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Rhythm of zoospore production of pythium on lettuce cultured hydroponically

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RHYTHM OF ZOOSPORE PRODUCTION OF PYTHIUM ON LETTUCE CULTURED HYDROPONICALLY

BY

YOUNESKHAN SULTAN

A THESIS SUBMITTED TO THE FACULTY OF THE

DEPARTMENT OF PLANT PATHOLOGY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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IN THE GRADUATE COLLEGE

THE UNIVERSITY OF ARIZONA

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This thesis has been approved on the date shown below:

Michael E. Stanghellini — April 18, 1988

MICHAEL E. STANGHELLINI — Date
Professor of Plant Pathology

ACKNOWLEDGEMENTS

In the name of God, Most Gracious, Most Merciful. Praise be to God, who Says " He who taught (the use of) the pen. Taught man that which he knew not. "

I am very gratefull to Dr. M. E. Stanghellini, under whose guidance this study was done, for his kindness and scientific wisdom.

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I would also like to express thanks to the faculty and students of the Department of Plant Pathology for their support and encouragement.

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ABSTRACT

Zoospore production of *Pythium dissotocum* Drechs. in the nutrient solution of hydroponically-grown lettuce, in the greenhouse, was shown to be cyclic. The number of zoospores detected in the nutrient solution was lowest around noontime, (11:00 - 14:00 hr) and highest around 20:00 hr.

Growth chamber studies were conducted to determine the effect of different light periods on zoospore production. Under continuous light or continuous darkness, the population of zoospores in the nutrient solution decreased. But under 12 hours light and 12 hours darkness or two periods of light each for 3 hours, zoospore populations decreased during the light period but increased during the dark period.

INTRODUCTION

Fungal spore liberation and/or sporulation is known to be rhythmic. Some fungi show a short period " 24-hour " rhythm or a diurnal liberation cycle which reflects the daily pattern of environmental factors.

Spore liberation of fungi on infected plants is governed by moisture, wind, temperature, and light. Meredith (6) in 1961 found that conidia of Deigtoniella torulosa and Cordana musae in the air above a banana field was highest just after dawn. The sharp decrease in the relative humidity at that time was necessary for the spores of these fungi to be liberated. Sreeramulu (8) showed that a high wind velocity around noon was responsible for the maximum concentration of the chalamydospores of loose smut of barley (Ustilago) in the air. The increase of conidia of Perenospora tabacina Adam in the air soon after sunrise was associated with an increase in air temperatures (7). Some fungi are diurnal in their spore liberation, e.g., Sordaria fimicola, whereas others are noctornal, e.g., Sordaria verruculosa (5).

Sporulation in fungi is controlled by moisture host condition, temperature and light. Sporulation of many *Peronospora spp.* decreased with relative humidities under 100% (7). Sporulation of the downy mildew of tobacco, *Peronospora tabacina* Adam, was related to the diffussion pressure dificit of host tissue and plant age (1). Different fungi have different temperature requirements for sporulation. Yardwood (1943) reported the minimum, optimum and maximum temperatures for sporulation of *Peronospora destructor* (Berk) as 7 C, 10-16 C, and 22 C respectively, whereas for *Peronospora tabacina* (2), the cardinal temperatures were 1-2 C, 15-23 C, and 27 C. In addition to moisture, host condition,and temperature,a moist dark condition following a period of light was shown to be essential for sporulation of many downy mildew fungi (9).

Most of the above studies were conducted on fungi which cause foliar disease. Nothing is known, however, concerning the existence of rhythms in spore production or liberation by root-infecting fungi. Many fungi, particularly those which belong to the Oomycetes, are known to cause root diseases of plants and zoospores have been implicated as the major propagule responsible for dissemination of these fungi, particularly in crops grown hydroponically. The objective of this study was to determine if there is a rhythm in zoospore production of *Pythium dissotocum* Drechs. in hydroponics. Experiments were conducted (1) to determine the rhythm of zoospore production in the nutrient solution of hydroponically - grown lettus in the greenhouse; (2) to study the effect of different light periods on zoospore production.

MATERIAL AND METHODS

Stock cultures of *Pythium dissotocum* originally isolated from asymptomatic lettuce feeder rootlets were maintained at 24 C on 10% V8 juice agar (VJA) medium.

Butterhead lettuce *Lactuca sativa* cv. Ostinata' seeds were germinated on water agar at room temperature (24 C). Five, two-week-old lettuce seedlings started in a nursery in peat pots, were transferred into holes cut into styrofoam flotation boards (32 x 27.5 x 2 cm.) . Boards were then placed in a 13.5-L plastic tubs (37.5 x 33 x 13.5 cm.) containing a continuously aerated nutrient solution. Tubs were placed in a temperature-controlled box (3), and the nutrient solution was equilibrated to desired temperature (22 C) before transplanting (Fig. 1). When the plants were 3 - 4 weeks old, they were inoculated by placing a petri dish (9 cm. diameter) of a one-week-old culture of *Pythium dissotocum* into each tub. The inoculum sources were removed from the tubs after 24 hours. After 48 hours, the roots of inoculated plants were assessed for infection by plating root segments on V-8 media.

GREENHOUSE STUDIES

The population of zoospores in the nutrient solution of the hydroponic system was monitored to determine if there was a rhythm to zoospore production. One week after the removal of the inoculum sources from tubs, samples of the nutrient solution were plated on a selective medium (cornmeal agar media containing 10 ug/ml pimaricin, 50 ug/ml Rose Bengal and 200 ug/ml Streptomycin sulfate). Samples were collected every 3 hours over a 24 hour period in the following manner: one end of a permanently placed tube (40 cm. in length) was inserted approximately 1 cm. into the nutrient solution. A Cornwall Continuous pipetting Syringe was attached to the opposite end (Fig.2). Five , 1ml. samples were deposited on selective media. Plates were incubated at 27 C and read at 24-36 hours by counting the number of Pythium dissotocum colonies. Three, 24 hour readings over a 5 - day - period from an individual tub was conducted. The experiment was repeated 6 months later with 2, 24 hour readings over a 2 day period.

GROWTH CHAMBER STUDIES

To study the effect of different light periods on zoospore production, some experiments were conducted in a growth chamber.

Lettuce plants, after being grown, inoculated, and checked for the presence of zoospores in tubs in the greenhouse, as explained earlier, were transferred to growth chambers. Growth chamber temperatures were set at 22 C. One growth chamber was set to a condition of continuous light. The second chamber was set to 3 hours light (05:00 to 08:00) + 9 hours dark (08:00 to 17:00) + 3 hours light (17:00 to 20:00) + 9 hours dark (20:00 to 05:00). The third growth chamber was set to 12 hours light (06:00 to 18:00) and 12 hours dark (18:00 to 06:00, but one of the tubs was covered with a box, so it will be in complete darkness. Plants were left in these conditions for two days before sampling. Sampling was conducted as previously described.

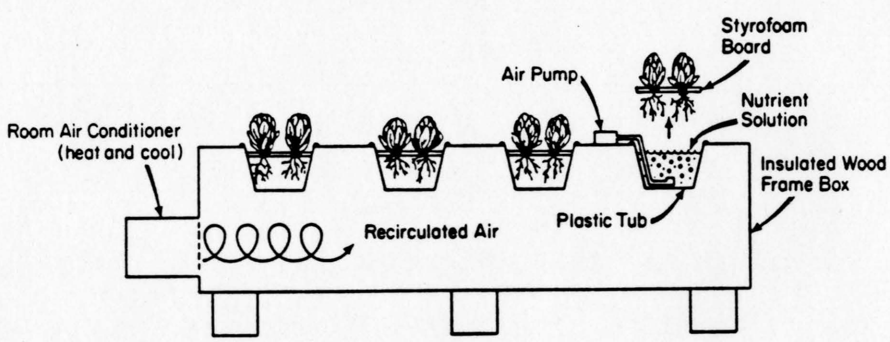

Fig.1. Diagramatic representation of a hydroponic cultural system used for studying zoospore liberation of Pythiumdissotocum on Lettuce. (after M. Stanghellini. Plant Disease 70: 1053-1056).

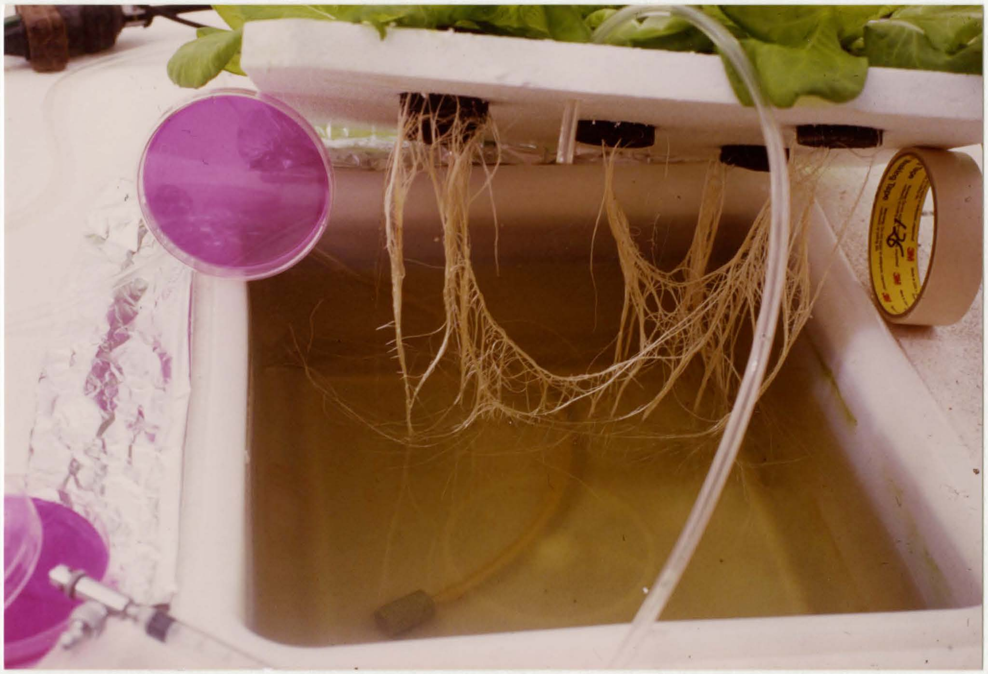

Figure 2. Method used for sampling the nutrient solution for the presence of zoospores.

RESULTS

GREENHOUSE STUDIES

In all five experiments conducted in the greenhouse, the number of zoospores detected in the nutrient solution was lowest around noon time, (11:00 - 14:00 hr) and highest around 20:00 hr (Fig. 3, 4, 5, 6, and 7). A composite of these five experiments is presented in Figure 8.

GROWTH CHAMBER STUDIES

Under continuous light (Fig. 9) or continuous darkness (Fig. 10, 11), the population of zoospore decreased in the nutrient solution and remained very low.

Under the conditions of 12-hour light (06:00-18:00 hr) and 12-hour darkness (18:00-06:00 hr), zoospore numbers in the nutrient solution decreased in the light period and reached its lowest around noon time (11:00 - 14:00 hr) and after that it increased (Fig. 10, 11).

Under the conditions of two periods of light (05:00 - 08:00 & 17:00 - 20:00 hr) and two periods of darkness (08:00 - 17:00 & 20:00 - 02:00 hr) in a 24 - hour period, the zoospores in the nutrient solution decreased in the first light period. There was no significant change after the first light period, but after the second exposure to light, the zoospore population increased (Fig, 12).

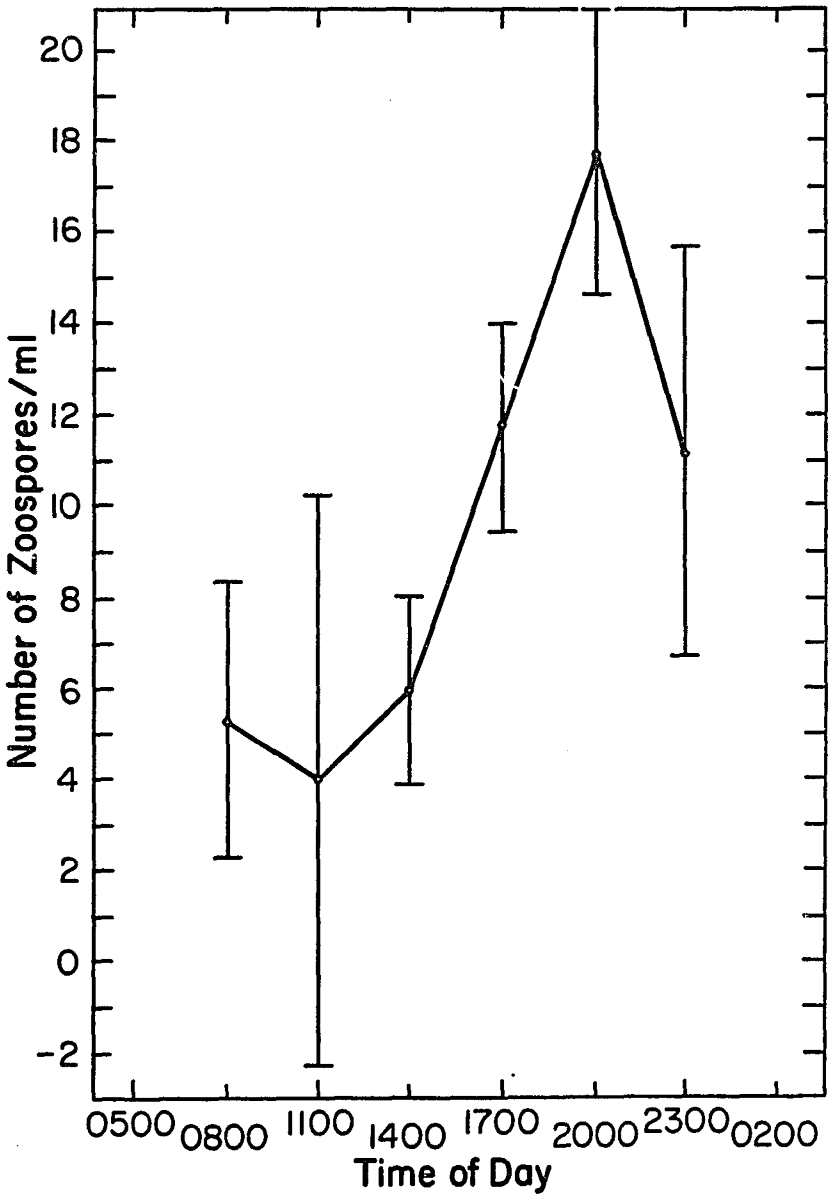

Fig. 3. Rhythm of *Pythium dissotocum* zoospores in the nutrient solution of hydroponically - grown lettuce in the greenhouse under normal light of day and night.

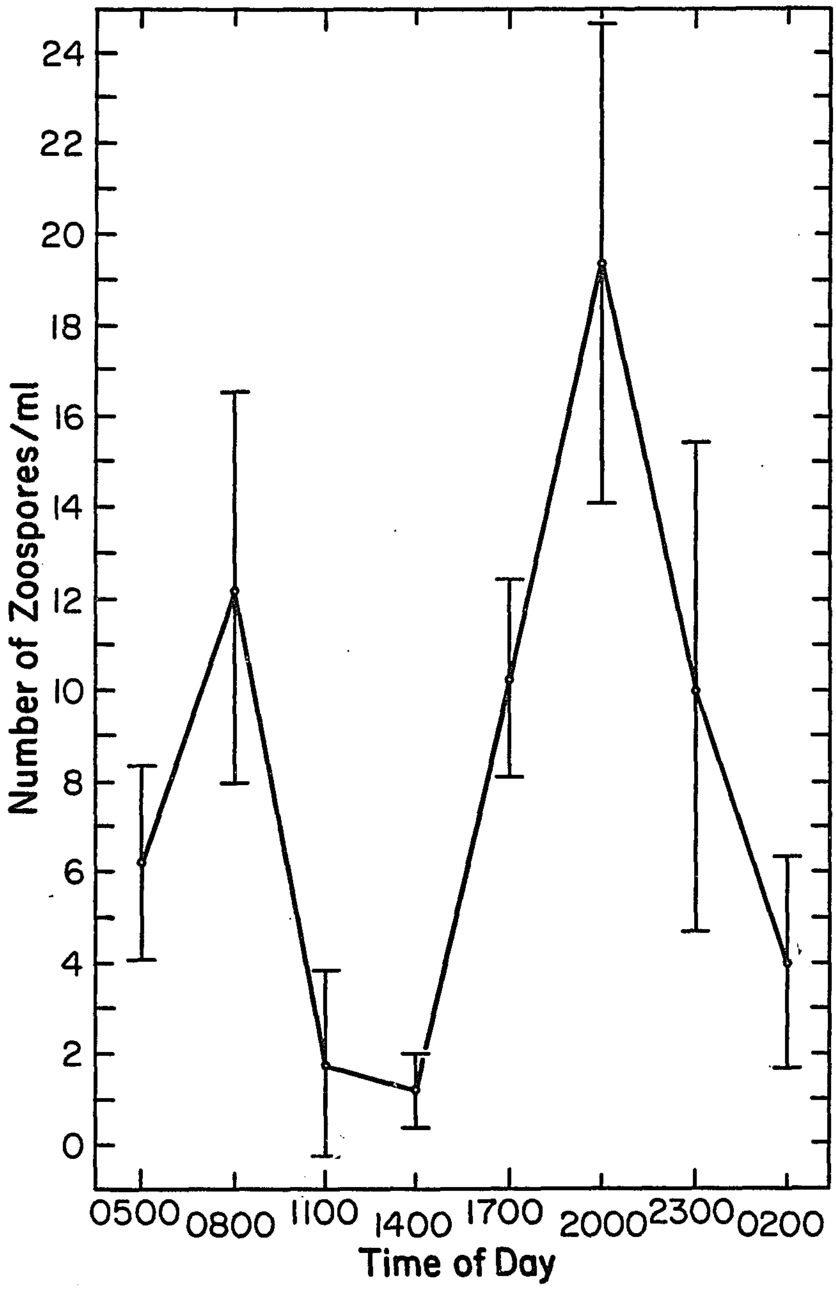

Fig. 4. Rhythm of *Pythium dissotocum* zoospores in the nutrient solution of hydroponically - grown lettuce in the greenhouse under normal light of day and night.

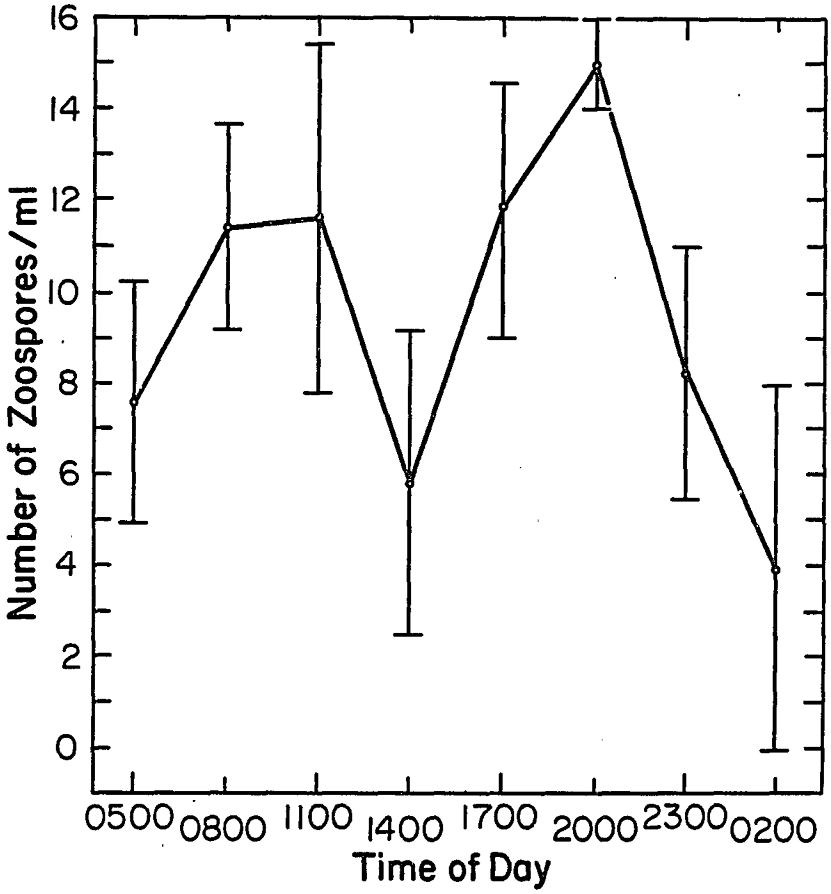

Fig. 5. Rhythm of *Pythium dissotocum* zoospores in the nutrient solution of hydroponically - grown lettuce in the greenhouse under normal light of day and night.

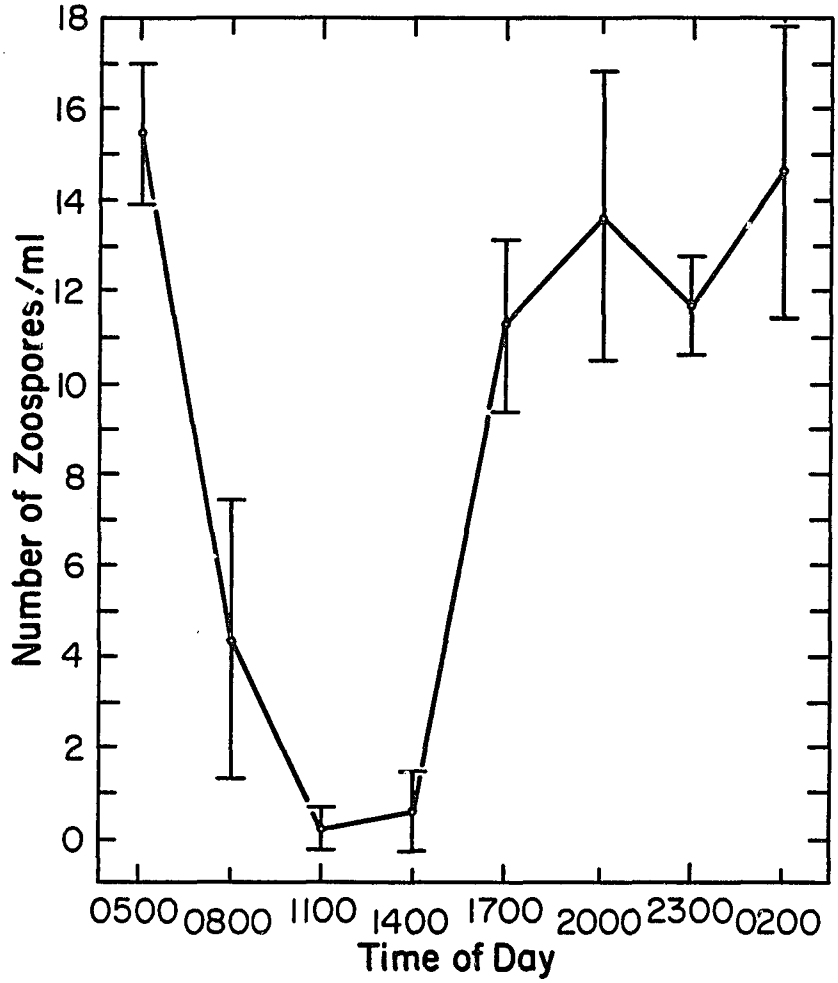

Fig. 6. Rhythm of *Pythium dissotocum* zoospores in the nutrient solution Of hydroponically - grown lettuce in the greenhouse under normal light of day and night.

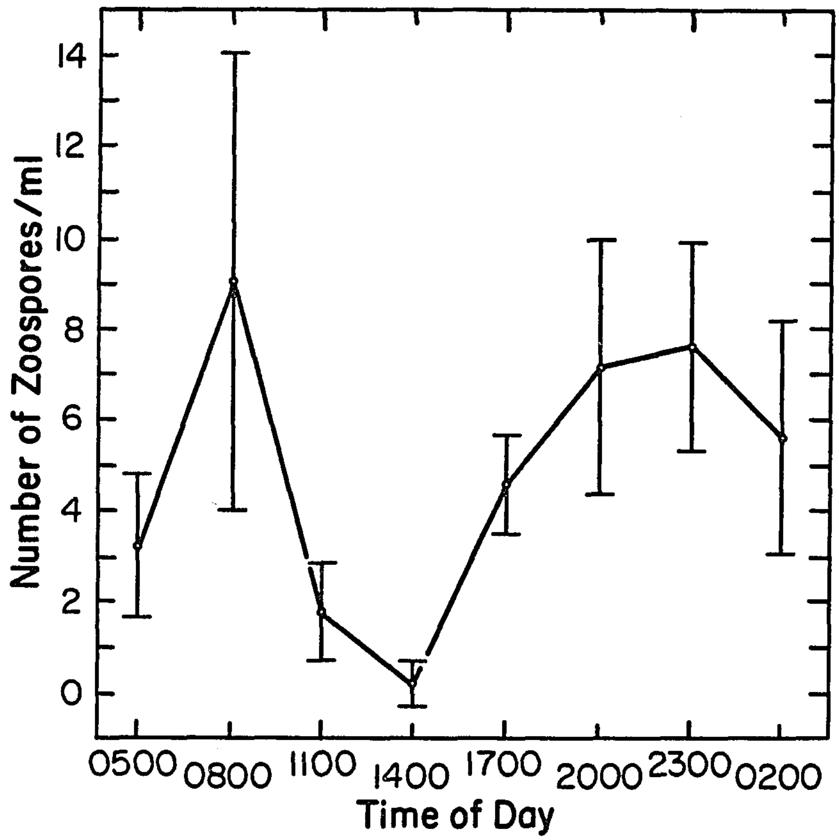

Fig. 7. Rhythm of _Pythium_ _dissotocum_ zoospores in the nutrient solution of hydroponically-grown lettuce in greenhouse under normal light of , day and night.

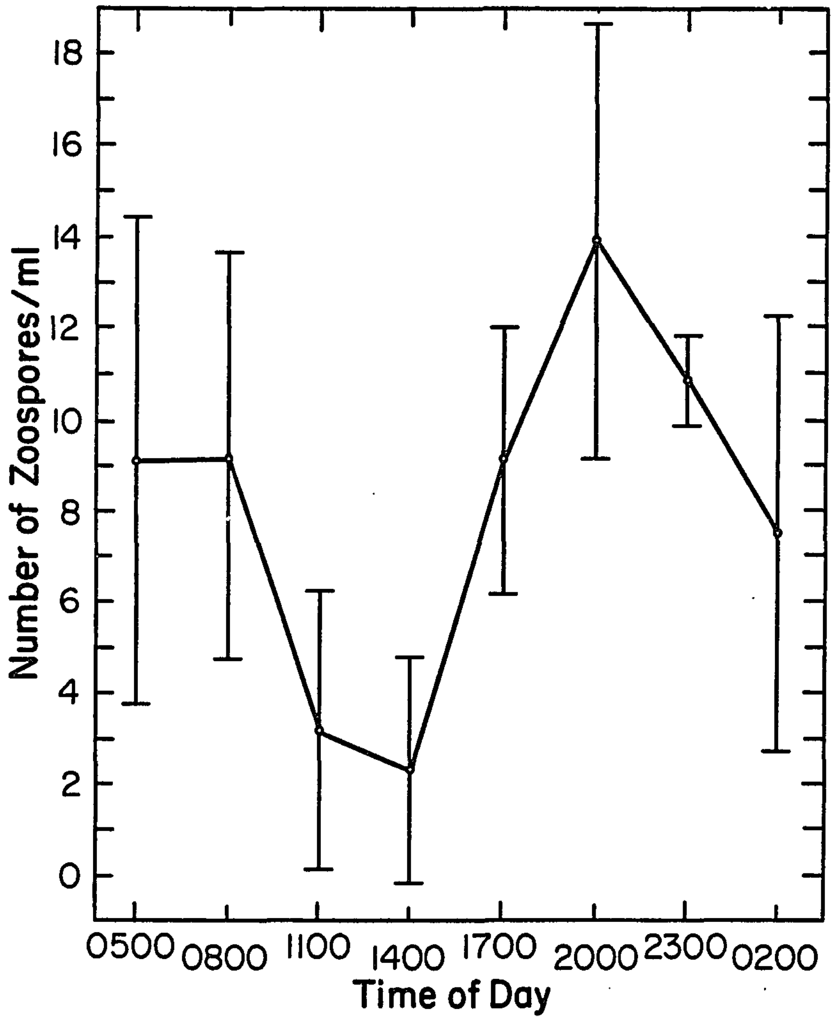

Fig. 8. The mean result of 5 experiments, showing the rhythm of Pythium dissotocum zoospores in the nutrient solution of hydroponically -grown ,lettuce in the greenhouse under normal light of day and night.

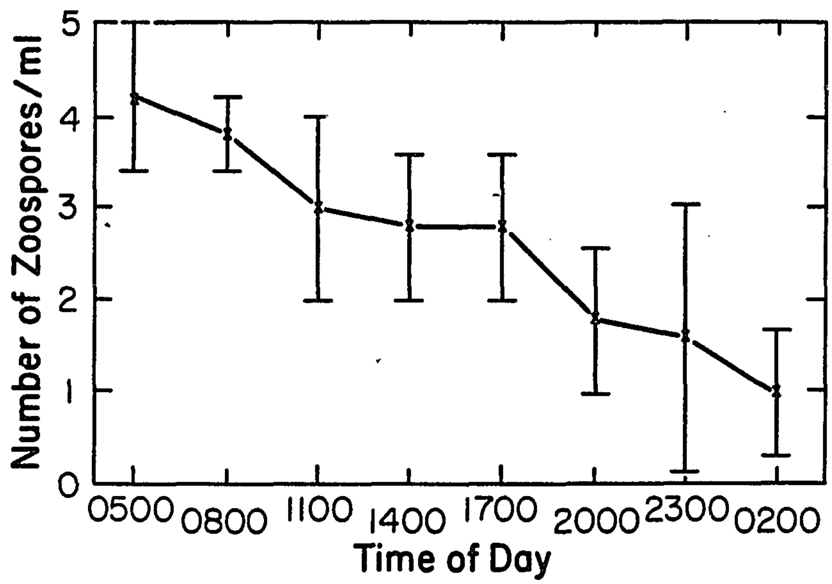

Fig. 9. Rhythm of *Pythium dissotocum* zoospores in the nutrient solution of hydroponically - grown lettuce in a growth chamber under constant temperature (22 C) and continuous light.

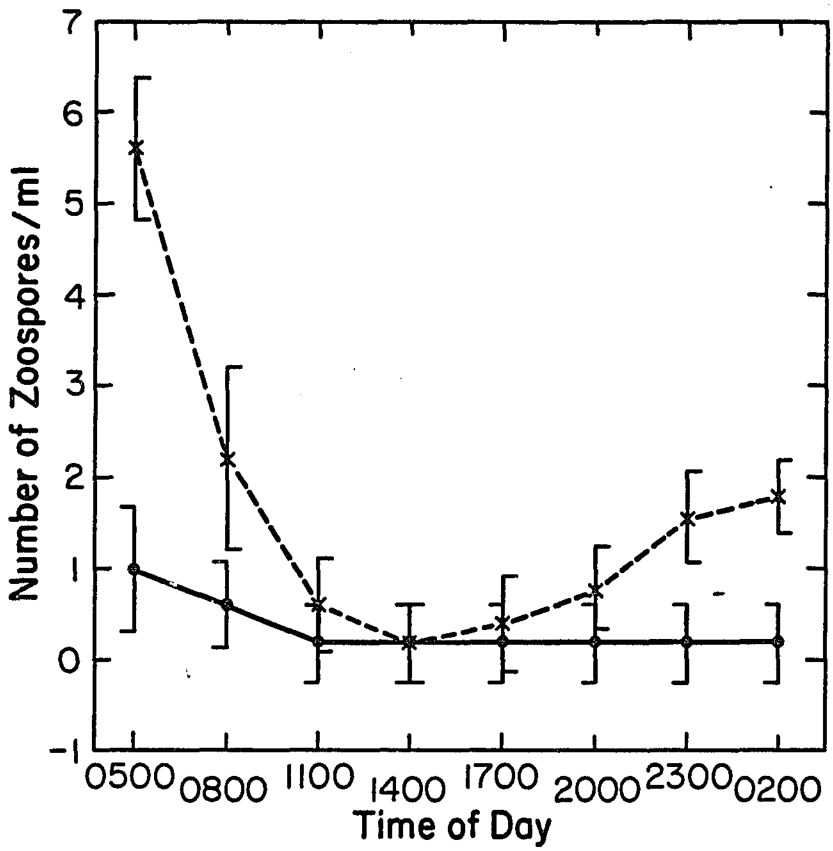

Fig. 10. Rhythm of Pythium dissotocum zoospores in the nutrient solution of hydroponically - grown lettuce in a growth chamber under constant temperture (22 C), and two light conditions; •——• continuous darknes, x——x 12 hours light (06:00 - 18:00) + 12 hours darkness (18:00-06:00).

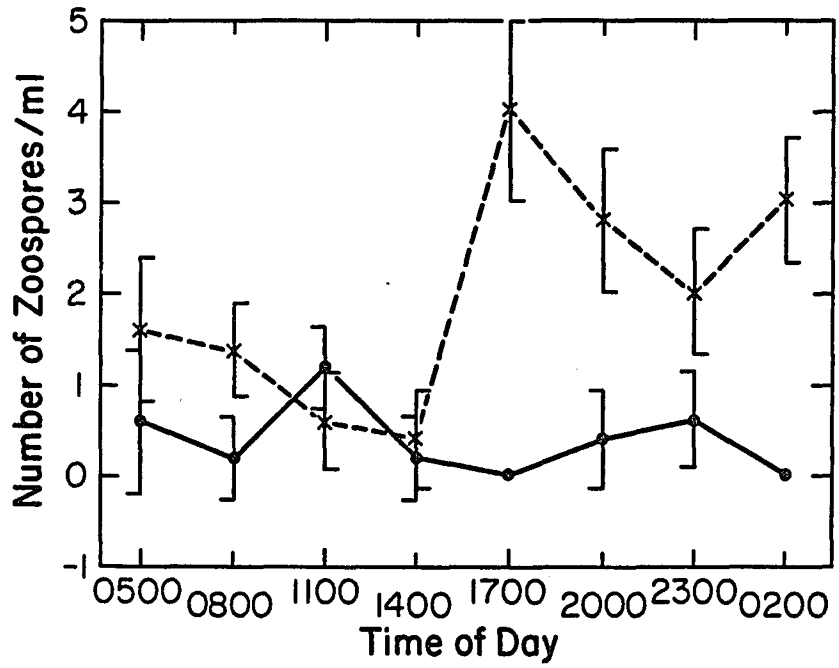

Fig. 11. Rhythms of Pythium dissotocum zoospores in the nutrient solution of hydroponically-grown lettuce in a growth chamberunder constant temperature (22 C), and two light conditions; continuous darkness, 12 hours light (06:00 - 18:00) + 12 hours darkness (18:00 - 06:00).

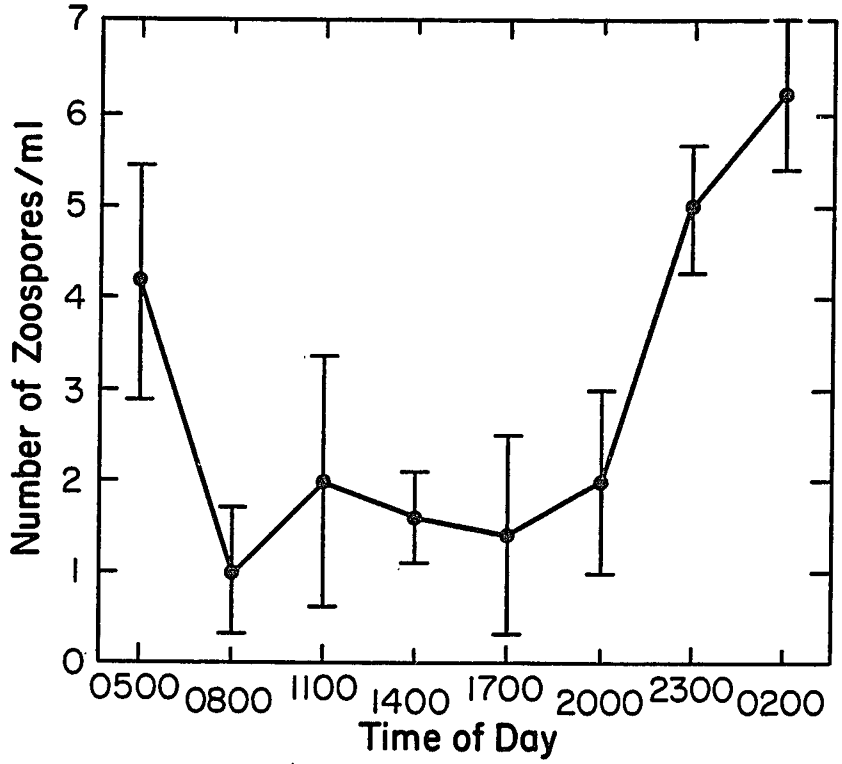

Fig. 12. Rhythm of *Pythium dissotocum* zoospores in the nutrient solution of hydroponically - grown lettuce in a growth chamber under constant temperature (22 C) and 3 hours light (05:00 - 08:00) + 9 hours darkness (08:00 - 17:00) + 3 hours light (17:00- 20:00) + 9 hours darkness (20:00-05:00).

DISCUSSION

The purpose of this study was to determine if there is a rhythm to zoospore production by *Pythium dissotocum* in the nutrient solution of a hydroponic system. The results of greenhouse studies showed that there is a rhythm in the production of zoospores and it was lowest at noon (11:00-14:00) {2.4/ml x 13,ooo ml/tub = 31,200 zoospores/tub (Fig.8)}, and highest about 20:00hr {13.88/ml x 13,000 m/tub = 180,440 zoospore/tub (Fig. 8) } (Fig.3, 4, 5, 6, 7 and 8). But these results did not indicate the mechanism governing this rhythm. Thus, growth chamber studies were conducted to determine the light effect on zoospore production. Under continuous light (Fig. 9) or continuous darkness (Fig. 10, 11) the population of zoospores in the nutrient solution decreased. But under 12 hours light and 12 hours darkness (Figs. 10, 11) and 2 periods of light each for 3 hours (Fig. 12), zoospore population decreased with light, and after 6 hours of light, it increased again and reached its peak in the dark period. So, we can conclude that the cyclic production of zoospores of

Pythium dissotocum on lettuce roots in hydroponics is dependent upon the alternation of light and dark conditions.

Although the cyclic production of fungal spores in response to light and dark is documented, the mechanism governing this phenomenon is not known. Yardwood (9) suggested that photosynthesis might have a role in the dirunal sporulation of hop mildew but he provided no evidence.

The cyclic behavior of zoospore production in hydroponics might be related to the physiology of the roots, but the particular mechanism involved is not known. Light affects root growth indirectly through regulating plant dry matter production and its partitioning between shoot and root (4). Hormones being translocated to the roots at certain times may have some role in the cyclic production of zoospore, or it could be related to the cyclic behavior of root cell division (4). Photosynthates are translocated to roots at night (4) and *Pythium* might depend on these energy sources for reproduction. The reduction in zoospore production under continuous light (Fig. 9) may be because lettuce plants remained in the synthetic phase and they need a reduction in light intensity or a dark period to translocate sugars to roots.

Knowing the rhythm of zoospore production in hydroponics may help to understand the epidimiology of disease, the timing of chemical application, and knowledge of when to sample to detect *Pythium*, other than isolation from root, in the nutrient solution.

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