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The respiratory metabolism of tobacco leaves infected with different strains of potato virus X

IV. Respiration rate in the early stage of infection

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INTRODUCTION

In previous papers of this series it was estabilished that the increased respiration of tobacco leaves, infected with different strains of potato virus X is related firstly to the appearance of symptoms of infection, and secondly to the severity of those symptoms, and that the most significant difference in the respiration between infected and healthy leaves is observed during the necrotisation process (Dwuraźna, Weintraub).

Recently Yamaguchi and Takahashi (1965) have studied the effect of systemic virus infection on the respiration of leaves. They could not detect any respiratory changes when whole-leaf tissue was used. A marked increase of respiratory rate was detected when epidermal tissue from inoculated leaves was used.

Because no increase of respiration rate was ever observed prior to the appearance of symptoms, either in localized or in systemic infection, the aim of the present work is to investigate further the respiratory changes in the early stages of infection in the epidermal tissue of tobacco leaves infected with different strains of potato virus X.

The polyphenoloxydase activity was also measured.

MATERIALS AND METHODS

In all experiments plants of *Nicotiana tabacum*, var. Haranova were used. The plants were grown in the greenhouse at a mean temperature of about 18°C, and given additional lighting to provide a 16 hour day. A week before inoculation they were put in a controled chamber at 20°C with 75% relative humidity and 16 hours of fluorescent light.

The plants, which were in the development stage of 4—6 leaves, were inoculated with different strains of PVX varying in virulence from latency, to mild mottle, severe mottle and ringspots.

The non-inoculated healthy plants were used as a control. The leaves were dusted with carborundum (600 mesh), inoculated on the lower

surface by brushing carefully with water-colour brush no. 6 soaked in purified virus preparations, and rinsed with running tap water for one minute. Then the inoculated and control plants were returned to the chamber and were kept there for the rest of the experiment.

The respiration rates were measured at 25°C by determining the oxygen uptake of the epidermal tissue, using the conventional Warburg manometric technique.

Epidermal strips were peeled away from the leaf at various intervals after inoculation. The method described by Welkie and Pound (1958) was used for stripping off the whole epidermis of the inoculated and control leaves. The epidermal layer was lifted from the end held by the forceps by means of a razor blade.

One part of the epidermal strips was put immediately into the main compartment of the Warburg vessel; the remnant of the epidermis was tested for its infectivity using the serological precipitin end point. All experiments were run in quadruplicate. Enzyme extract was prepared, and rapid spectrophotometric method for determining polyphenoloxydase activity was used as described by Van Kammen and Brouwer (1964).

RESULTS

The respiration of epidermal tissue of tobacco leaves infected with four different strains of potato virus X was measured at daily intervals during a period of 7 days.

The results were compared with similar epidermal tissue from the healthy control leaves (Fig. 1).

It is evident from fig. 1 that during the period of 7 days there was no striking differences with latent and mild mottle strains in the oxygen uptake values of the control and infected tissue. An increased respiration was observed only in the leaves infected with the two severe strains.

The initial increase in respiration (48 hours after inoculation) was concurrent with the first detectable synthesis of virus in tissue and coincided with first appearance of symptoms. The greatest increases in the respiration occurred on the fourth day after inoculation, when the symptoms were well developed and the necrotisation process had begun.

These findings indicate that the increased respiration rate in infected epidermal tissue is related to the development of symptoms.

Parallel to the measurements of respiration, polyphenoloxydase activity was measured every day for seven days. The results are given in table I.

An increase in polyphenoloxydase activity was found only in the leaves infected with the two severe strains of potato virus X.

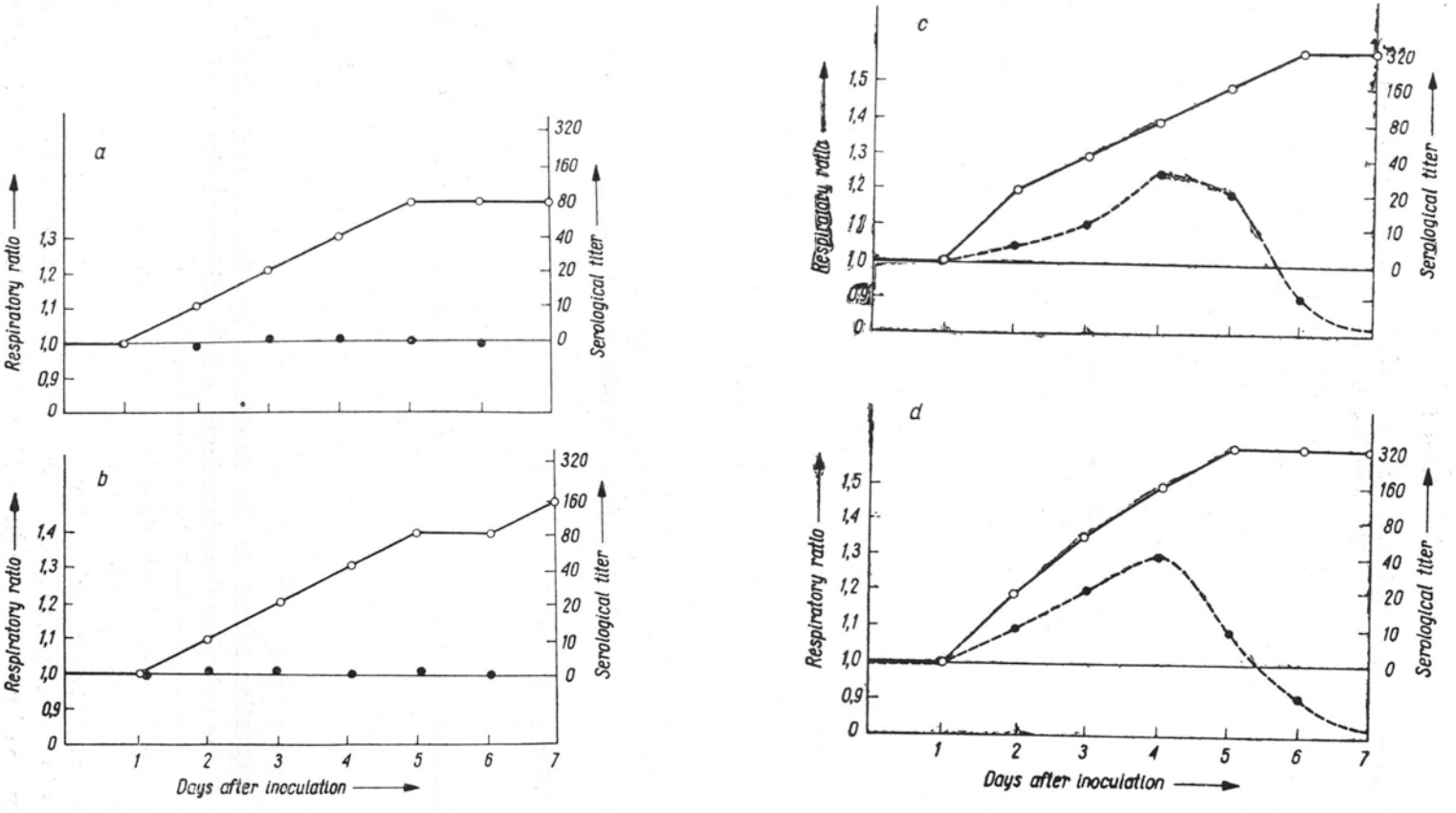

Fig. 1. Serological assays of virus concentrations and respiratory ratios between healthy tobacco leaf epidermis and infected with potato virus X strains. *a* — latent, *b* — mild mottle, *c* — severe mottle, *d* — ringspots, at different times after inoculation

Table I

Polyphenoloxydase activity in the healthy and in the infected tobacco leaf epidermis at different times after inoculation

Days after inoculation	Activity in the*:				
	healthy leaf	infected with potato virus X strains:			
		latent	mild mottle	severe mottle	ringspots
1	50	51	53	50	52
2	52	52	53	52	64
3	60	62	61	82	99
4	63	62	63	101	116
5	64	62	65	115	120
6	60	61	64	112	104
7	59	60	59	98	89

* The reaction mixture contained 0.6 ml chlorogenic acid at a concentration of 0.1 mg/ml, 0.8 ml enzyme extract, and 1.6 ml buffer.

The increase began on the 3rd day after inoculation, and reached a maximum after about 5 days.

This result suggests that the activity of polyphenoloxydase increases during the development of symptoms.

DISCUSSION

The results of the experiments reported here do not indicate that the process of infection with potato virus X during the early stage of infection alters the metabolism which is demonstrable as a change in the respiration rate.

The failure to find in the epidermal tissue significant differences between the respiration of healthy control leaves and those infected with latent and mild mottle strains is not consistent with the observations of Yamaguchi and Takahashi (1965) Takahashi and Hirai (1964).

The only increased respiration in epidermal cells infected with two severe strains of potato virus X 2—4 days after inoculation indicates that the differences in the oxygen uptake of healthy control and infected tissue are a secondary effect of the virus on the tissue.

This suggestion seems to be supported by the findings that the activity of the polyphenoloxydase increased in epidermal tissue infected with the severe strains 3—5 days after inoculation.

Although the role of polyphenoloxydase in cellular respiration is not as yet clear (Yamaguchi and Takahashi 1965) it seems that the enhanced activity of this enzyme mainly reflects the biochemical processes in necrotisation rather than those in virus synthesis (Farkas, Kiraly, Solymosy 1960; Kikuchi and Yamaguchi 1960).

It is possible, of course, that the increase in respiration is not solely related to the necrotisation process. But, as long as we have so little information on how an infecting virus particle, devoid as it is of metabolic activity, can interfere with the metabolism of the cell-initiated physiological abnormalities which lead to characteristic macroscopic symptoms (Diener 1963), explanation of results of experiments will continue to differ.

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Metabolizm oddechowy liści tytoniu zakażonych różnymi szczepami ziemniaczanego wirusa X.

IV. Oddychanie we wczesnym stadium infekcji

Streszczenie

Badano intensywność oddychania w liściach tytoni *Nicotiana tabacum* L. var. *Haranova* zdrowych i zakażonych różnymi szczepami wirusa X, we wczesnym stadium infekcji. Pomiary przeprowadzono w tkance epidermalnej liści. Podobnie jak w poprzednich badaniach zaobserwowano zwyżkę w oddychaniu w liściach zakażonych silnie wirulentnymi szczepami ziemniaczanego wirusa X, w porównaniu ze zdrowymi. Zwyżka w oddychaniu pojawiała się od momentu wystąpienia objawów zewnętrznych choroby i pozostawała w korelacji z ostrością objawów oraz procesem nekrotyzacji tkanki. Badano również aktywność oksydazy polifenolowej. Stwierdzono wzmożoną aktywność tego enzymu jedynie w liściach zakażonych silnie wirulentnymi szczepami wirusa X, wywołującymi nekrozę tkanki.

Uzyskane wyniki sugerują, że tak zwyżka w oddychaniu, jak i wzmożona aktywność oksydazy polifenolowej są raczej skutkiem procesu nekrotyzacji tkanki, a nie skutkiem syntezy samego wirusa.