Dispersal of Phytophthora palmivora Sporangia by Wind-Blown Rain

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ABSTRACT

Sporangia of *Phytophthora palmivora* produced on papaya could not be recovered on Hirst spore trap slides, even though infected fruits surrounding the trap or in wind tunnels were subjected to a wide range of meteorological conditions suitable for release of dry sporangia of *Phytophthora infestans* from potato leaves. However, wind-blown rain collected from severely diseased orchards contained sporangia. Rain-splash experiments showed that sporangia are readily released in splash-droplets formed when rain drops impact on disease lesions. Detached sporangia held at relative humidities lower than 100% dehydrated in 2-4 min and failed to germinate when placed in water. There was an inverse relationship between temp and survival of detached sporangia. Sporangia attached to papaya fruits can survive drying conditions, therefore these spores serve as a source of inoculum which can be dispersed by wind-blown rain. Intermittent showers capable of detaching sporangia are common during the night in Hawaii and survival of the sporangia would be expected because the humidity is usually 100% for 8-9 h each night of the year. Thus, wind-blown rain is an ideal spore release and dispersal mechanism for survival of this species.

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Additional key words: Phytophthora colocasiae, Colocasia esculenta, epidemiology.

In Hawaii and other wet tropical areas, *Phytophthora palmivora* (Butl.) Butl. (previously identified as *Phytophthora parasitica* Dast.) causes an aerial blight in addition to seedling root rot of papaya (*Carica papaya* L.) (5, 11, 13). Most aerial infections first occur on nearly mature fruits on the lower portion of the acropetally developing fruiting cluster. Some initial infections also occur on the immature portion of the upper part of the stem. Aerial infection of the lower portion of the trunk occurs primarily through wound sites or bud scars (11). Nearly complete destruction of orchards not receiving fungicide sprays can occur within 1-2 mo.

Epidemics occur only during periods of extremely wet weather. In Hawaii, rainfall during such periods may average tens of cm (several inches) per wk for several wk (10). Infected fruits become covered with a mycelial mat, sporangia, and chlamydospores. In previous studies with a Hirst spore trap, we failed to recover sporangia in air sampled in heavily infected fields (10). A preliminary study showing rain-splash and wind-blown rain as spore release and dispersal mechanisms was published previously in abstract form (9). These conclusions are substantiated by the laboratory studies reported herein. Moreover, field studies with a trap designed to collect wind-blown rain confirm this method of dissemination.

MATERIALS AND METHODS.—To determine whether dry, air-borne sporangia are released and disseminated by wind, ca. 200 papaya fruits infected with *P. palmivora* were placed on a coarse, wire-mesh screen held in an upright position in a circle around a Hirst spore trap. The screen, which was 0.91 m (3 ft) in height, was positioned so that diseased fruits were equally distributed above and below the orifice of the trap (Fig. 1). During some tests, the entire area was covered with a Saran-cloth screen to simulate shading of fruits by papaya leaves. A Rotorod spore sampler was used occasionally in place of the Hirst spore trap. The experiment was conducted in an open area exposed to natural environmental conditions. A rain gage and hygrothermograph were used to record precipitation, temp, and humidity. Further attempts to trap dry, air-borne sporangia were made in a simple wind tunnel. The tunnel was ca. 25.4 cm (10 inches) in height and 0.61 m (2 ft) in length with the front end funneled toward the orifice of a Hirst spore trap. Several infected fruits covered with sporangia were placed throughout the tunnel. Although the wind-tunnel experiments were conducted outdoors to expose the sporangia to natural changes in temp and humidity, a roof was used to prevent rain-splashing. A fan was used to blow air at speeds from 3.2-48.3 km/h (2-30 mph) over the fruits in the direction of the spore trap orifice. The experiment was repeated in a chamber in which the temp and humidity could be changed rapidly. To insure that the experimental conditions were suitable for spore release and recovery, potato leaves infected with *Phytophthora infestans* (Mont.) d By. were used as a control. With rising temp and falling humidity, this pathogen is known to release sporangia in the air (6).

A trap was improvised to determine whether sporangia were present in wind-blown rain. A sheet of corrugated plastic 0.61 × 1.52 m (2 × 5 ft) was mounted in a vertical position in a wooden frame of adjustable height. The top of the trap was partially covered with a plastic sheet to restrict collection primarily to rain being blown in a horizontal direction. The bottom of the plastic sheet was cut in a V-shape and a large Tygon tubing attached to form a trough for collecting the water running down the plastic sheet. A funnel was used to collect the water in a narrow-mouth storage container (Fig. 2). Sodium hypochlorite was used to inhibit microbial growth. The water was centrifuged and the pellet examined microscopically for sporangia. Collections were made for several wk in an orchard with a high incidence of disease. The base of the trap was ca. 1.53 m (6 ft) above the ground. The trap was placed in a position normally

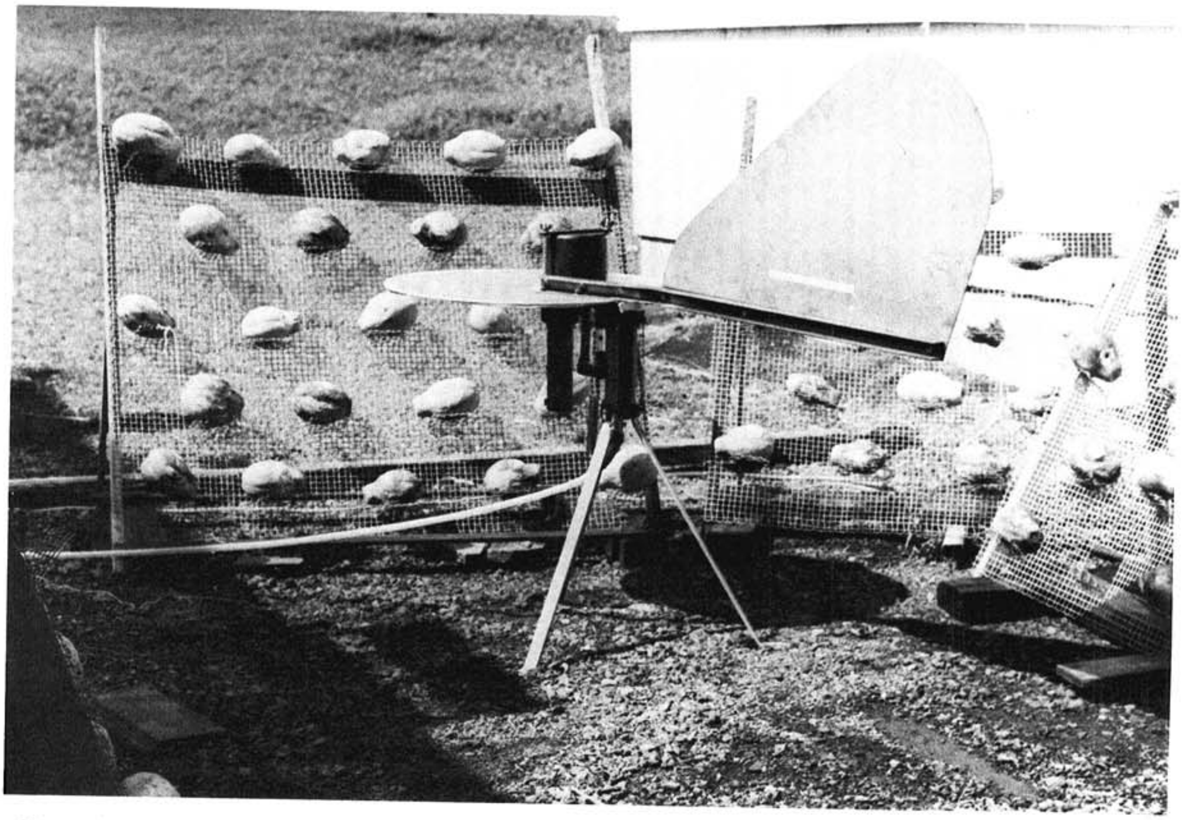

Fig. 1. Hirst spore trap surrounded by papaya fruits infected with *Phytophthora palmivora* to determine if sporangia become airborne.

occupied by a papaya tree to insure that the distance sporangia traveled to reach the trap was at least equivalent to the distance required to spread from a diseased tree to the fruiting clusters of a healthy one.

Rain-splash experiments were conducted in the laboratory to determine the feasibility of this method for releasing sporangia of *P. palmivora* growing on papaya fruit. Artificial rain drops were released from a syringe and needle mounted at a fixed angle on the end of a 7.62 cm (3-inch) diam, 1.52 m (5-ft) length of aluminum rain-spout tubing. Drop size was varied by using needles ranging in size from 18-26 gauge. Crystal violet dye was used to color the rain drops and facilitate detection of sporangia, which readily absorbed the dye. The tubing was positioned so that drops fell from a height of 1.52 m (5 ft) onto the edge of a *P. palmivora* lesion on a papaya fruit. The fruit was placed in a small box free of air currents. A 2.5 × 12.7 cm (1 × 5-inch) Vaseline-coated glass slide extending outward from the base of the lesion was placed on the bottom of the box. After 10 rain drops had fallen upon a lesion, the slide was examined microscopically to determine the incidence of splash-droplets of 25-50, 51-100, 101-200, 201-400, 401-800, and 801-1,600 μm in diam. The number of sporangia in each splash-droplet and the incidence of sporangia free of any detectable splash-droplet was recorded. The splash dispersal experiment was replicated five times with 18- and 22-gauge needles.

The effect of relative humidity (RH) and temp on survival of detached sporangia was determined under controlled conditions in the laboratory. The glycerol-water system used by Trujillo (12) to obtain the desired relative humidity was used in these experiments, except that small containers (made of soft plastic) with snap tops were used in place of plastic petri dishes. The RH control system was small enough to be placed inside of refrigerated incubators in an air-conditioned room maintained at ca. 24 C.

Sporangia were produced by incubating inoculated papaya fruits at 24 C, 100% RH. Spores were detached by gently rubbing the surface of the lesion with cheesecloth strips previously conditioned to the RH test condition. The cheesecloth strip containing the detached sporangia was placed immediately in the RH chamber. A separate chamber was used for each strip so that each chamber was only opened once. After the desired length of treatment, the strip was removed and the sporangia immediately washed-off into 1 ml of distilled water (24 C) in a 50-ml beaker. The sporangial suspension was then poured over the surface of a petri dish containing 1% water agar. All plates were incubated at 24 C for 12 h. The percentage germination (direct and indirect) was determined by observing 300 sporangia. The effect of RH on survival was studied at 60, 80, and 100% RH at 24 C. Survival in relation to temp was studied at 16, 24, and 32 C at 80% RH. Treatments were replicated twice and all

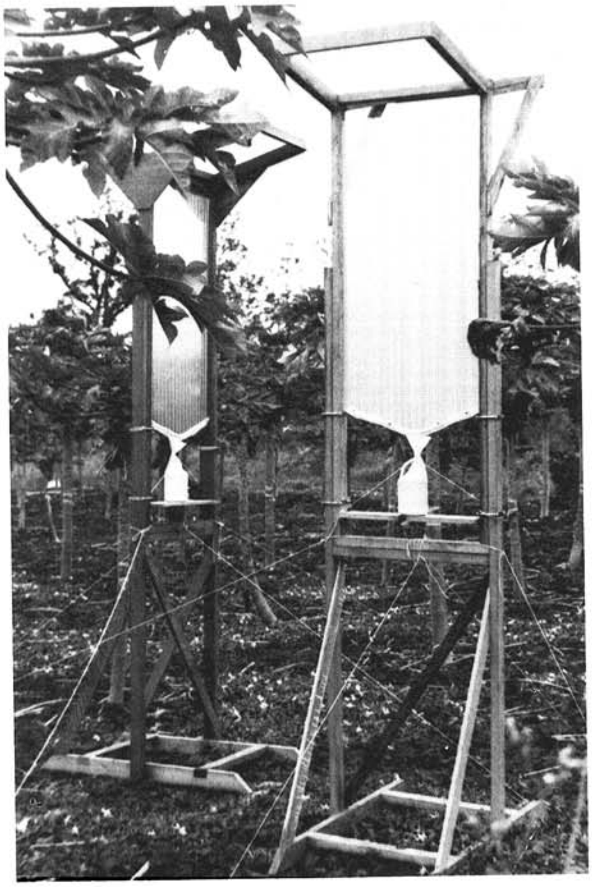

Fig. 2. A trap improvised for recovering wind-blown rain in papaya orchards infected with *Phytophthora palmivora*.

experiments were repeated at least once.

Survival of sporangia attached to mycelial mats growing on papaya fruits was studied under field conditions. To remove spores of unknown age, lesions on diseased papaya fruits attached to the trees were scraped in the afternoon. Throughout the following day, when the temp reached a high of 32 C and the humidity fell to 50%, sporangia which had formed overnight were removed with cheesecloth strips and washed-off in 1 ml of 25% papaya extract (1). The sporangial suspension was poured over the surface of a petri dish containing 1% water agar and allowed to germinate for 5 hr at ambient temp before determining the percentage germination. The experiment was replicated six times.

RESULTS.—*Spore-trapping experiments.*—Hirst spore trap slides collected over a period of several wk remained free of *P. palmivora* sporangia even though the trap was surrounded by 200 infected fruits. Likewise, sporangia also were not recovered on a Rotorod spore trap positioned at various heights in the center of the ring of diseased fruits. However, sporangia were found in rain water collected near the base of the fruit racks. Dry, air-borne sporangia also could not be recovered in wind-tunnel experiments, even through several fruits covered with spores were placed in the wind tunnel and subjected to marked changes in RH and temp as well as wind speed. Wind-tunnel experiments were effective, however, in recovering *P. infestans* sporangia from diseased potato leaves, thus the experimental conditions were adequate for recovery of dry sporangia released in air.

Wind-blown rain collected from a height of 1.8-3.4 m (6-11 ft) in papaya orchards severely infected with *P. palmivora* frequently contained sporangia. No effort was made to quantify spore recovery but the number of sporangia was adequate to provide convincing evidence of this method of spore dispersal.

Rain-splash experiments.—Sporangia were released readily when artificial rain drops, falling from a height of 1.52 m (5 ft) struck a *P. palmivora* lesion on a papaya fruit. Upon impact with the lesion, individual drops split into many smaller droplets commonly referred to as splash-droplets (4). Splash-droplets on the Vaseline-coated slide left a crystal violet-stained outline of the original size of the droplet before evaporation, although slides were maintained at 100% RH to minimize evaporation before being counted. The greatest number of sporangia were found in splash-droplets of 801-1,600 μm diam, even though droplets of this size were the least common. Approximately half as many sporangia were recorded in the 401-800 μm splash-droplets. In comparison, only a relatively few were observed in smaller droplets. A few sporangia containing the crystal violet dye used in the artificial rain drops were found free of any detectable splash-droplet on the slide (Fig. 3). These sporangia were stained by the crystal violet dye but apparently they were liberated from the mycelial mat without being trapped in a splash-droplet.

The number of sporangia released in a splash-droplet of given size depended upon the size of the artificial rain

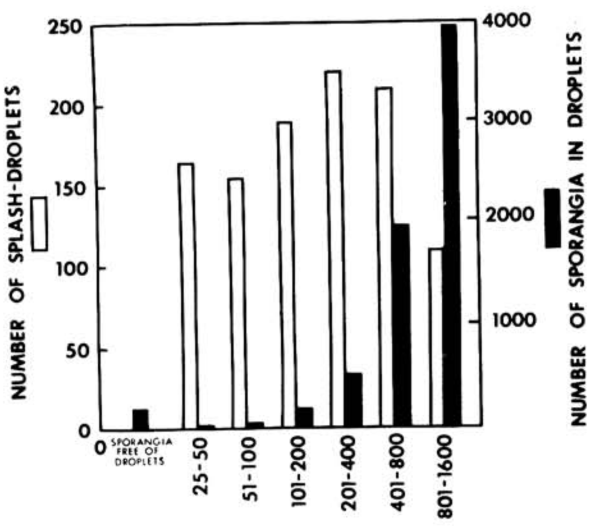

Fig. 3. Number of *Phytophthora palmivora* sporangia released from lesions on papaya fruits in relation to number and size of splash-droplets produced by impaction of artificial rain drops falling 1.52 m (5 ft). Data are total number of splash-droplets and sporangia recovered in five replicates; 10 drops containing crystal violet dye were released per replicate.

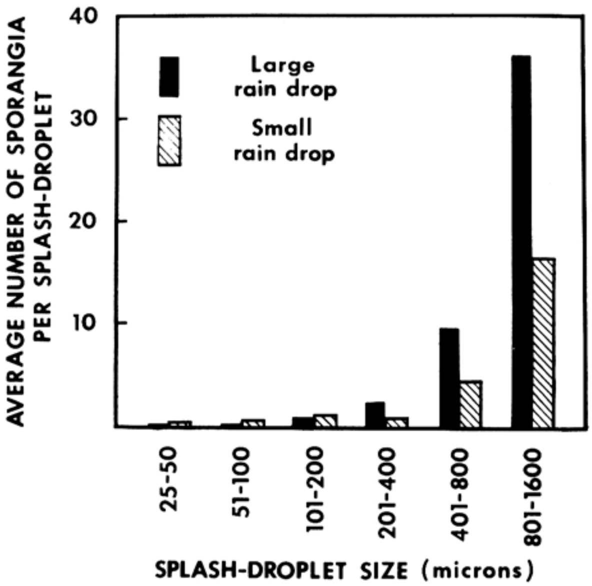

Fig. 4. Effect of size of rain drop on number of sporangia released in splash-droplets of any given size. Large and small rain drops were released from 18- and 22-gauge needles, respectively, from 1.52 m (5 ft) above a *Phytophthora palmivora* lesion on a papaya fruit.

drops. Splash-droplets produced from large drops released from an 18-gauge needle contained approximately twice as many sporangia as splash-droplets of the same size produced from small drops released from a 22-gauge needle (Fig. 4).

Survival of attached and detached sporangia.—Sporangia produced on the surface of papaya fruits at night survive drying conditions in the field during the following day, provided they remain attached to hyphae. Fifty percent of the sporangia collected early in the morning germinated within 5 h and approximately the same percentage germination occurred with spores removed from lesions late in the afternoon. However, detached sporangia subjected to RH of 90% or lower at 24 C, rapidly dehydrated and failed to germinate when placed in water. At 100% RH there was no decrease in percentage germination within the 8 min test period (Fig. 5A). Survival also is affected by temp. At 80% RH, an increase in temp was associated with a decrease in percentage germination (Fig. 5B).

DISCUSSION.—Unlike *P. infestans* (6), *P. palmivora* sporangia on papaya fruits are not released in moving air under drying conditions. Lesions on individual papaya fruits contain thousands of sporangia but all efforts have failed to liberate them by blowing air over sporulating lesions; whereas every method used with *P. infestans* sporulating on potato leaves has resulted in an abundant release of spores. Dry sporangia released into moving air are considered to account for long-distance spread of *P. infestans,* but sporangia carried in water dripping from leaves or splashed about in rain are thought to be the means of close-range dispersal (7, 8). The latter method,viz., release by impact of falling water drops, is considered to be the most important method for air-borne release of *P. palmivora* sporangia from fruit lesions in papaya orchards. In a pilot study, sporangia of *Phytophthora colocasiae* Rac., the causal agent of leaf blight of taro, *Colocasia esculenta* Schott, also were not released into moving air under drying conditions, but were readily released by rain-splashing (J. Hunter and R. Kunimoto, *unpublished*). Other aerial *Phytophthora* species need to be studied to determine if release of dry sporangia into air, as in the case of *P. infestans,* is the exception for this genus rather than the common method of spore release? It should be noted that only sporangia are readily released by rain drops falling on mycelial mats on papaya fruits. Chlamydospores appear to be deeply embedded in the mycelial mat and held more firmly to the hyphae, thus they rarely, if ever, become air-borne.

Papaya leaves tend to shield fruits from direct impact with falling rain drops, although some drops do fall

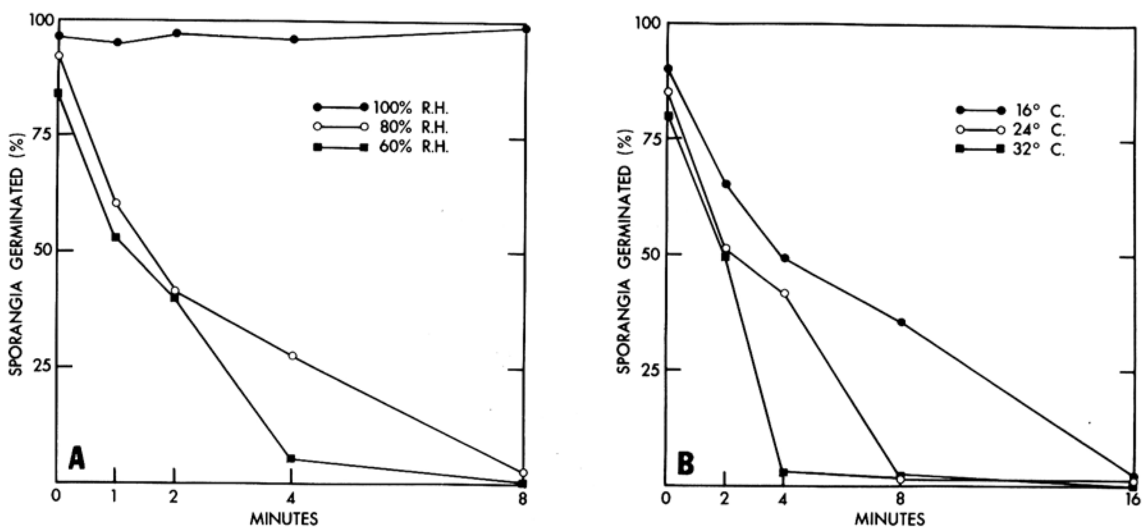

Fig. 5. Effect of relative humidity **(A)** and temperature **(B)** on survival of detached sporangia of *Phytophthora palmivora.*

through the canopy and make direct contact with fruits. However, rain drops caught on leaves subsequently fall onto fruit below. Although our splash experiments were conducted from a height of 1.52 m (5 ft), simple tests with drops falling only a few cm indicate that this distance is adequate to cause release of sporangia. This is in agreement with previous studies on spore dispersal by water dripping from vegetation (4). Rain drops also frequently are blown under the canopy and make direct contact with lesions on infected fruits. When the apical portion of trunks become infected, leaves shrivel and open the canopy to falling rain. Thus, there are several ways for rain drops to penetrate the canopy of trees and release sporangia of *P. palmivora* from lesions on papaya fruits.

Most of the sporangia are liberated in splash-droplets of 400 μm diam or larger. Such large droplets probably fall from the air very rapidly or are removed from the air by rain. However, large splash-droplets will fracture into smaller droplets upon impact with a solid surface. Moreover, under some conditions the size of splash-droplets are rapidly diminished by evaporation (3), hence sporangia initially liberated in large splash-droplets may ultimately become air-borne in very small droplets. In any case, some are immediately liberated in very small droplets or with only a film of water around them. Regardless of the size of the splash-droplet, presumably most sporangia released during heavy rain are quickly removed from the atmosphere. In Hawaii, however, intermittent passing showers and winds are very common, thus providing a mechanism for spore release by rain without subsequent removal from the atmosphere by continued rain. Furthermore, rain dripping from the canopy could liberate sporangia after a shower. Parenthetically, we should mention that our data from splash dispersal studies in still-air agree with Gregory et al. (4) who reported that, "droplets of diameters between 164 and 655 μ tended to travel further than either smaller or larger droplets."

Rain in the Puna district of Hawaii occurs most frequently in the early evening hours and continues intermittently throughout the night. Regardless of the occurrence of rain, RH usually reaches 100% for several hr each night throughout the year. Thus, although sporangia only survive a few min at RH below 100%, the high RH at night is conducive to survival for several hr. Sporangia liberated in water droplets immediately initiate indirect germination. Aragaki et al. (1) showed that 62% of the sporangia germinated indirectly in water within 10 min at 24 C. Doo (2) reported that zoospores could infect mature fruits within 15 min; i.e., zoospore suspensions washed-off fruits after 15 min exposure, infected fruits incubated at 100% RH. Inoculation experiments in our laboratory (J. Hunter and R. Kunimoto, *unpublished*) indicate that even surface sterilization of fruits after a few hr exposure to zoospore suspensions failed to prevent infection.

Because sporangia attached to mycelial mats on infected fruits can survive drying conditions during the day, sporangia released by night rains are viable and can germinate and infect papaya fruits before onset of adverse environmental conditions in the morning. Thus, rain-splash and wind-blown rain are not only feasible spore release and dispersal mechanisms, but actually insure ideal conditions for survival of this organism.

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