Phytochemistry, 1966, Vol. 5, pp. 819 to 822. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

THE EFFECT OF THREE STRAINS OF TOBACCO MOSAIC VIRUS ON PEROXIDASE AND POLYPHENOL OXIDASE ACTIVITY IN *NICOTIANA TABACUM*

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(*Received* 12 *October* 1965)

Abstract—Polyphenol oxidase activity, peroxidase activity and virus titer were determined at 2-day intervals for 22 days in tobacco leaf tissue singly infected by three strains of tobacco mosaic virus (TMV). The aucuba and yellow strains induced large diffuse chlorotic lesions on inoculated leaves. Small necrotic areas occasionally developed on young leaves inoculated with the aucuba strain. The green strain did not induce readily distinguishable symptoms on inoculated leaves. Peroxidase and polyphenol oxidase activity increased significantly in leaves infected by the aucuba strain or yellow strain, but not in leaves infected by the green strain.

INTRODUCTION

THE visible symptoms induced in plant tissues infected by viruses are manifestations of disturbances in host metabolism, but the nature and direct causes of these disturbances are mostly unknown. Disturbances in growth-regulating hormones and accumulation of oxidized phenolic derivatives are possibly involved.[1] Alterations in the metabolism of tobacco following infection by tobacco mosaic virus (TMV) include decreased photosynthetic activity,[2,3] increased respiration,[4] increased activity of polyphenol oxidase and possible accumulation of oxidized phenolic compounds,[5–7] and an increase in peroxidase activity.[5,8]

The present investigation was undertaken to determine the effect of three strains of TMV which differ in the type of symptoms they produce in tobacco on polyphenol oxidase and peroxidase activity in inoculated tobacco leaf tissue during the period of virus multiplication and symptom development. The three strains selected were the aucuba strain, which causes chlorosis, some necrosis, and distorting of leaves; a strain which causes yellow mottling (yellow strain); and a strain which causes a mild green mottling (green strain).

RESULTS

Inoculation of susceptible tobacco leaves with the three strains of TMV resulted in the production of distinct symptoms 8 days after inoculation. Virus titer rose rapidly during this

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period, reaching a maximum about 12 days after inoculation. Between 14 and 22 days after inoculation, virus titer decreased. Virus multiplication and titer in leaves inoculated with the aucuba or yellow strain did not differ significantly from each other but were much higher than in leaves inoculated with the green strain (Fig. 1).

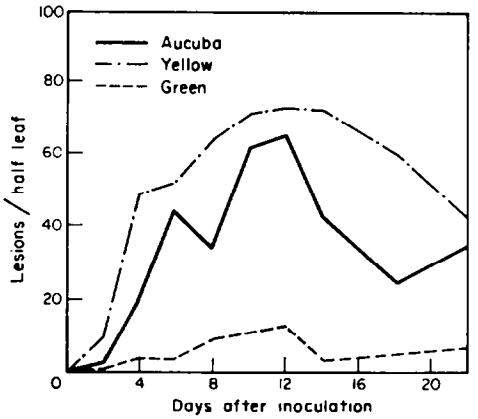

FIG. 1. VIRUS TITER IN TOBACCO LEAVES 0–22 DAYS AFTER INOCULATION.

Peroxidase activity did not increase until after symptoms were present, after which it increased sharply in leaves infected by the aucuba or the yellow strain, reaching a maximum at 14 days after inoculation (Fig. 2). At this time, leaves infected by the aucuba strain had a higher peroxidase activity than leaves infected by the yellow strain (Fig. 2). After 14 days,

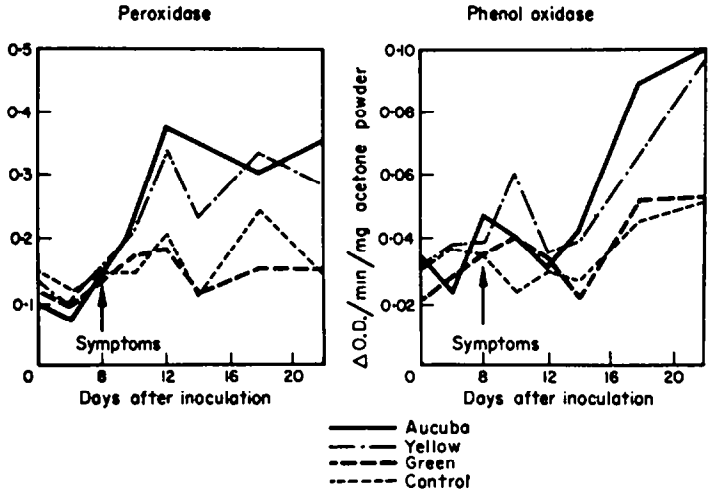

FIG. 2. PEROXIDASE AND POLYPHENOL OXIDASE ACTICITY IN TOBACCO LEAVES INOCULATED WITH THREE STRAINS OF TMV 0–22 DAYS AFTER INCULATION.

peroxidase activity in leaves infected with the aucuba or the yellow strain decreased slightly and became about equal, but both were still higher than uninfected leaves. No increase in peroxidase activity occurred in leaves inoculated with the green strain (Fig. 2).

Polyphenol oxidase activity followed a similar pattern (Fig. 2) reaching a maximum

(180–260 per cent of control) 10 days after inoculation and began to decline slightly, whereas peroxidase activity reached a maximum (200–300 per cent of control) after 14 days. No clear-cut relationship between virus strain and extent of increase in polyphenol oxidase activity was evident, except that no pronounced increase was caused by the green strain.

DISCUSSION

A close correlation between increased peroxidase activity and appearance of symptoms in virus infected plants has been reported by several investigators.[9–11] Schwarze[12] reported that disturbances in chlorophyll synthesis were accompanied by high peroxidase activity. Our observations support these observations in that infection by the aucuba or the yellow strain, which produces pronounced chlorosis in the inoculated leaves, resulted in the greatest increase in peroxidase activity, and that infection by the green strain, which interferes only slightly with chlorophyll synthesis, resulted in no significant increase in peroxidase activity. Polyphenol oxidase activity was stimulated slightly or not at all by infection with the green strain of TMV, but was stimulated by infection with the aucuba and yellow strains beginning 14–16 days after inoculation.

It has been suggested that the increase in phenol oxidizing enzymes such as polyphenol oxidase and peroxidase following infection may account in part for the production of symptoms resulting from the accumulation of polyphenol oxidation products. This subject has recently been reviewed.[1]

However, two objections to this hypothesis might be raised. One is that accumulation of polyphenol oxidation products *in vivo* in virus-infected plants has not been conclusively demonstrated, even though activity of polyphenol oxidizing enzymes increases markedly *in vitro*. Secondly, it must be assumed that if oxidized polyphenols resulting from high activity of polyphenol oxidase or peroxidase have a causative relationship to symptom expression, the increase in activity of these enzymes following infection should precede symptom appearance. Our data suggest, however, that stimulation of the two enzymes beings only at the time of symptom appearance and that they reach their maximum activity 2–6 days after the appearance of symptoms. On the other hand, the data presented above show that there is a relationship between symptom severity, virus titer, and increased peroxidase and polyphenol oxidase activity.

EXPERIMENTAL

The tobacco plants used in this study were Ky Iso 1 Ky 16, a true homozygote of *Nicotiana tabacum* var. Ky 16 developed by Stokes.[13] Plants were grown in the greenhouse in a soil sand-peat mixture in 4-in. clay pots and were watered with Hoagland's nutrient solution at weekly intervals. When they reached the 7–9 leaf stage, uniform plants were selected for studies of changes of enzyme activities during symptom development, enzyme activities at different leaf ages, and virus multiplication. Leaves were inoculated with one of the three TMV strains by rubbing carborundum-dusted leaves with a cheesecloth pad saturated with inoculum. Inoculum was prepared by grinding infected tobacco leaf tissue in 0·05 M phos-

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phate buffer at pH 6·0. Leaves of control plants were treated identically except that phosphate buffer alone was substituted for the inoculum. The experiments were set up in a randomized complete block design with three single plant replications. Leaf samples were harvested separately as blades or as 7-mm discs between 9.00 and 11.00 a.m. at 2-day intervals or at a predetermined number of days after inoculation and stored at $-15°$ until used.

For enzyme assays, extracts of acetone powder or leaf discs were used. Acetone powders of the frozen samples were prepared and 100 mg aliquots and the powders were incubated in 5 ml of 0·05 M phosphate buffer at pH 6·0 for 20 hr at 5°. Centrifugation at 5000 rev/min for 15 min resulted in a clear supernatant. Acetone powder extract or leaf disc extract was assayed for peroxidase and polyphenol oxidase as described previously.[14]

Virus titer was determined by the local lesion assay method, using *Nicotiana tabacum* var. Ky 12 as an assay host. Tissue was collected from inoculated leaves of three plants at 2-day intervals with a 7 mm cork borer, ten discs comprising a sample. The discs were frozen and assayed at the same time, thus minimizing the effect of environmental fluctuations on the assay host. Three replicate samples were used for the assays and the lesion counts of the three samples were averaged.

Acknowledgements—This investigation was supported in part by National Science Foundation Grant GB-2972 and is published with approval of the director of the Kentucky Agricultural Experiment Station.

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