

Leaf Movement Alterations on Bean Plants with Common Bacterial Blight

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ABSTRACT

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Rhythmic movements of unifoliolate leaves of *Phaseolus vulgaris* 'Bush Blue Lake' that develop wilt following inoculation with *Xanthomonas campestris* pv. *phaseoli* continue for several days following inoculation. Three types of patterns have been identified in movements of diseased

leaves that distinguish them from the normal rhythmic patterns of healthy leaves. These special patterns occur during the light span, are absent in darkness, and continue for various durations of time prior to death of the plant.

Additional key words: chronobiology, rhythm.

The study of biological rhythms has become an important area of research in human pathology and medicine (8,9) but it is practically nonexistent in the area of plant pathology (13). Fusaric acid, a fungal phytotoxin (7), causes phase shifts in the leaf movements of cotton plants maintained under continuous illumination (12) and this implies that altered leaf movement patterns may be expressed in a diseased plant.

Our experiments were designed to evaluate the effects of a systemic wilt disease on rhythmic behavior of leaf movements. Since wilt is a prominent clinical feature of common bacterial bean blight, we hypothesized that disruptive physiological events leading to wilt probably have significant effects on a component of temporal organization (leaf movements) as well. If such is the case, analysis of leaf movements could be useful in early diagnosis of disease.

MATERIALS AND METHODS

Young plants of *Phaseolus vulgaris* L. 'Bush Blue Lake,' raised from seed in a greenhouse, were transferred to controlled-environment chambers at least 3 days before their leaf rhythms were monitored. This duration of time before treatment was used to synchronize plants to the regime of the controlled environment (1,6). Temperature was maintained at 28 ± 0.5 C and the plants were subjected to cycles of 15 hr of light (intensity about $145 \mu\text{Ein} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$) followed by 9 hr of darkness. Although the leaves of *Phaseolus* continue their movements under constant conditions (e.g., continuous illumination) and display a circadian rhythm (5), a synchronized light/dark cycle was used to more closely duplicate natural field conditions. Plants having visible disease symptoms on their leaves were monitored for leaf movements. From a total of approximately 70 plants, the patterns of two representative leaves on plants in separate experiments were traced from the original strip chart, photographically reduced and illustrated for this publication (Fig. 1a and b). Initially, each experiment included six plants, three serving as controls and three inoculated with the bacterial pathogen. Later, only one control was used because patterns of control plants were similar and because the same patterns occurred in the inoculated plants prior to onset of disease symptoms. In these later efforts, four experiments were made using the same equipment under similar conditions but emphasizing an

analysis of different types of movement patterns which usually occurred on the day of permanent wilting.

Plants were inoculated at the cotyledonary node when the first unifoliolate leaves were unfolding (7-9 days after planting). Bacteria were introduced in either of two ways: a droplet of suspension containing approximately 10^8 CFU/ml was formed on the tip of a 27-gauge hypodermic needle and the needle subsequently was thrust through the stem; or a spear-shaped scalpel was dipped in a 48-hr surface growth of bacteria on nutrient agar (Difco) and thrust horizontally halfway through the stem. With either method, unifoliolate leaves of infected plants wilted 4-10 days following inoculation. Movements of individual unifoliolate leaves on inoculated and control (water only) plants were monitored continuously by an electromechanical device consisting of a lever, magnet, fulcrum, and Hall effect sensor (4). Only one leaf was monitored on any given plant. The leaf blade was connected to the tip of the lever by a silk thread so that the lever would move when the leaf changed position. Changes in angular position of the lever were converted into voltage variations and recorded on a strip chart. To determine if the pattern changed within a given time span (phase) or if the event had a random distribution, the data from Table 1 were converted to angular position; the nonrandomness of the circular distribution (24-hr cycle) was tested by using the Raleigh test (2).

RESULTS AND DISCUSSION

The horizontal "day" and vertical "night" leaf positions plus the profile displayed by a plant in a normal synchronized day/night environment are illustrated in Fig. 1a. Approximately 3-7 hr were required for the leaf to reach its maximum horizontal position but only 1-2 hr to reach its maximum vertical position. Generally, the pattern traced by the pen on a strip chart recorder during the dark span was relatively flat, while the pattern in the light span was characterized by irregular fluctuations that varied widely from day to day and from plant to plant. From 3 to 9 days after inoculation, the leaf of a diseased plant begins to rise as usual near the beginning of the light span, but often this upward movement aborts before completion and the leaf falls rapidly downward (Fig. 1b and c). In seven of 12 infected plants, this sudden alteration of the leaf movement pattern was preceded the day before by a steady decline of amplitude. After the appearance of the disease pattern type A (Fig. 1c), five of the leaves showed a pattern that was flat, while seven leaves displayed a residual circadian leaf movement pattern with an amplitude reduced to about one-tenth its normal range. Results from six separate experiments are presented in Table 1. The average delay was 84 ± 17 min between the beginning of the light

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span and the time at which leaves on diseased plants surged upward and reached maximum amplitude. Results from the statistical analyses of these data ($P = 0.01$) strongly support the hypothesis that the rhythm in the diseased plants either changed, dampened out, or stopped during the early portions of the light span.

In experiments that focused on different types of movement patterns, leaf movements of eight of 11 plants again followed the typical A-type pattern (Fig. 1c) that had been observed earlier. In

these experiments, the duration of recording was extended to cover 10–12 days beyond the time that disease symptoms were first observed. Patterns displayed by diseased leaves generally appeared to fit into three groups (Fig. 1c). How often the pattern appeared first and last in these experiments is illustrated in Table 2. There were a few instances (listed as “other” in Table 2) in which the movements simply ceased after a previously typical pattern. In some instances, leaves were not killed by disease and rhythmic

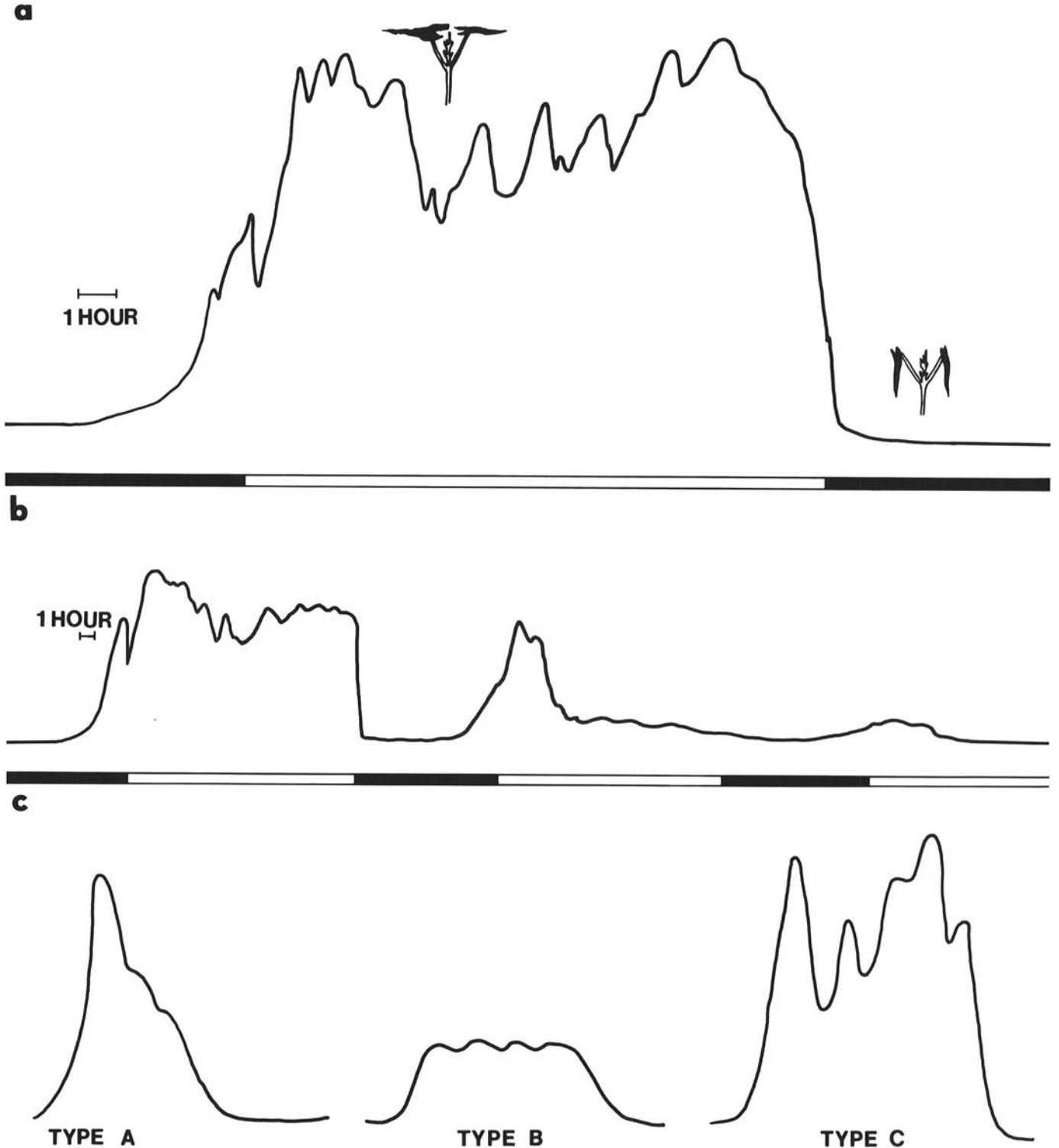


Fig. 1. Leaf movement patterns (recorded electromechanically) for a unifoliolate leaf of a plant of *Phaseolus vulgaris* a, maintained in a synchronized light/dark environment (□ = light span, ■ = dark span) with two diagrams of a bean plant inserted to illustrate the horizontal (light span) and vertical (dark span) positions of the unifoliolate leaves; b, infected with *X. campestris* pv. *phaseoli* (data from Experiment 2, plant 3, of Table 1); and c, diagrammatic illustration of three patterns (types A, B, and C) observed in diseased plant leaves.

TABLE 1. Delay in minutes (± 5 minutes) between beginning of the light span and the time when leaves on bean plants infected by *Xanthomonas campestris* pv. *phaseoli* showed type A leaf movement (Fig. 1c) and reached maximum amplitude (3–9 days after inoculation)

Experiment	Replicates (leaf)		
	1	2	3
1	30	R ^a	R
2	144	165	69
3	118	R	R
4	0	R	R
5	54	114	73
6	177	25	40

^aSymbol R indicates that the leaf recovered and movements continued without a pronounced change in pattern.

TABLE 2. Occurrence^a of the three leaf movement patterns (illustrated in Fig. 1c) observed in the unifoliolate leaves of 11 bean plants inoculated with *Xanthomonas campestris* pv. *phaseoli*

a. First pattern		b. Last pattern	
Type	Percentage of plants	Type	Percentage of plants
A	46%	A	55%
B	18%	B	27%
C	9%	C	9%
Other	27%	Other	9%

^aPercent of each type observed as (a) the first observed pattern differing from that of a healthy leaf, and (b) the last pattern before the movement ceased for at least 24 hr.

leaf movements reappeared following days of complete immobility (or of movements of small amplitude).

An account of underlying mechanisms regulating these patterns of movement is beyond the scope of this paper. However, one could readily hypothesize that alterations in turgor or water potential play an important role since certain leaf movements are in part regulated by such factors (10), and because the bacteria function as a causal agent of wilt (14). Since daily rhythmic cycles for the times of birth and death in animals and human beings are known (3,11), death of plants, including death caused by certain pathogens, may

also have a rhythmic feature. How pathogens, environmental agents, aging, or senescence interact with rhythms are currently being examined in our laboratory.

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