. TNV inocula were diluted to give different numbers of lesions on the primary leaves of a number of seedlings. Extracts were made 4 days after inoculation of 0.6 g of discs respectively containing 1, 5, 10 and 20 lesions/disc. Controls were extracts from 0.6 g of discs cut from leaves 'inoculated' with distilled water and from the non-necrotic areas of virus-inoculated leaves. Peroxidase activity increased with lesion number (Table) and any increase seemed to depend upon the presence of virus in an active state in the leaf cells. Approximately the same differences in relative activity were found between results on leaf area basis and fresh weight basis.

Peroxidase activity increase following infection. Aliquots of a concentrated TNV inoculum were rubbed on the primary leaves of seedlings at varying times before extraction of the enzyme. The relative peroxidase activity was greater the longer the period of virus infection but there was a detectable increase at 24 and 48 h after inoculation although no lesions could be seen at these stages (Figure 2).

With TMV infection of *N. glutinosa*, YAMAGUCHI and HIRAI[7] suggest that the increase in respiration is related to the necrotic process, but other workers[2,8] found that respiration increases before the appearance of local lesions. The latter would appear to be true for TNV infected *P. vulgaris*, i.e. respiration increases during the period of virus synthesis. The increase in peroxidase activity also began before there was any visible sign of lesions but further work is required to establish whether this increase is involved in the glycolytic pathway. An increase in polyphenoloxidase activity has been found in a number of necrotic virus infections in *N. glutinosa* and *Datura stramonium*[8] but I found very little activity of this enzyme in either healthy or infected French bean seedlings. One of the roles of peroxidase is to catalyse the oxidation of phenolic substances to quinones in the presence of hydrogen peroxide[9]. It is possible that the necrotic reaction in TNV-infected *P. vulgaris* is a result of such activity. It may be that peroxidase is connected with the hexose monophosphate shunt in that it may oxidise aromatic compounds such as coumarin and polyphenols produced in this pathway.

Zusammenfassung. Atmungsgeschwindigkeit und Peroxidasetätigkeit mit Tabak-Nekrose-Virus eingespritzter Primärblätter des *Phaseolus vulgaris* L. nehmen bei 26 °C vor dem Erscheinen der lokalen Wunden zu, und zwar proportional zur Wundenzahl und ebenso in Abhängigkeit vom Abstand der Periode vom Zeitpunkt der Einspritzung an.

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Eye of the Cockle, *Cardium edule*: Anatomical and Physiological Investigations

The cockle responds defensively to shadow, the reflex consisting of siphon withdrawal and shell closure. The sense organs which probably mediate the reflex are a series of about 60 small eyes at the apices of tentacles, which arise from around the base of each siphon.

The structure of these eyes has been described by light microscopists[1]. Each eye consists of a cup of reflecting material enclosing 12–20 receptor cells. Nerve fibres arising from these cells leave the eye in a single bundle at the lowest point of the reflector cup (Figure 1). This nerve runs down the tentacle, and joins with those from other tentacles in the external pallial nerve. A semi-circle of brown pigment runs round the side of the eye nearest to the siphon.

Eyes were fixed for electron microscopy as in our previous study on *Pecten*[2]. The receptor cells are irregularly arranged, some in the centre and some in contact with the walls of the reflector cup. They are unusual in possessing large numbers of cilia (Figure 2). We estimate that there are of the order of 100 cilia per cell. The cilia have a 9 + 0 filament content and have basal bodies but no roots. Over much of the cell the cilia form an intertwining tangle, but in the part of the cell nearest to the reflector they are often flattened, with many cilia forming a regular parallel array. No synaptic structures have been seen in the eye, and so we presume that the optic nerve fibres arise directly from the receptors and that there are no synaptic interactions between the cells.

The only important optical structure in the eye is the reflector. It is not possible that the 'lens', an ill-defined region of soft tissue overlying the receptors, exerts any significant convergent effect on the incident light over so short a distance. A crude image formed by reflection is visible when the eye is viewed from the apex of the tentacle. This image, located by optical construction, does lie in the region occupied by the receptors. However, as each receptor also occupies regions of the eye-cup other

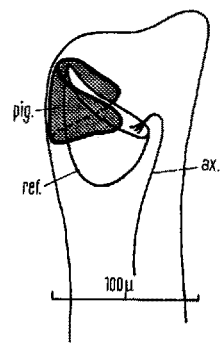

Fig. 1. Drawing of an eye of *Cardium edule* from living material, showing the reflector cup (ref.), which encloses the receptor, the brown pigment band (pig.), and the optic nerve (ax). The siphon would be to the left.

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