Techniques

A Sequential Sampler for Monitoring Water-Disseminated Pathogens from Trees

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ABSTRACT

Olson, B. D., and Jones, A. L. 1983. A sequential sampler for monitoring water-disseminated pathogens from trees. Phytopathology 73:922-925.

An apparatus for studying the dissemination of waterborne pathogens from trees is described and illustrated. It is used for monitoring a population of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* in rainwater collected under a cultivar Montmorency sour cherry tree. The apparatus collects rain from a 5,026-cm² area and saves 5- to 8-ml subsamples for each 0.5 mm of rainfall. Simplicity of design and a sequential sampling mechanism operated solely by the weight of the collected rainwater make this apparatus useful for certain phytopathological studies.

Many bacterial pathogens of fruit trees are water-disseminated during part of their life cycle (eg, *Pseudomonas syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum*, the cause of bacterial canker on cherry) (2,4). Splashing and windblown rain are considered important in the spread of these bacteria (2,4). Bacterial canker occurs sporadically; therefore, to comprehensively study the role of rain in removing and disseminating *P. syringae* pv. *morsprunorum* from leaves, we needed an apparatus that sequentially samples rain dripping from the canopy of infected *Prunus cerasus* L. 'Montmorency' sour cherry trees. Sequential rain samplers are used for air pollution studies (1,7,8), but due to large size, limited sampling capacity, and high cost they are not practical for most plant disease epidemiological studies or for widespread monitoring of chemicals.

This paper describes the design and use of a tipping-bucket

sequential rain sampler for monitoring bacterial pathogens in runoff rainwater.

MATERIALS AND METHODS

Instrument design and operation. The design of this self-advancing sequential rain sampler combines a fraction collector and tipping-bucket rain gauge (Figs. 1 and 2). A large funnel collects the rainwater that fills and tips a divided bucket. The tipping action mechanically advances the turntable one position and the collected subsample of water is siphoned into a 9-ml collection tube.

The funnel is made of 0.20-mm (8-mil) polyethylene and has an 80-cm-diameter opening with a rim formed from 1.9-cm-diameter rigid plastic tubing (Fig. 1). The rim is supported 92 cm above the ground with four 51-cm-lengths of 1.9-cm-diameter steel pipe attached to the sampler cover. A collar placed on the sampler cover is constructed from an inverted 20-cm-diameter plastic funnel, with a 10-cm-diameter orifice. The bottom of the polyethylene funnel is fitted into the collar and is held in place by inserting a 10-cm-diameter plastic funnel inside the polyethylene. The bottom of the

10-cm-diameter plastic funnel directs water into the tipping bucket and is held in place with a metal pin.

The sampler cover is made of a 25-cm-diameter × 31.7-cm-high metal can (Fig. 1). The sampler is mounted on two 1.9-cm-thick plywood boards spaced 10 cm apart with three 0.95 × 16-cm leveling bolts. The upper and lower boards are 55- and 77-cm equilateral triangles, respectively. The lower board is fastened to the ground with three 0.95 × 30-cm steel spikes.

The tipping bucket and sampling mechanism are constructed of 3.17 and 6.35-mm-thick Plexiglas (Fig. 2). The tipping bucket has two compartments, each with a capacity of 280 ml. Tipping-bucket volumes are adjusted by placing counterweights on a metal rod suspended below the tipping bucket. The axis for the tipping bucket is supported on both ends with removable Teflon (E.I. du Pont de Nemours & Co., Wilmington, DE 19898) bearing supports.

The tipping-bucket mechanism also advances the rack of collection tubes. A Plexiglas arm, reinforced with a 2.17-mm steel shaft, is attached near the top center of the tipping-bucket mechanism. A Teflon sliding bearing at the end of the steel shaft is inserted into a pivoting arm connected to the advancing mechanism located at the base of the sampler.

Each compartment of the tipping bucket empties into a separate drain. The floor of each drain slopes down 6.15 mm from the side walls to a center outlet. A 2.5-cm-long × 2-cm-wide × 2.5-cm-high Plexiglas box with an open top is placed on the floor of each drain directly above a row of collection tubes. Each box has a siphon made of 5-mm-diameter glass tubing for transferring water from the box to a collection tube each time the tipping bucket empties.

A turntable and rack of collection tubes are located underneath the floor of the drains. The bottom of the turntable consists of three 21.6-cm-diameter plates made from 3.17-mm-thick Plexiglas. The top and bottom plates are notched with 30 teeth pointing clockwise and counterclockwise, respectively, and are cemented to the center plate. The turntable revolves on a 10.16-cm-diameter Lazy Susan bearing (Triangle Manufacturing Co., Oshkosh, WI 54901). A removable rack for holding 13 × 100-mm test tubes is rotated by the turntable. The rack is made of three 20.5-cm-diameter Plexiglas plates, two of which are 3.15-mm-thick and drilled with holes in two concentric circles that hold 30 tubes each. The bottom plate is 6.35 mm thick with correspondingly positioned depressions drilled into it 3.2 mm deep for stabilizing the tips of the tubes. Collection tubes in the outside circle are filled by the right siphon, those in the inside circle by the left siphon.

Two consecutive bucket tippings fill a collection tube in each row of tubes and also advance the turntable one notch. The back-and-forth movement of the tipping bucket pivots the advancing arm and slides a Teflon advancing block back and forth in a partially enclosed box. An angled piece of flexible metal, protruding from the inside surface of the advancing block, catches a clockwise tooth on the turntable. When the left bucket tips, the advancing block moves from left to right, rotating the turntable counterclockwise one notch. As the advancing block moves from left to right, a second angled piece of flexible metal is exposed and catches a counterclockwise tooth, stopping the advancement of the turntable. The metal stop is pulled away from the turntable as the advancing block moves from right to left. Clockwise movement of the turntable is prevented by a stationary stop piece.

To record when rain samples are collected, an electronic switch (MICROswitch, Freeport, IL 61032) is attached to the advancing arm just below the Plexiglas arm coming from the tipping bucket. Each tipping of the bucket causes the switch to complete an electronic circuit that sends a pulse to an event recorder (WEATHERtronics, Inc., West Sacramento, CA 95691).

Evaluation of instrument. To measure the volume of water needed to tip each bucket, known quantities of water were poured into the sampler until the bucket tipped. This was repeated 20 times per bucket for each of the three samplers. The volumes of water
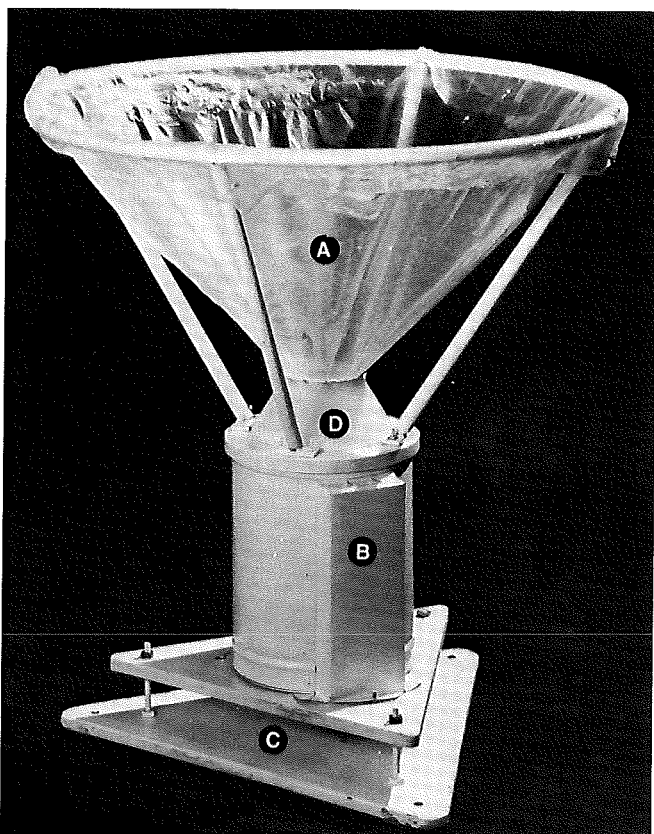

Fig. 1. Sequential rain sampler. A = funnel, B = cover, C = platform with three leveling bolts, and D = collar.
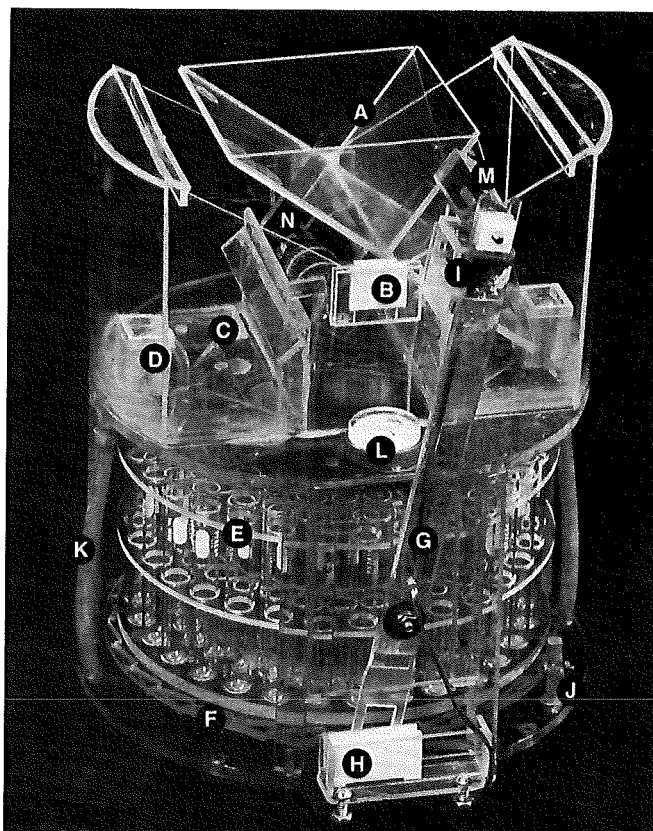

Fig. 2. Mechanism for the sequential rain sampler. A = tipping bucket, B = bucket bearing, C = drain, D = Plexiglas box and siphon, E = collection tube rack and tubes, F = turntable, G = pivoting arm, H = advancing block, I = electronic switch, J = stationary stop piece, K = drain tube, L = level, M = Plexiglas arm, and N = counterweights.

were multiplied by 1.99×10^{-3}, the millimeters of rain necessary to collect 1 ml of water in the sampler, to obtain the amount of rain needed to tip each bucket.

Redistribution of bacteria within each sampler was tested by pouring a bacterial suspension into the samplers, then removing bacteria that adhered to each sampler with simulated rain. A rifampicin-resistant strain of *P. syringae* pv. *morsprunorum* (PsmR) (6) grown on King's medium B (5) for 2 days incubation at 22 C was suspended in 0.01 M phosphate buffer adjusted to pH 7.2. The sampler was washed thoroughly with buffer before pouring in enough bacterial suspension for two bucket tips. This was immediately followed by pouring in enough phosphate buffer for six bucket tips. Bacterial suspensions and phosphate buffer were poured around the perimeter of the funnel. From each of the eight collection tubes, duplicate 0.1 ml subsamples were pipetted with a Finnpipette (Finnpipette, Helsinki, Finland) onto King's medium B amended with 50 μg/ml rifampicin (Calbiochem-Behring Corp., La Jolla, CA 92037) and 25 μg/ml cycloheximide (Sigma Chemical Co., St. Louis, MO 63178). Colonies were counted after 5 days of incubation at 22 C, and the colony-forming units (cfu) per milliliter of sample were computed. The concentration of bacteria in each collection tube was expressed as a percentage of the concentration of bacteria in the initial suspension (bacterial frequency). This experiment was replicated three times per sampler.

In the field, a sequential rain sampler was positioned under the drip line of a cultivar Montmorency sour cherry tree at East Lansing, MI, that was spray inoculated with PsmR at sunset on 17 and 29 April and on 8 and 29 May 1980. Inoculum was prepared from 2-day-old cultures of PsmR grown on King's medium B and incubated at 22 C. The cultures were suspended in phosphate buffer to give a final concentration of 10^8 cfu/ml. The Montmorency sour cherry leaves were lightly misted with 1.5 L of inoculum applied with a handgun sprayer operated at 28 kg/cm^2. Concentrations of PsmR in rainwater were determined from each collection tube by plating duplicate 0.1 ml subsamples onto King's B medium amended with rifampicin and cycloheximide. Colonies were counted after 5 days of incubation at 22 C, and the number of cfu per milliliter of rainwater were computed. Rainwater was plated within 12 hr after the end of each rain period.

RESULTS AND DISCUSSION

The amount of rain needed to tip each bucket varied slightly among samplers and ranged from 0.533 ± 0.23 mm to 0.452 ± 0.023 mm.

When suspensions of bacteria were added to samplers that were still wet from being washed with buffer, there was a mean reduction of 16 and 9% in the concentration of bacteria in collection tubes 1 and 2, respectively, compared to the initial bacterial suspension (Fig. 3A). When sterile phosphate buffer was added to the sampler (Fig. 3A, samples 3–8), the concentration of bacteria in successive collection tubes declined until no bacteria were detected.

The results fit a distinct pattern that was reconstructed with the following equation:

$$M_n = 0.9(0.9 \ I_n + 0.1 \ I_{n-1}) + 0.1 \ M_{n-2} \qquad (1)$$

in which M = concentration of bacteria in collection tube number n, I = concentration of bacteria in the solution entering the sampler, and n = collection tube number. This equation assumes that the funnel and sampling mechanism each retain 10% of the bacteria from the previous sample. Therefore, the quantities $0.1 \ I_{n-1}$ and $0.1 \ M_{n-2}$ represent the numbers of bacteria remaining on the funnel and sampling mechanism, respectively. To reconstruct the experiment using equation 1, we assumed $I_{n-1} = 0$ and $M_{n-2} = 0$ for sample 1 and $M_{n-2} = 0$ for sample 2, since the sampler was cleaned with phosphate buffer prior to the experiment. When the equation was solved with known concentrations of bacteria entering the sampler, the predicted values were similar to the empirical data points (Fig. 3B). A correlation coefficient of $r = 0.96$ was obtained when the empirical data were compared with the predicted data.

Equation 1 was used to estimate the concentration of bacteria in rain entering the sampler from the concentration of bacteria detected in the collection tubes. If the sampler was dry before a rain period, the concentration of bacteria in the first collection tube would be equal to the concentration of bacteria in the water entering the sampler. The concentration of bacteria in the second collection tube would be corrected for retention of bacteria on the funnel and the concentration of bacteria in the third to nth collection tubes would be corrected for retention of bacteria on the funnel and sampling mechanism. The prediction equations were:

1st tube, $I_1 = M_1$ (2)

2nd tube, $I_1 = (M_2 - 0.1 \ I_1)/0.9$ (3)

3rd–nth tubes, $I_n = ((M_n - 0.09 \ I_{n-1}) - 0.1 \ M_{n-2})/0.81$ (4)

in which I = concentration of bacteria in the rain entering the sampler, M = measured concentration of bacteria in the collection tube, and n = collection tube number. Retention of bacteria on the funnel was accounted for by $0.1 \ I_1$ and $0.09 \ I_{n-2}$ in equation 3 and 4, respectively, and retention of bacteria on the sampling mechanism was accounted for by $0.1 \ M_{n-2}$ in equation 4.
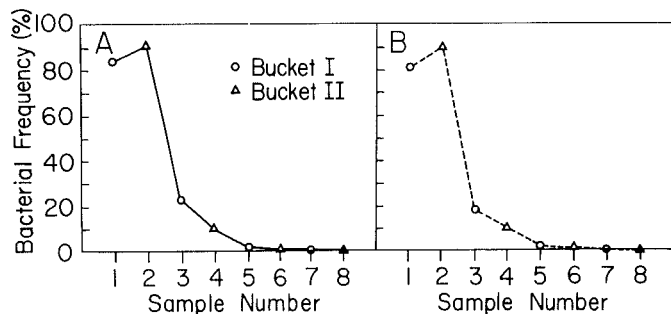

Fig. 3. Bacterial frequency (%) in collection tubes after sufficient quantities of bacteria in suspension were added to the sampler for two bucket tips, followed by sufficient quantities of phosphate buffer for six bucket tips. Prior to sampling, samplers were thoroughly rinsed with phosphate buffer. **A,** Empirical data were the means of three replicate experiments from three samplers. **B,** Predicted values were from the sampler retention equation.
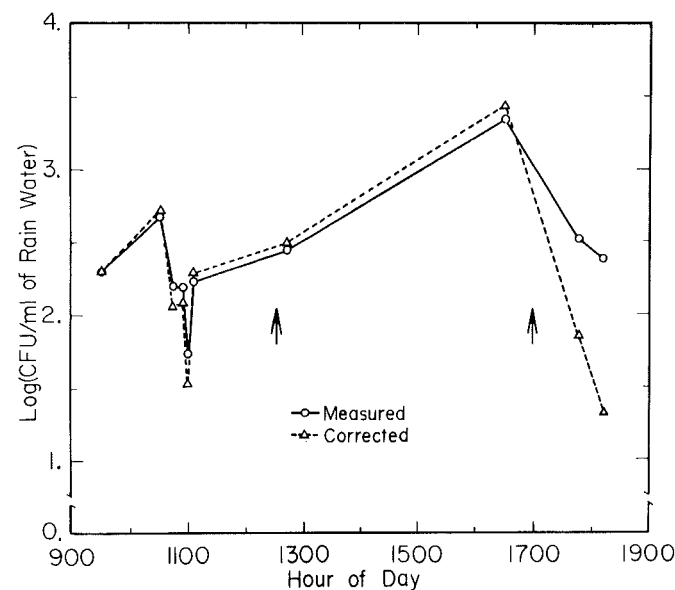

Fig. 4. Concentration of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* in samples of rainwater collected under a cultivar Montmorency sour cherry tree with a sequential sampler during a rain on 8 October 1980. Arrows indicate the beginning and end of a period of light mist.

During a rain period on 3 October 1980, the sampler collected rainwater from a cultivar Montmorency sour cherry tree previously inoculated with PsmR. Actual concentrations of PsmR detected in the collection tubes and corrected concentrations were plotted over time (Fig. 4). High concentrations of bacteria were detected in the first two samples collected after the onset of the rain. As the intensity of the rain increased, concentrations of bacteria in the samples decreased. However, when the intensity of the rain decreased, concentrations of bacteria in subsequent samples increased with the maximum concentration being detected at 1630 hours. This may be explained if we consider that during a rain period the leaves are saturated with water and the bacteria in the leaves move out of the leaves through the stomata at a constant rate (cfu per unit of time). When the rain intensity increases, the concentration per unit volume of water decreases and vice versa. After 1630 hours, there was a decline in the concentration of bacteria in the rain samples. Corrected concentrations of PsmR were similar to measured concentrations except in the last two samples. Corrected values were lower because many of the bacteria recovered were retained from the previous sample at 1630 hours.

Crosse (3) measured the inoculum potential of *P. syringae* pv. *morsprunorum* on sweet cherry leaves in vitro by washing detached leaves in water. The sequential sampler allows researchers to associate in vivo inoculum concentrations of pathogens in foliar-runoff rainwater with temporal environmental events.

One sampler functioned for two growing seasons and two samplers functioned for one growing season without problems.

There were no clocks to wind or batteries to replace except on an event recorder. The samplers were easily transported, disassembled, and cleaned in the field. The sequential sampler may also be useful for monitoring fungal spores and pesticide runoff in rainwater from trees.

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