Basidiospore Discharge in Gymnosporangium

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ABSTRACT

Basidiospore discharge in *Gymnosporangium juniperi-virginianae, G. globosum,* and *G. clavipes* was studied under controlled conditions with a Kramer-Collins spore sampler. Telia were soaked in water and suspended over a cool-mist vaporizer in an ISCO E-1 growth chamber. Discharge began within a few hours and continued for 2-3 days. In *G. juniperi-virginianae* and *G. globosum* under alternating conditions of light and dark, discharge was periodic with peak maxima occurring in the dark whether soaking and initial period were in light or dark. Continuous dark resulted in a single peak after 6 hr; in continuous light, the pattern was similar but the peak was after 20 hr. In *G. globosum,* under alternating light-dark periods, discharge peaked in the first 12 hr irrespective of conditions; but on the 2nd and 3rd days, the pattern was similar to that in the other species. Phytopathology 61:951-953.

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The genus *Gymnosporangium* contains over 50 species with pycnial and aecial stages on Malaceae and a few other families, and telial stages on Juniperaceae, chiefly *Juniperus virginiana* L. The telium is remarkable in its capacity to expand greatly during spring rains, with the teliospores germinating immediately to produce basidiospores. This occurs in April and May in Kansas where three species are present on *J. virginiana: G. juniperi-virginianae* Schw. with alternate host *Malus,* and *G. globosum* Farl. and *G. clavipes* C. P. both with *Crataegus* as alternate hosts.

The development and germination of the teliospores of *G. juniperi-virginianae* have been studied thoroughly (1, 2, 4, 10, 12), but little is known of possible periodicity in basidiospore discharge. Only two reports are known of periodicity in basidiospore discharge in rusts. In *Puccinia malvacearum* (3), and *Cronartium ribicola* (11) discharge is periodic and nocturnal. This paper on basidiospore discharge in the three Kansas species of *Gymnosporangium* is based on studies made under controlled conditions.

MATERIALS AND METHODS.—Galls of *G. juniperi-virginianae, G. globosum,* and *G. clavipes* were collected in late April and May when they were mature enough to germinate. The mature galls could be stored in a refrigerator for 8 weeks without losing viability. All experiments were done within this time period.

To induce germination, galls were soaked in water for 30 min. The galls were enclosed in nylon net and suspended ca. 4 inches above the nozzle of a cool-mist vaporizer. Telia expanded rapidly under these conditions and remained so for 2-3 days. Six to eight large galls of *G. juniperi-virginianae* were sufficient to give a good spore sample; the numbers were doubled for *G. globosum,* and doubled-to-tripled for *G. clavipes.* The growth chamber was an ISCO E-1, operated at 21 C with 90% relative humidity. Alternating periods were 12-hr light:12-hr dark (LD 12:12), or dark-light (DL 12:12).

Spores were collected with two Kramer-Collins samplers (6) during four periods/hr of 6-8 min each. The samplers were placed on a shelf 25 cm above the galls, with the inlets of each sampler at least 20 cm from the flow stream of the vaporizer. One sampler was wired so that when its vacuum pump was started, the vaporizer motor was shut off. A sampling series consisted of 2- to 3-day runs.

RESULTS.—*Gymnosporangium juniperi-virginianae.*—In preliminary studies, 12 3-day series were run, the galls being placed in the chamber 4 hr before the dark period. Basidiospores began to be discharged during the first 2-3 hr, increased rapidly to a peak about 6 hr later, then rapidly declined (Fig. 1-A). Similar but smaller peaks occurred in the dark on the 2nd and 3rd days. These experiments indicated that conditions in the growth chamber were satisfactory for spore discharge for several days, and that discharge was periodic.

In subsequent studies, galls were soaked in water in the dark and placed in the chamber at the beginning of the dark period. Spore discharge began during the 2nd hr, increased steadily to a peak on the 5th hr, then declined rapidly (Fig. 1-B). Similar but smaller peaks occurred in the dark on the following 2 days (Fig. 1-B).

Galls, soaked in water in the light and placed in the chamber at the beginning of the photoperiod, began to discharge spores after 3 or 4 hr. Numbers increased gradually until the 10th hr, when there was a rapid increase to a climax after 1 hr in the dark (Fig. 1-C). Numbers were lowest during the light, with smaller but distinct peaks in the dark on the 2nd and 3rd days (Fig. 1-C).

Continuous dark (Fig. 1-D) or light (Fig. 1-E) resulted in a single peak of spore discharge after 8 hr in the dark and 20 hr in the light. Light during the first 12 hr thus retarded discharge, but there is no evidence that germination or spore formation is inhibited. There were as many spores on the slides in continuous light as on those in continuous dark.

Gymnosporangium globosum.—Thirty series were run: eight beginning in the light; four beginning in the

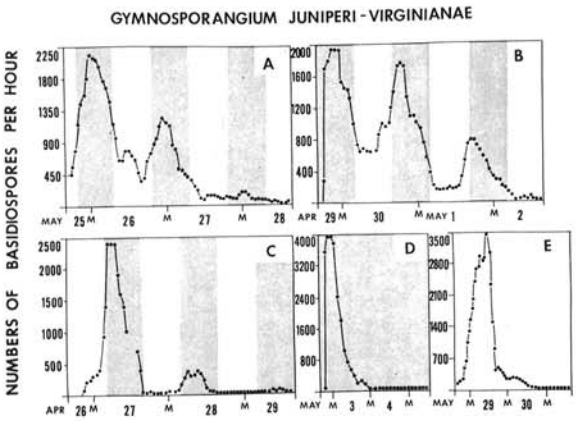

Fig. 1. Spore release patterns in *Gymnosporangium juniperi-virginianae*. Stippled areas indicate darkness. Sampling began at 1600. Photoperiod 12 hr in A, B, C. **A)** Photoperiod beginning 0800, 12 hr light:12 hr dark (LD 12:12). **B)** Photoperiod beginning 1600, DL 12:12. **C)** Photoperiod beginning 0400, LD 12:12. **D)** Continuous dark. **E)** Continuous light.

dark; eight in continuous dark; and ten in continuous light. Spore discharge was usually completed in 48 hr, but sometimes continued into the 3rd day. Under DL 12:12, the pattern was similar to *G. juniperi-virginianae* with peaks after 6-7 hr in the dark, and few-to-no spores in the light (Fig. 2-A). But when sampling began in the light LD 12:12, the first peak occurred after 8 hr, then declined sharply until the beginning of the dark period when spore numbers increased for 2 hr to a second peak, followed by a steady decline to zero in the light (Fig. 2-B). In the next dark period, discharge resumed for 7 hr and then ceased. Single discharge peaks occurred within 7-11 hr under continuous dark (Fig. 2-C) and continuous light (Fig. 2-D).

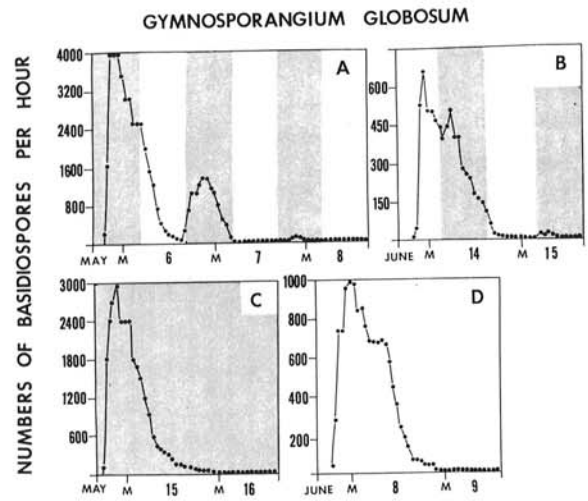

Fig. 2. Spore release patterns in *Gymnosporangium globosum*. Stippled areas indicate darkness. Sampling began at 1600. Photoperiod 12 hr in A and B. **A)** Photoperiod beginning 0400, 12 hr dark:12 hr light (DL 12:12). **B)** Photoperiod beginning 1600, LD 12:12. **C)** Continuous dark. **D)** Continuous light.

Gymnosporangium clavipes.—Basidiospore discharge occurred readily, but numbers were frequently too few for satisfactory counts. Under DL 12:12, peaks occurred during the 8th hr in the dark, while under LD 12:12, discharge gradually increased to peaks during the 17th and 18th hr. A single peak appeared after 8 hr in continuous dark and after 20 hr in continuous light. All data indicated that the discharge pattern was similar to that of *G. juniperi-virginianae*.

Discussion.—Moisture is clearly a limiting factor in the development and discharge of *Gymnosporangium* basidiospores. The gelatinous telial horns and similar gelatinous structures in other fungi are capable of absorbing water rapidly, thereby facilitating germination of the teliospores and the formation and discharge of the basidiospores within a few hr (9). Buller (1) found that in the tremellaceous fungi, *Dacrymyces* and *Calocera*, only 50 and 80 min, respectively, were required for maturation and discharge of basidiospores after soaking in water. He sprayed water on the dried telial horns of *G. juniperi-virginianae*, and basidiospore discharge followed within 6 hr (2). Parmelee (8) observed that when galls were placed in water, basidiospores were produced in such large numbers that water became yellow. In our experiments, galls of *G. juniperi-virginianae* soaked for 30 min and placed in the mist stream produced basidiospores after 2-3 hr, and continued to do so for at least 72 hr (Fig. 1).

Production of basidiospores is probably continuous in the field during periods of cool weather with frequent rains and high relative humidity. MacLachlan (7) reported that during a period of intermittent rain in May, basidiospores were released almost continuously for 60 hr. Reed & Crabill (10) thought it necessary to dry the telial horns for 24 hr for spore dissemination. Our studies indicate that discharge begins in the rain and continues as long as moisture is available, usually 3 days, but sometimes 4 days. Accidental desiccation in the chambers caused cessation of spore discharge, but discharge resumed as soon as the telia reabsorbed moisture.

Coons (4) found that basidiospore discharge in *G. juniperi-virginianae* could take place anytime during the day or night, and concluded that light did not affect discharge. Our work supports his observations but not his conclusion, as light does have an initial retarding effect when discharge begins in the light in *G. juniperi-virginianae* and *G. clavipes*. In *G. globosum* this initial effect is less pronounced. Maximum numbers in all three species on the 2nd and 3rd days were always in the dark.

All three species exhibited a distinct, periodic pattern of spore discharge under DL 12:12, with characteristic peaks in the dark on the 1st, 2nd, and often the 3rd day (Fig. 1-B, 2-A). Under continuous conditions (Fig. 1-D, E, 2-C, D), only one peak was found, suggesting that most teliospores germinated during the first 24 hr. Light retarded spore discharge, and perhaps also teliospore germination, when the initial period was in the

light (Fig. 1-A, C, E) in *G. juniperi-virginianae* and *G. clavipes,* but this effect was much less in *G. globosum* (Fig. 2-B). Darkness usually stimulated discharge; on many days, as on 28, 29 April (Fig. 1-C), increases began after 1 or 2 hr in the dark, reached a peak in the next few hr, then subsided. On some days, however, as on 30 April to 1 May (Fig. 1-B), the increase began in the light 4 or 5 hr before the dark period, and continued into the dark. This suggested an endogenous, circadian rhythm, but the experiments under continuous conditions (Fig. 1-D, E, 2-C, D) gave no supporting evidence. *Sordaria verruculosa* has a similar pattern with discharge stimulated by the change from dark to light, but with a delay of 8 to 12 hr before the response (5). The spore release pattern in *G. juniperi-virginianae* in the light (Fig. 1-B) indicates that the same mechanism may be operating here.

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