

## A 24-Hour Deposition Sampler for Spores of *Heterobasidion annosum*

R. L. Edmonds, R. W. Hinshaw, and K. B. Leslie

Professor, electronics technician, and graduate student, respectively, College of Forest Resources, University of Washington, Seattle 98195. This work was supported by a Cooperative Aid agreement between the University of Washington and the U.S. Forest Service Pacific Northwest Forest and Range Experiment Station, Portland, OR; Earl Nelson is especially thanked for his support. Accepted for publication 26 April 1984.

### ABSTRACT

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A 24-hr deposition sampler for airborne spores of *Heterobasidion annosum* (*Fomes annosus*) is described. Twenty-four 60-mm-diameter disposable petri plates are mounted on a circular Plexiglas sheet. Once an hour the petri plates filled with selective medium are rotated beneath a 60-mm-diameter opening in the sampler cover. The Plexiglas sheet is rotated by a 24-position rotary solenoid (stepping motor) driven by two 6V

light weight rechargeable gel batteries in series. Electronic circuitry for the sampler is described. The sampler was used in a western hemlock (*Tsuga heterophylla*) stand in western Washington to examine diurnal spore deposition patterns in June and October 1982. In October, most spores were deposited during the night and early morning, but in June there was no distinct pattern.

*Additional key words:* root disease, spore sampler.

Volumetric spore traps are commonly used for studying inoculum levels of fungal pathogens (4,6,9). In some situations, however, deposition traps provide more useful information than volumetric traps. Such is the case for *Heterobasidion annosum* (Fr.) Bref. because spores of this fungus are deposited on cut stump surfaces after forest thinning operations.

Spore deposition by *Heterobasidion annosum* has typically been studied by exposing wood disks for a short time period. Disks are incubated and the surface is then examined microscopically for the conidial (*Oedocephalum*) stage (1,13,15). It is difficult, however, to obtain spore deposition patterns throughout a 24-hr period by using this method because exposure is not automated.

Wood and Schmidt (17) developed a 24-hr spore trap for *H. annosum* involving deposition on a moving glass slide. This sampler, however, was designed for studying spore release from basidiocarps and is not suitable for studying spore deposition away from fruiting bodies of *H. annosum*. Edmonds and Driver (2) developed an automated spore deposition sampler which exposed fresh petri plates containing a selective medium for *H. annosum* every 3 hr over a 24-hr period. An improved version of this sampler, which samples every hour, is described here, including electronic circuitry.

### MATERIALS AND METHODS

The sampler (Fig. 1) consists of a circular Plexiglas sheet (67.5 cm in diameter and 0.3 cm thick) to which 24 60-mm-diameter disposable plastic petri plates are attached with double-sided tape. The center of each plate is located 40 mm from the edge of the sheet and the plates are 25 mm apart.

A rotary solenoid (stepping motor) (Ledex, Inc.; Series 50-L 1/4 Duty 24 steps per revolution) is mounted in the center of a 13-mm-thick plywood base with the shaft extending upward. A rubber universal coupling, to absorb shock, attaches the shaft to the lower side of the circular Plexiglas sheet on which the petri plates are mounted. A 33-cm-diameter circular Plexiglas sheet is mounted in the center of the larger sheet to give stability. The weight of the large

sheet is supported at the outside edge by three 35-mm-diameter rubber model aircraft wheels mounted upside down.

A 74 × 74 × 11.5-cm Plexiglas cover, in which a 60-cm-diameter hole was drilled near the center of one side, is placed over the top of the sampler. The cover is shown in place in Fig. 1a and removed in Fig. 1b. One petri plate is exposed for an hour immediately below the hole and then moved under the cover. Thus, after 24 hr each petri plate has been exposed for 1 hr. Clearance between the petri plate top and sampler cover is 3 mm. In operation, plates were filled to the rim with a selective medium (Kuhlman and Hendrix [11] plus 300 ppm Rose Bengal to slow growth). After 24 hr, tops are placed on the petri plates. They are incubated for 10 days at 25 C and examined for the presence of colonies of the conidial (*Oedocephalum*) stage of *H. annosum*. The colonies are counted on each plate assuming that each colony arose from a single deposited spore, and the number of spores deposited per square meter per hour is calculated.

The circuitry for the sampler is shown in Fig. 2. Two 6V light-weight rechargeable gel batteries (Power Sonic, model PS 6200, 20 ampere hours) were used in series in the field. Only one battery is shown in Fig. 1. These batteries are more convenient to use than a single 12 V auto battery. The circuit activates the rotary solenoid advancing the collector one position each time the magnetic reed switch, which is activated by a magnet mounted on the clock hour hand, is closed.

The sampler was used to determine diurnal spore deposition patterns of *H. annosum* in a 60-yr-old western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) stand 16 km northwest of Hoquiam, WA, near the Pacific coast. Sporophores of *H. annosum* were present on large stumps and logs. The sampler was placed on the forest floor with the top of the exposed plate located 18 cm above ground level.

### RESULTS

Hourly spore deposition data for 7-8 June and 11-12 October 1982 are shown in Fig. 3. These dates generally represent high and low periods of spore deposition, respectively (3,12). The highest deposition rates in October occurred at night with the maximum rate at 0600 hours (23,500 spores m<sup>-2</sup>·hr<sup>-1</sup>). The lowest was in the afternoon at 1500 hours (0 spores m<sup>-2</sup>·hr<sup>-1</sup>). Spore deposition rates were much lower in June (range, 0-9,100 spores m<sup>-2</sup>·hr<sup>-1</sup>), and there was no distinct diurnal pattern. The highest rate occurred in the afternoon at 1400 hours.

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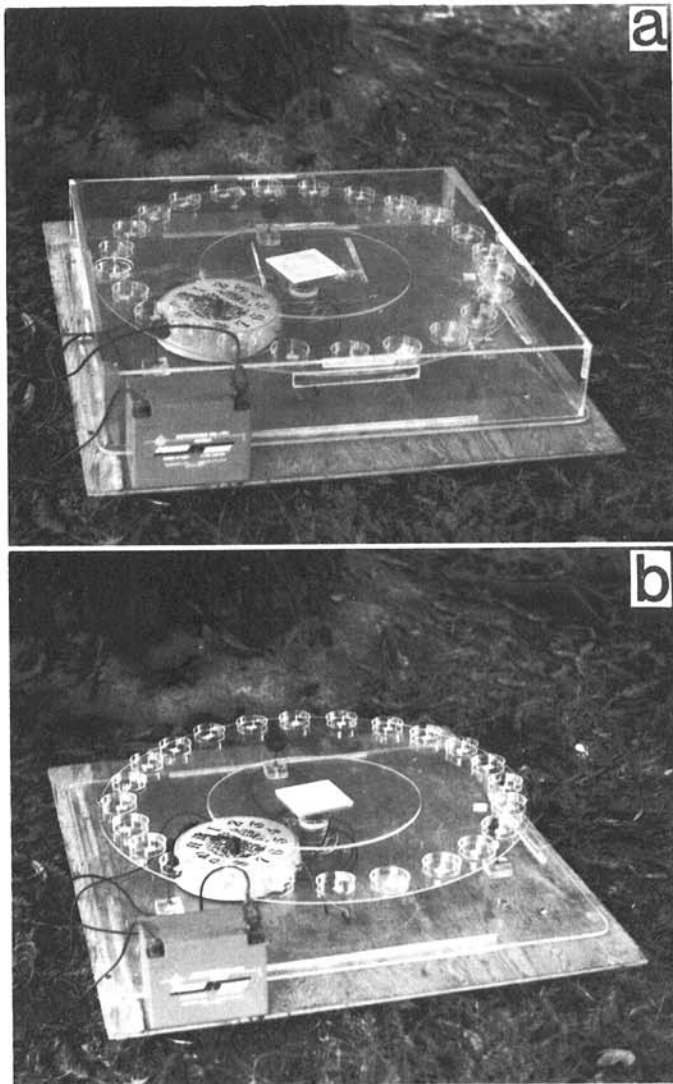


Fig. 1. Spore deposition sampler showing the sampler cover a, in place and b, removed. The deposition opening is in the left edge of the cover. Petri plates are shown empty.

## DISCUSSION

The automated spore sampler described here allows investigators to examine diurnal deposition of spores of *H. annosum* at a relatively fine level of resolution (ie, hourly) and also allows sampling in remote forest stands. The sampler is inexpensive and can be easily constructed.

Use of the sampler in a western hemlock stand in Washington has given some insights into diurnal deposition patterns of spores of *H. annosum* in this area. Typically, *Heterobasidion annosum* has been found to deposit its spores at higher rates in the night or early morning than during the day (2,7,8,14). The data in Fig. 3, however, indicate that in Washington this pattern may depend on the season of year, since the typical pattern was very strong in October, but not at all distinct in June.

One concern in using this sampler is possible contamination of plates when they are not in the exposed position. However, because there is only 3 mm of clearance between the sampler cover and the top of the petri plates the chance of such contamination is small, particularly if the sampler is located on the forest floor where wind speeds are very low. If plates are filled to the rim with selective medium, edge effects are reduced (5). This condition also more nearly represents stump surfaces. Two selective media (10,11) have been used for detecting spores of *H. annosum*. Our experience is that both work equally well, although the Kuhlman (10) medium has been used more often (14,16).

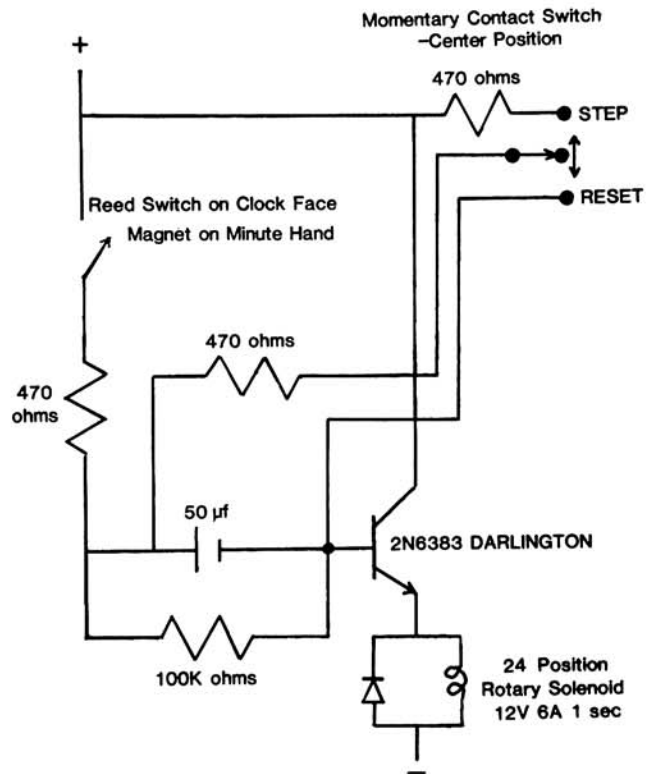


Fig. 2. Electronic circuitry for the sampler. All resistors are rated at 0.25 W.

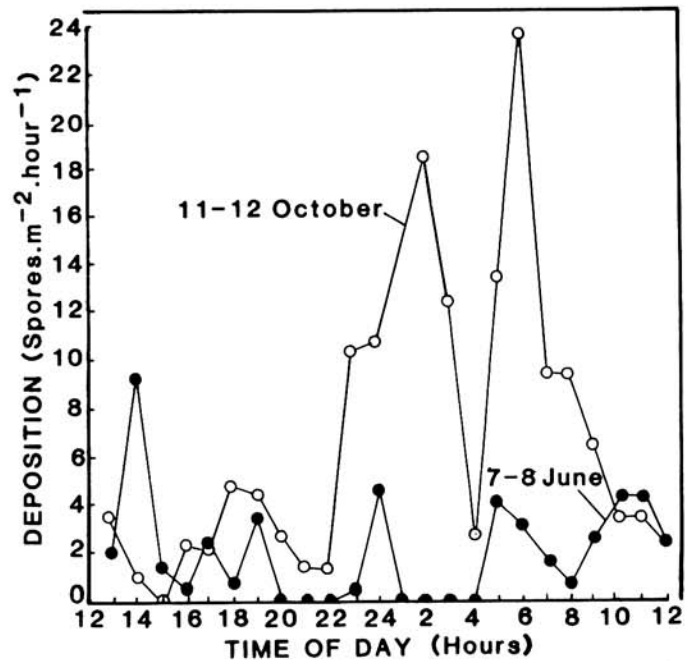


Fig. 3. Hourly deposition rates of spores of *Heterobasidion annosum* in June and October 1982, in a western hemlock stand near Hoquiam, WA. Time is 24-hour Pacific Standard Time.

Data obtained from this sampler used in combination with microclimatic data (temperature, relative humidity, and wind speed) can give important insights into the epidemiology of infection caused by *H. annosum*. It can also be used for other species of fungi, such as *Armillaria mellea*.

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