UNPUBLISHED PRELIMINARY DATA

FINAL REPORT TO

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Research Grant NsG - 528

Effect of Space Environment on Circadian Rhythms of Plants, For the Purpose of Defining and Verifying an Experiment Suitable for Use in a Biosatellite.

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Final Report
Grant NsG 528

This is the final report of research performed under NASA Grant 528-63, for the purpose of defining and verifying an experiment suitable for use in a Biosatellite. The experiment is to test the effect of space environment on the circadian rhythms of plants: leaf movements of the Pinto bean plant (Phaseolus vulgaris) and growth bands of fungus (Neurospora crassa 21863). 23742

Our work has resulted in (1) the development of the NOGRAVATRON, an apparatus to simulate weightlessness condition for plants, (2) the development of an infra-red time lapse photographic system, (3) the development of a strain gauge recording system which measures the movements of Pinto leaves and whose signal is suitable for telemetry, (4) the development of prototype flight package for Neurospora experiments, (5) finding response differences in growth rates and growth directions in Barley seedlings when they were grown in a simulated weightlessness condition, (6) confirmation and delineation of the leaf movement when the plant is stationary and grown under constant conditions, (7) the reduction in the size of the experimental plant material, (8) finding that the leaf movements of Pinto beans respond to a simulated weightlessness condition by the loss of leaf movements or by a phase shift of the leaf movements, (9) confirmation of the basic 27 hour leaf movement rhythm by computer analysis of photographic time lapse data. author

The early results which have been presented in the previous reports will be summarized in this report and results that were obtained in the recent experiments will be discussed in some detail. The results to be reported are from experimental work done in the Space Biology Laboratory at the University of California, Los Angeles and at the Ames Research Center, Moffett Field, California. Further work is anticipated for the latest series of experiments before the publication of the findings.

DEVELOPMENT OF SPECIAL APPARATUS

During the initial six month period of the grant, most of the effort was directed toward the development of the experimental apparatus and the selection of the appropriate experimental methods and the refinement of the experimental techniques.

The Nogravatron - A Weightlessness Simulator.

The Nogravatron was developed and the simulated weightlessness environment was tested by growing Atlas barley seedlings on the Nogravatron. The simulated weightlessness condition is achieved by rotating the plant simultaneously about two axes perpendicular to each other. The plant is rotated at the rate of one revolution per minute around one axis and one revolution in four minutes around the second axis.

Time Lapse Photographic System

The time lapse 16 mm camera and accessory equipment were adapted to the Nogravatron to expose one frame every time the rotated plants reached an upright position. In order to take pictures during both the dark phase and the light phase in the various experimental regimes used in our experiments, special infra-red lamps were designed, fabricated and adapted for this particular purpose. The infra-red photographic record obtained by this system were of high quality.

Strain Gauge Leaf Movement Recording System

In addition to the time lapse photographic method, a strain gauge leaf movement recording system was developed as an alternative telemetry system. This system which is small and light weight consists of a micro-strain gauge glued to a brass shim stock measuring 1/16 inch wide by 1 1/4 inch long. This shim stock is glued to the blade and to the petiole. The strain resulting from the leaf movement is amplified and recorded by appropriate instruments.

Prototype Flight Package for Neurospora crassa 21863

In addition to the development of ground based experimental equipment, a two pound prototype flight package was developed at the Space Biology Laboratory for orbital flight. This unit was flight stressed to $7\frac{1}{2}$ g's at 2 KC. The package with its environmental controls and the Neurospora fungi were not damaged by these conditions. The subsequent growth of the Neurospora fungi appeared normal. In later experiments, variation in the growth pattern was noted and further studies showed that these variations could not be eliminated by standard isolation techniques. Due to this variation problem in the Neurospora stock culture and due to the shortage of help and time, the Neurospora experiment was set aside for the time being and our efforts were placed on the leaf movement experiments.

SIMULATED WEIGHTLESSNESS STUDIES USING ATLAS BARLEY SEEDLINGS

To test the simulated weightlessness conditions created by the 2 axes rotations of the Nogravatron, barley seedlings were germinated and grown on the apparatus. The Nogravatron grown seedlings initially grew faster than the stationary control seedlings indicating a growth stimulation effect of the simulated weightlessness environment. The growth direction of the Nogravatron grown shoot is determined by the orientation of the embryo. Upon germination in the simulated weightlessness environment, the shoot will grow out following a line coincident with the direction of the original embryo shoot structure. On the other hand, the growth direction of the roots appears to depend on the initial spatial relationship i.e. a crowding effect that develops as the roots are emerging from the seed and expanding in girth. Thus the Nogravatron does indeed produce an ageotropic condition for the Barley seedlings.

PINTO BEAN LEAF MOVEMENT STUDIES

Confirmatory tests

In the early part of this grant, leaf movement experiments were run at the Ames Research Laboratory in order to determine the environmental limits within which the Pinto bean would continue to exhibit its leaf movements. At the same time these experiments confirmed the earlier leaf movement studies and established firmer base line data for Pinto bean leaf movements.

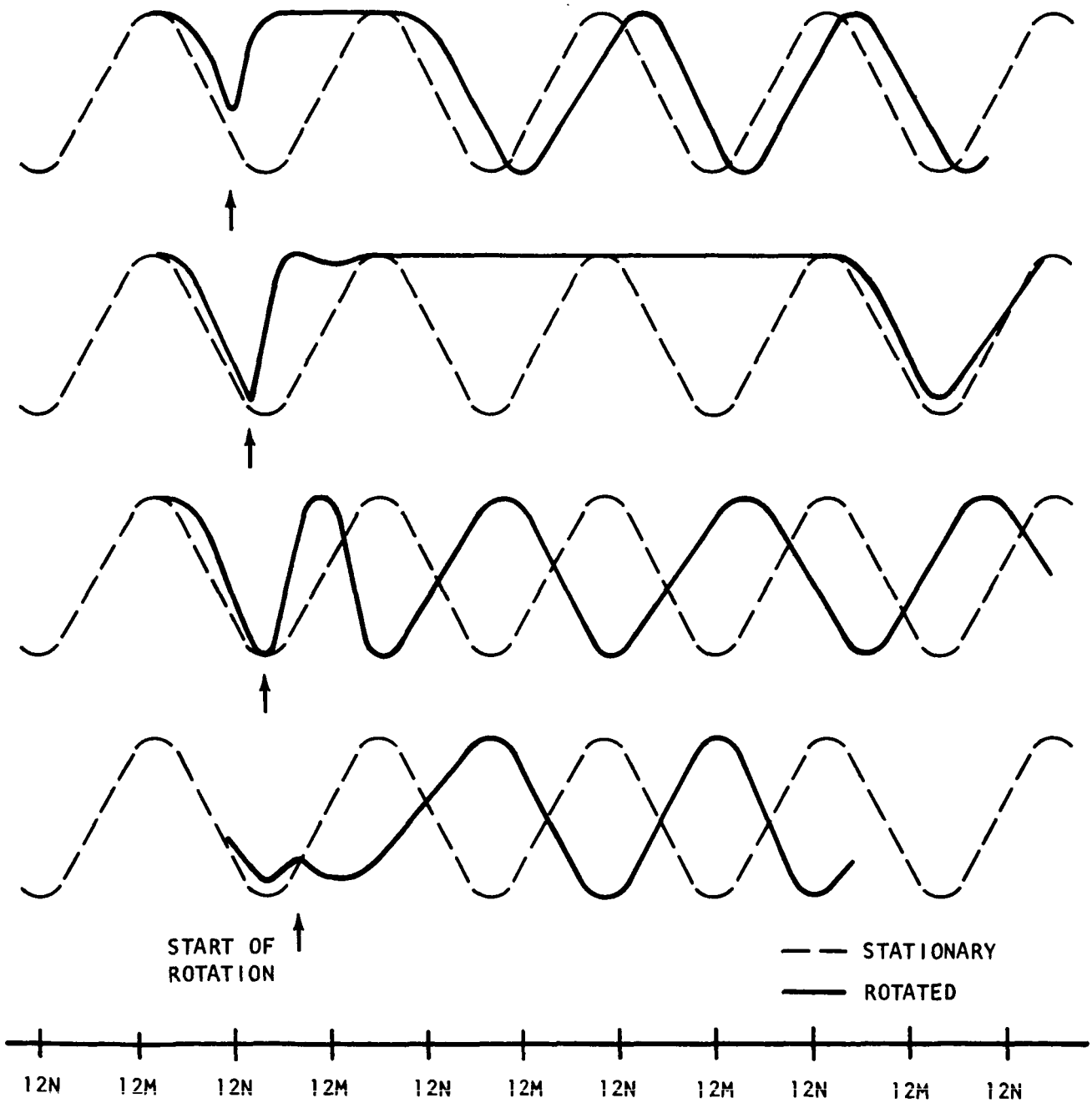
Reduction of Experimental Plant Size

The size of the experimental material was substantially reduced due to the work done at the Ames Research Laboratory. The plant leaf material has been reduced to a petiole and leaf midrib both of which are 1 cm in length. These very reduced plant materials still exhibit circadian leaf movements. A report on this phase of work was made at the 1964 American Institute of Biological Sciences meeting held at Boulder, Colorado.

Simulated Weightlessness Effects on Pinto Bean Leaf Movements

Pinto bean plants were grown in the green house until the primary leaves were well developed. These plants were then placed in a growth chamber with constant temperature and continuous light. Four plants were used as stationary controls and four plants were placed on the Nogravatron. Time lapse movie records were taken for three days during the pre-rotation period for base line data. At the beginning of the fourth day, the Nogravatron was started and the four mounted plants began their simulated weightlessness existence. Time lapse movie records were obtained during the whole course of the experiment which consisted of 3 stationary days followed by 7 days of simulated weightlessness conditions.

PINTO BEAN LEAF MOVEMENT AS AFFECTED BY THE ONSET OF ROTATION AT DIFFERENT PHASES OF THE CIRCADIAN RHYTHM



Preliminary analysis of the data (Fig. 1.) indicates that if rotation is started when the leaves are in their opening or morning phase, the leaves appear to lose their rhythmic movements and may not recover their rhythmicity for as long as 60 hours. Upon recovery, the leaves were found to be in synchrony with the normal stationary control leaves. If the rotation is initiated during the early closing or the afternoon phase, there is an immediate shift in the rhythmic pattern of the leaves and the leaves do not appear to lose their rhythmic movements.

It appears that a change in the gravitational environment has the same effect on the circadian rhythm as those of light and of temperature. Further experiments are presently being conducted to look more closely at this particular response.

Computer Analysis of Leaf Movement Data

With the cooperation of the Health Science Computing Center at UCLA, analysis of bean leaf movement data have been made. From the data generated by analysis, the Pinto bean leaves have a basic 27 hour periodicity in their leaf movements. Other rhythms of shorter periodicity do not appear to be present in leaves grown under constant conditions. Further analyses are being made to determine the presence of other rhythms shorter or longer than the 27 hour rhythm which has been proven to be so prevalent. In addition, analysis will be made on leaf movement data obtained from plants grown under a simulated weightlessness condition.

ACKNOWLEDGEMENTS

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Reports

French, J. D. and T. Hoshizaki. Semi-annual Report for the period ending March 1, 1964. Effect of Space Environment on Circadian Rhythms of Plants, For the Purpose of Defining and Verifying an Experiment Suitable for Use in a Biosatellite.

French, J. D. and T. Hoshizaki. Semi-annual Report for the period ending September 1, 1964. Effect of Space Environment on Circadian Rhythms of Plants, For the Purpose of Defining and Verifying an Experiment Suitable for Use in a Biosatellite.

Publications

Hoshizaki, T., Yokoyama, K., and Jones, W. H. Observations of the Rhythmic Movements of Excised Phaseolus Leaf. Plant Physiology 39 (Suppl.) XXXVIII

Hoshizaki, T., and Yokoyama, K. Recording Leaf Movements with a Strain Gauge (Submitted for publication to Nature)

Hoshizaki, T. Effects of Rotation Around Two Axes on the Growth of Atlas Barley Seedlings. (in manuscript form)