Proc. Indian Acad. Sci., Vol. 81 B, No. 4, 1975, pp. 170–173

Respiratory and enzymatic changes in the *Colletotrichum* leaf spot of turmeric

M. CHANDRASEKHARAN NAIR*

AND

K. RAMAKRISHNAN, F.A.SC.†

*Department of Plant Pathology, College of Agriculture, Vellayani, Kerala

†University of Agricultural Sciences, Bangalore

MS received 28 November 1974

ABSTRACT

Turmeric leaves infected by *Colletotrichum capsici* exhibit a higher respiratory rate with augmentation of the terminal oxidative system. A similar effect is brought about by the treatment of the turmeric leaves with toxins isolated from culture filtrate and mycelium of the pathogen. Ascorbic acid oxidase and polyphenol oxidase activity register the maximum increase both under infection and toxin treatment.

INTRODUCTION

THE leaf spot disease of turmeric caused by *Colletotrichum capsici* (Syd.) Butl. & Bisby is characterised by the formation of a central necrotic area surrounded by a yellow halo. It has been demonstrated that similar symptoms could be induced by the application of extremely low concentrations of the toxic metabolite secreted by the pathogen to the leaf (Nair and Ramakrishnan 1973). We present, in this paper, the changes in respiratory rate and in the oxidative enzymes of the tissues both under infection by *Colletotrichum capsici* and by treatment with the toxin.

MATERIALS AND METHODS

The toxin produced by *Colletotrichum capsici* was isolated as per the procedures described elsewhere (Nair and Ramakrishnan 1973). This toxin (2 mg) dissolved in sterile water (1 ml) formed the stock solution. This toxin was diluted with sterile water to contain 2 and 0·2 μg/ml of the toxin. The stock toxin solution containing 2000 μg/ml when bioassayed

* Forms part of a thesis submitted by the first author to the Tamil Nadu Agricultural University for the award of a Ph.D. degree.

against turmeric leaves by applying drops of 0·05 ml of the test solution on the leaves produced a characteristic necrotic spot having an average diameter of 20 mm in 24 hr. The lowest concentration, *viz.*, 0·2 μg/ml was not sufficient to form any visible changes on the host leaves. Turmeric plants were raised in pots under glass house conditions and leaves of uniform age were treated with drops of the test solution at the rate of 0·05 ml per drop by a micropipette and lightly pricked under. While inoculating, one half of the leaf, either on the left or right side of the mid rib was inoculated by the toxin or by the organism and the other half treated as control by applying sterile water. Samples were collected from the inoculated area at appropriate time intervals. The respiratory rate was studied manometrically as per the procedures outlined by Umbreit *et al.* (1964). We detected ascorbic acid oxidase by the method of Hampton (1963), catalase by the method of Dekock *et al.* (1960), polyphenol oxidase by the method of Matta and Dimond (1963) and peroxidase activity by the method of Mudd *et al.* (1959).

Results

Changes in respiratory rate brought about by different dilutions of the toxin at various time intervals are shown in figure 1. It may be seen that the respiratory rate in the toxin treated tissues was higher than in the healthy controls, which was true even with the highly diluted toxin solution. Both the toxin solutions having the concentration of 2000 μg/ml and 2 μg/ml caused the highest rate of respiration 8 hr after treatment. Thereafter the respiratory rate decreased and was lower than that of the control in 2 days. Infection by *C. capsici* also produced a similar pattern in respiratory changes (figure 2). But the maximum increase was 4 days after inoculation.

Activity of all the oxidative enzymes tested was found to be higher in treated plants than in the healthy tissues (figure 3). The maximum increase

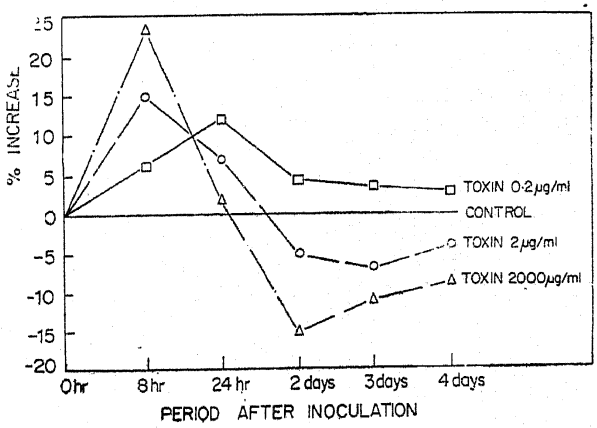

Figure 1. Percentage increase or decrease in respiration over healthy tissues after treatment with toxins of various dilutions.

was noted in ascorbic acid oxidase and polyphenol oxidase activity, and the minimum increase in the activity of catalase and peroxidases. Ascorbic acid oxidase activity was highest during the early stages but decreased later both in inoculated and toxin treatment. Polyphenol oxidase activity was high even 24 hr after toxin treatment. In inoculated leaves polyphenol oxidase activity remained high up to 8 days, and thereafter decreased.

DISCUSSION

The sharp rise in respiration noted in the present study coincided with the first appearance of the symptoms. Thereafter the respiration rate declined. Increase in respiratory rate under pathogenesis is a well-documented phenomenon. Wood (1967) after critically reviewing this stated that in almost all cases the respiration increased sharply at the time of first appearance of symptoms and declined as necrosis progressed. Treatment of the tissues with the toxin also caused a pattern of respiratory changes similar to that produced by infection. It has also been observed that the toxin at a concen-

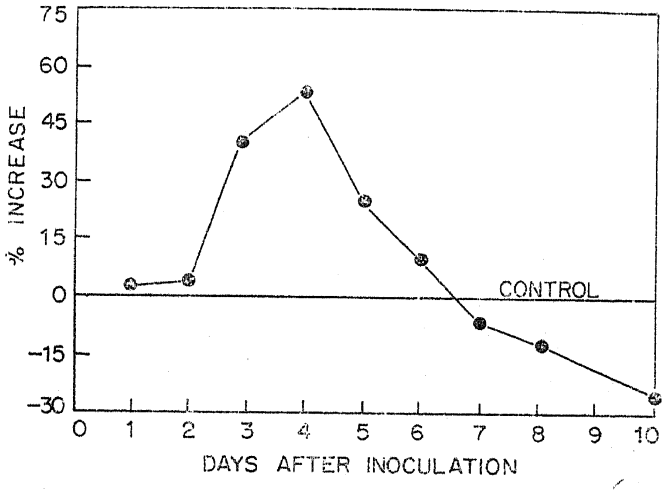

Figure 2. Percentage increase or decrease in respiration after inoculation with *C. capsici*.

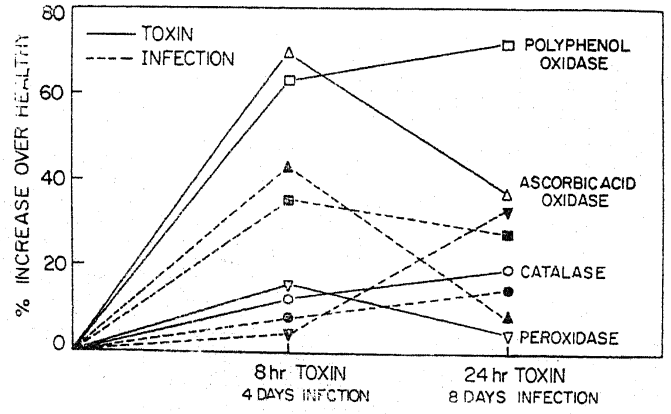

Figure 3. Percentage increase of different oxidative enzymes on turmeric leaves as a result of toxin treatment and infection.

ration of 0·2μ g/ml, which is not capable of producing any visible symptoms, enhanced respiration. The pathotoxin victorin produced by *Helminthosporium victoriae* has also been demonstrated to enhance respiration (Krupka 1959; Scheffer and Pringle 1963; Luke and Freeman 1965; Amador and Wheeler 1966). The results obtained by the above workers have led to suggestions that changes in oxidative pathways or partial uncoupling of oxidative phosphorylation might account for the respiratory rise induced by victorin. In the present study how far the latter mechanism was in operation has not been examined but there was evidence to show that the first mechanism was in operation in the toxin-treated as well as infected tissues of the turmeric leaves. The most striking observation made in the present study in respect of the terminal oxidative system was the highly activated ascorbic acid oxidase activity in both toxin-treated and infected leaves. Ascorbic acid oxidase activity is considered to be unimportant in healthy plants, but in many cases under pathogenesis this is augmented (Kiraly and Farkas 1975; Krupka 1959; Heitefuss *et al.*, 1960). There was a shift in the terminal oxidative system in the infected tissues as well as in those treated with toxin. This points to a definite role of the toxin in pathogenesis.

References

Amador, E. J. and Wheeler, H., Effect of leaching on victorian induced respiration of oat tissue. *Phytopathology* **56** 1196–1200 (1966).

Dekock, P. C., Commsiong, K., Farmer, V. C. and Inkson, R. H. E., Interrelationships of catalase, peroxidase, hematon and chlorophyll. *Plant Physiol.* **35** 599–604 (1960).

Hampton, R. E., Activity of some soluble oxidase in carrot slice infected with *Thielaviopsis basicola*. *Phytopathology*, **53** 497–499 (1963).

Heitefuss, R., Stahmann, M. A. and Walker, J. C., Production of pectolytic enzymes and fusaric acid by *Fusarium oxysporum*, *F. conglutinans* in relation to cabbage yellows. *Phytopathology* **50** 367–370 (1960).

Kiraly, Z., and Farkas, G. L., On the role of ascorbic oxidase in the parasitically increased respiration of wheat. *Archs. Biochem. Biophys.* **66** 474–485 (1957).

Krupka, L. R., Metabolism of oats susceptible to *Helminthosporium victoriae* and victorin. *Phytopathology* **49** 587–594 (1959).

Luke, H. H. and Freeman, T. E., Effects of victorin on Krebscycle intermediates of a susceptible oat variety. *Phytopathology* **55** 967–969 (1965).

Matta, A. and Dimond, A. E., Symptoms of fusarium wilt in relation to quantity of fungus enzyme activity in tomato stem. *Phytopathology* **53** 574–575 (1963).

Mudd, J. B., Johnson, B. G., Burris, R. H. and Buchholts, K. P., Oxidation of indole acetic acid by quack grass rhizomes. *Plant Physiol.* **34** 144–148 (1959).

Nair, M. C. and Ramakrishnan, K., Production of toxic metabolites by *Colletotrichum capsici*. *Curr. Sci.* **42** 362–363 (1973).

Scheffer, R. P. and Pringle, R. B., Respiratory effects of the selective toxin of *Helminthosporium victoriae*. *Phytopathology* **53** 465–468 (1963).

Umbreit, W. W., Burris, R. H. and Stauffer, J. F., *Manometric techniques* (Burges Publishing Co., Minnesota) 305 pp. (1964).

Wood, R. K. S., *Physiological Plant Pathology* (Blackwell Scientific Publications, Oxford) pp. 570 (1967).