Planta 144, 111–112 (1978)

Planta

Short Communication

Influence of Fusaric Acid on Circadian Leaf Movements of the Cotton Plant, *Gossypium hirsutum*

K.S. Sundararajan, R. Subbaraj, M.K. Chandrashekaran, and S. Shunmugasundaram

School of Biological Sciences, Madurai University, Madurai 625 021, India

Abstract. The cotton plant, *Gossypium hirsutum* L. (cv. Lakshmi) exhibits circadian leaf movements. Fusaric acid (5-n butyl pyridine 2-carboxylic acid), an in vivo toxin shown to be produced during the pathogenesis of the wilt disease in cotton, causes phase shifts of the leaf movement rhythm that varied in degree and magnitude as a function of the treated phases. The data support the hypothesis that membranes play a vital role as a pacemaker in circadian rhythms.

Key words: Circadian rhythms – Fusaric acid – *Gossypium* – Membrane permeability.

Introduction

Results from studies on circadian rhythms in plants and animals lend credence to the view that membranes play a vital role in the generation of such rhythms (cf. Hastings and Schweiger, 1976). In cotton plants wilt disease is caused by a parasitic fungus, *Fusarium oxysporum fsp vasinfectum Akt.*, wherein fusaric acid has been shown to be produced in vivo during pathogenesis (Lakshminarayanan and Subramanian, 1955). Impairment of semipermeability by fusaric acid in the plasma membrane leading to large amounts of K^+ in cuticular excretion in cut shoots of tomato has been reported. Spectrochemical analysis of infected cotton plants indicates a significant derangement in the ionic balance of these plants (Sadasivan, 1961). The fusaric acid by acting on the membrane causes the observed pathological symptoms in diseased plants. In view of this rather direct action of fusaric acid on the membrane we considered it worthwhile to investigate the influence of fusaric acid on the circadian rhythm in the leaf movements of cotton plants. We report here results of experiments in which the circadian rhythm of leaf movements in cotton plant was successfully phase shifted by short exposures to fusaric acid.

Materials and Methods

The plants, *Gossypium hirsutum* L. (cv. Lakshmi) were grown in greenhouses under normal day and night cycles. Experiments designed to evoke phase shifts were carried out in an experimental cubicle at a constant temperature (22° C ± 1°). Six week old plants were exposed to continuous white light from fluorescent lamps (Philips) of 600–2,700 lx. Under these conditions the rhythm in the leaf movements persisted for a period of two weeks and revealed a free running rhythm with a period of about 22.5 to 26.4 h. For the purpose of recording leaf movements on a mechanically wound 7-day kymograph drum, a well expanded terminal leaf positioned just below the developing young leaf was selected. Fusaric acid (5-n butyl pyridine 2-carboxylic acid; Sigma Chemical Company, USA) was dissolved in distilled water to a final concentration of 10^{-3} M · 0.06 ml of fusaric acid from the stock solution (10^{-3} M) was applied on the upper (adaxial) side of a leaf around the prominent mid vein at the basal portion of its blade and brushed over uniformly. The same procedure was repeated with leaves of different plants at required phases. 5 h after the time of application, the fusaric acid was washed off from leaves using distilled water. Control plants were treated in a similar manner with distilled water. The period length just prior to the fusaric acid treatment was taken into account and the circadian phase was calculated in degrees, 0° designating maximum night position. The differences between the predicted time of maximal night position and observed maximal night position following treatment was a measure of phase shift.

Results

Figure 1 shows the phase response curve constructed from the phase shifts that resulted from application of fusaric acid along the several phases of the circadian cycle. The data are based on the phase shifts observed in the directly influenced cycle (first cycle, ● – ●) and the third cycle (▲ – ▲) that ensues after treatment. Phase advances occurred during the sub-

0032-0935/78/0144/0111/$01.00

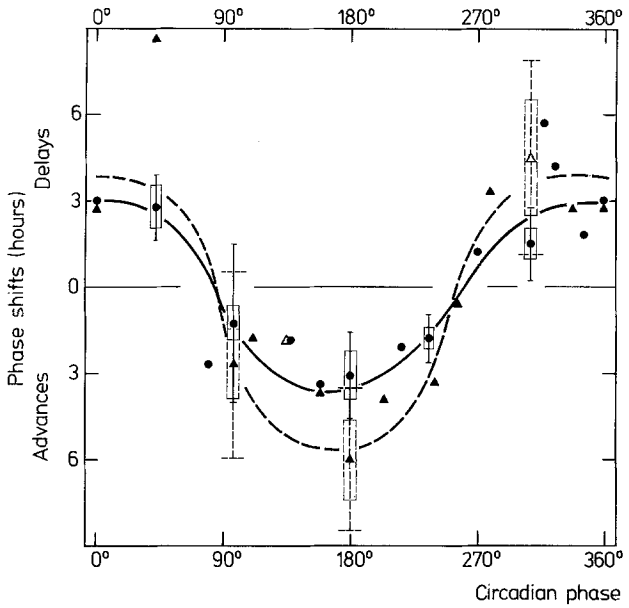

Fig. 1. Phase response curves for 5 h chemical (fusaric acid) pulses. The shifts were measured in hours and averaged over 1 hourly (15° degrees) bins of circadian cycle. Vertical lines indicate standard deviation on both sides of the means. Rectangles indicate standard error. Values plotted without standard deviation or standard error are single determinations. Ordinate: Measure of advance and delay phase shifts in the first cycle ( ) and third cycle ( ) following fusaric acid perturbations. Abscissa: Circadian cycle in degrees. 0° denotes the maximum night position of the leaves. The phase shift values are plotted against the beginning of time of application of fusaric acid

jective day (90–270°) while phase delays were observed in subjective night cycle (270–90°) of the observed rhythm. The transition from delay to advance responses occurred near 90° while the transition from advance to delay occurred near 270°.

In some experiments in which the leaves were left unwashed after fusaric acid treatment the resultant phase shifts were similar.

Discussion

Phase response curves have been predominantly constructed using pulsed light and temperature on plant and animal systems (Aschoff, 1965). Many attempts have been made to elicit similar phase response curves by treating the organisms with various biologically active chemicals for a short time to gain a better understanding of the components and the mechanism(s) involved in the basic oscillator. Most metabolic inhibitors proved to be ineffective in this respect (Bünning, 1971). The effectiveness of valinomycin in phase shifting both the circadian leaf movements of *Phaseolus* (Bünning and Moser, 1972) and stimulated bioluminescence in *Gonyaulax* (Sweeney, 1974) suggests the implication of K^+ in the generation of rhythmicity. That fusaric acid in the concentration used in our experiments could affect the permeability of the membrane as well as the ionic balance is evident by its specific effect on membrane systems in various plants. In this context it is noteworthy that fusaric acid also impairs the active absorption of water (Sadasivan, 1961).

Recently membrane related circadian rhythms, all of which persist in continuous light of low intensity, have been reported (Sweeney and Herz, 1975). Fusaric acid has a direct effect on the phospholipids of membranes (enzyme induction studies in the diseased cotton plants by Lakshmanan and Shunmugasundaram, unpublished). Further fluorescence techniques indicate that fusaric acid brings about conformational changes in phospholipid membranes (Gauesan, unpublished). Apparently topical application of fusaric acid elicits qualitatively and quantitatively varying phase responses depending on the state of the membrane during the different phases of the circadian oscillation. Nevertheless to the best of our knowledge this is the first report of its kind in which a phytotoxin is shown to cause a phase shift implicating the role of membrane as forming a part of the pace-maker complex regulating circadian rhythms.

We are grateful to Professor E. Bünning, Professor W. Engelmann and Dr. M. Schrempf for suggesting critical improvements and to Professor M. Lakshmanan for permission to cite some unpublished work.

References

Aschoff, J.: Response curves in circadian periodicity. In: Circadian Clocks. Aschoff, J., ed. pp. 95–111. Amsterdam: North Holland Publ. Co. 1965

Bünning, E.: Symptoms, problems and common features of circadian rhythms in plants and animals. Proc. Int. Symp. Circadian Rhythmicity, Wageningen Centre for Agricultural Publishing and Documentation. 11–31 (1971)

Bünning, E., Moser, I.: Influence of valinomycin on circadian leaf movements of *Phaseolus*. Proc. Nat. Acad. Sci. USA **69**, 2732–2733 (1972)

Hastings, J.W., Schweiger, H.G.: The Molecular Basis of Cricadian Rhythms: Report of the Dahlem Workshop on the Molecular Basis of Circadian Rhythms, Berlin: Abakon (1975)

Lakshminarayanan, K., Subramanian, D: Is fusaric acid a vivo toxin? Nature **176**, 697 (1955)

Sadasivan, T.S.: Physiology of wilt disease. Ann. Rev. Plant. Physiol. **12**, 449–468 (1961)

Sweeney, B.M: The potassium content of *Gonyaulax polyedra* and phase changes in the circadian rhythm of stimulated bioluminescence by short exposures to ethanol and valinomycin. Plant Physiol. **53**, 337–342 (1974)

Sweeney, B.M., Herz, J.M.: Evidence that membranes play an important role in circadian rhythms. Proc. XII Int. Conf. Int. Soc. Chronobiol. Washington. «Il Ponte» Italy 1975

Received 7 August; accepted 7 September 1978