Quality of Light Required for Sporulation by Leptosphaerulina

K. T. Leath

Research Plant Pathologist, U.S. Regional Pasture Research Laboratory, Crops Research Division, ARS, USDA, University Park, Pennsylvania 16802.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 10 August 1970.

ABSTRACT

The effect of light quality on the sporulation of *Leptosphaerulina briosiana* was investigated. Cultures grown under cool-white fluorescent lamps did not sporulate when light of wavelengths shorter than 340 nanometers (nm) was excluded by filters. The generality of this requirement within the genus was determined by growing 1 isolate of *L. americana,* 6 of *L. arachidicola,* 1 of *L. argentinensis,* 2 of *L. australis,* 49 of *L. briosiana,* and 2 of *L. trifolii* under cool-white, fluorescent light, with half of each culture covered with plexiglass to exclude light of wavelengths shorter than 340 nm. One isolate each of *L. arachidicola* and *L. briosiana* sporulated normally throughout the culture. All other isolates sporulated normally in the culture half that received unfiltered light, but formed black, sterile ascostromata in the culture half from which light below 340 nm was excluded. Formation of sterile ascostromata also occurred in cultures grown in total darkness.

The quality of light essential for sporulation was more precisely defined for two isolates of *L. briosiana.* When cultures were irradiated with narrow bands of light throughout the range of 290-340 nm, light of 310-330 nm inclusive stimulated ascospore production. No requirement for light outside this range was observed for any of the fungi tested. Phytopathology 61:70-72.

Additional key words: ascospores, fungus, photo-induction, irradiation, near-ultraviolet light.

The failure of *Leptosphaerulina briosiana* to produce ascospores in a lighted growth chamber prompted this investigation of the effect of light quality on sporulation. It is known that light is necessary for reproduction in many ascomycetes, and that either quality, intensity, or a light-temperature interaction may be critical (1). *Pleospora herbarum,* a fungus closely related to *Leptosphaerulina,* requires near-ultraviolet light for sexual reproduction (5, 6). Thomas (10), however, reported that visible light stimulated *L. australis* to produce ascospores.

This paper reports a broad requirement for near-ultraviolet light by a diverse group of isolates, and more precisely defines the quality of light essential for sporulation of two isolates of *L. briosiana.* A portion of this study was previously reported (7).

MATERIALS AND METHODS.—All reference to species is as proposed by Graham & Luttrell (3) for the genus *Leptosphaerulina* McAlp. Cultures were grown on V-8 juice agar (9) in plastic petri dishes at 20 C, and were illuminated with approx 400 ft-c of cool-white fluorescent light unless otherwise specified.

The effect of light quality on spore production was investigated by growing cultures under various filters. The relative light transmittal property of each filter was determined in a spectrophotometer at 10-nm intervals throughout the spectral range of 260-600 nm. The filters used, along with the shortest wavelength in nanometers transmitted by each, are as follows: soft glass, 340; borosilicate glass, 275; thermal-barrier plastic, 340; petri-dish plastic, 285; and the photographic filters, 340. The photographic filters had peak transmission properties at one of the following wavelengths: 425, 475, 510, or 550 nm.

Generality of the requirement for near-ultraviolet light within the genus *Leptosphaerulina* was investigated by placing plexiglass over half of each culture to exclude light of wavelengths shorter than 340 nm. Alternating 12-hr dark and light periods were used during the first trial and continuous light during the second trial. A total of 61 isolates was tested, including one of *L. americana* (Ell. & Ev.), six of *L. arachidicola* Yen, Chen & Huang, one of *L. argentinensis* (Speg.), two of *L. australis* McAlp., 49 of *L. briosiana* (Poll.), and two of *L. trifolii* (Rost.) Petr. Each isolate was replicated 3 times in at least two experiments. Petri dish lids were examined for ejected ascospores under ×25 magnification after 72 hr of growth.

The quality of light essential for sporulation was determined for two isolates of *L. briosiana.* Strips were cut from ascospore-seeded agar and placed aseptically against the rear side of a silica, spectrophotometer absorbance cell having a light path of 1 cm (Fig. 1-A, B). This facilitated the use of a Beckman DU spectrophotometer equipped with a hydrogen lamp as the monochromatic light source for irradiation of cultures. The temp within the cell was maintained at 22 C during the light treatment. Peak wavelengths used were 290, 300, 310, 320, 330, and 340 nm. Total irradiation time at each peak wavelength was 18 hr composed of a 6-hr period after 24, 48, and 72 hr of growth. This photoperiod regime was adopted after shorter exposures failed to induce sporulation. All irradiations were made with the exit slit opened to 2 mm to provide max intensity. This increased band-widths, which was undesirable, but a narrower slit width reduced intensity to a level insufficient to stimulate sporulation at any of the wavelengths tested. Cultures were kept in total darkness at 20 C except during irradiation periods. All spectrophotometric determinations were performed 3 times. The glass surface of the absorbance cell opposite to the culture was examined for ejected ascospores after the third light period.

RESULTS.—All of the filter materials tested had ap-

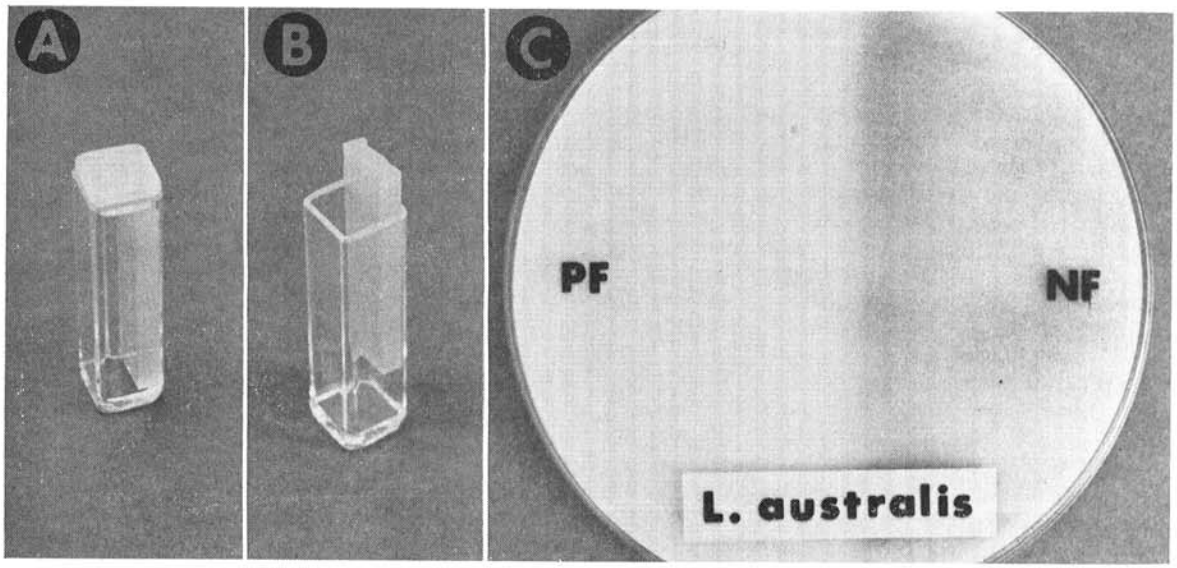

Fig. 1. Spectrophotometer absorption cell **A)** closed with seeded agar strip inside; **B)** opened with agar strip partially inserted; **C)** ascospores deposited on a petri-dish lid by *Leptosphaerulina australis* grown under cool-white fluorescent light. One half (PF) was covered with plexiglass to exclude light shorter than 340 nm; the other half (NF) was not covered by a filter.

prox equal transmittal properties for light of wavelengths above 340 nm. *Leptosphaerulina briosiana* sporulated normally under all filter materials that passed light shorter than 340 nm; conversely, the fungus did not sporulate normally under any filter that did not transmit light shorter than 340 nm. No filter passed much light shorter than 300 nm; therefore, the light between 300 and 340 nm stimulated sporulation. The plastic thermal barrier material, plexiglass, and the photographic filters did not transmit light shorter than 340 nm. Petri-dish plastic and borosilicate glass passed light shorter than 340 nm.

Of the 61 isolates screened for the near-ultraviolet light stimulus, 59 sporulated profusely in that half of the culture not covered by plexiglass, but produced only black, sterile ascostromata under the plexiglass. One isolate of *L. arachidicola* and one of *L. briosiana* produced fertile ascocarps throughout the culture and were not affected by the light treatments. No isolate required light other than that in the near-ultraviolet portion of the spectrum. Occasionally, a culture growing under a plexiglass filter produced a few spores, but even these cultures showed a marked increase in sporulation from the near-ultraviolet light stimulus as shown in Fig. 1-C for *L. australis*.

Two isolates of *L. briosiana* responded similarly to irradiation with monochromatic light of 290-340 nm. Table 1 shows the development of ascocarps, asci, and ascospores in response to differences in light quality. The production of mature, viable ascospores occurred only when cultures were irradiated with light of 310- to 330-nm peak wavelengths. Cultures that received irradiation at 340 nm were indistinguishable from cultures grown in the dark. Although irradiation of cultures with light of 310 nm resulted in ascospore production, these spores were not ejected as were spores produced at 320 and 330 nm. The ejection of spores from the 310-nm treatment did occur, however, if the cultures were subsequently exposed to approx 50 ft-c of cool white fluorescent light.

DISCUSSION.—The requirement of light for sporulation by *Leptosphaerulina* was first noted by Jones (4). Graham & Luttrell (3) observed that, although most isolates of *Leptosphaerulina* needed light for sporulation, others sporulated profusely in the dark. Ascospores were produced by *L. briosiana* cultures in a greenhouse only when sunlight was supplemented with fluorescent light (8), but the effect of light quality on sexual reproduction in *Leptosphaerulina* has received scant attention. Thomas (10) reported that ascospore production by *L. australis* was stimulated by visible

TABLE 1. Effect of light quality on the sexual reproduction of *Leptosphaerulina briosiana*. Irradiation consisted of a 6-hr photoperiod after 24, 48, and 72 hr of growth. Observations were made after 78 hr of growth

Light wavelength	Ascocarps	Asci	Ascospores	Ascospores ejected
nm				
290	Normal	Few; small	Few; atypical	None
300	Normal	Few; small	Few; immature	None
310	Normal	Many; normal	Many; mature	Few
320	Normal	Many; normal	Many; mature	Many
330	Normal	Many; normal	Many; mature	Many
340	Sterile	None	None	None
Dark	Sterile	None	None	None

light, but that *L. trifolii* required light shorter than 400 nm for sporulation. Trione et al. (11) extracted a sporogenic substance from both *L. australis* and *L. trifolii* that absorbed near-ultraviolet light.

The light-induced sporulation found for all but two of the isolates tested agrees with earlier observations (3, 8) on the cultural requirements of *Leptosphaerulina*. The lack of response to visible light is quite similar to the reported behavior of *P. herbarum* (6). The light factors of wavelength, duration, and intensity, complexed with the biological factors of culture age and isolate variation and with environmental factors of culture media, temp, and undoubtedly others, make comparison with other results difficult.

The general area of the spectrum that stimulated perithecial initials in *P. herbarum* (5) is the same area that stimulated perithecial, ascus, and ascospore formation in *L. briosiana*. Leach & Trione (6) found 290 nm to be the most effective wavelength for inducing perithecial initials in *P. herbarum* under their test conditions. They also reported an unexplained shoulder that occurred at 310 nm in the action spectrum curve for this fungus. It is impossible to state from the results presented here that only the wavelengths from 310-330 nm stimulate sexual reproduction in *L. briosiana*. If photoperiods and intensities were varied, the fungus might respond to other wavelengths. Dosages of light energy necessary for perithecial initiation as well as perithecial inhibition in *P. herbarum* were less at shorter wavelengths (5). The stimulation of sexual reproduction in *L. australis* by irradiation with visible light (10) was not duplicated in these tests, but test conditions varied greatly. It was suggested by Leach (5) that wavelengths longer than 400 nm might induce sporulation in *P. herbarum* if intensities were increased. Since in this study energy levels were not known, comparisons of these results on this basis with earlier reports could not be made.

The 310- to 330-nm portion of the spectrum that induced sporulation of *L. briosiana* spans the spectral absorption range of the P-310 sporogen extracted from *L. australis* and *L. trifolii* (11), and it is entirely possible that this or a similar, sporogenic substance is involved in the sexual reproduction of *L. briosiana*.

Many steps make up the sexual reproductive process of a fungus, e.g., perithecial initiation, ascus and ascospore formation, and spore ejection. These individual steps may have different light requirements. Much more definitive investigation is needed before specific, sporulative requirements for *L. briosiana* can be categorized. These results do, however, provide a working knowledge for use in the production of inoculum.

The role of light in the natural survival and reproduction of pathogenic isolates of *Leptosphaerulina* is not known. Near-ultraviolet light reaches the earth's surface (2) and may have ecological significance, or the light-induced sporulation common to cultures on prepared media may have no relevance to the natural life cycle.

LITERATURE CITED

1. Carlile, M. J. 1965. The photobiology of fungi. Annu. Rev. Plant Physiol. 16:175-202.
2. Gates, D. M. 1966. Spectral distribution of solar radiation at the earth's surface. Science 151:523-529.
3. Graham, J. H., & E. S. Luttrell. 1961. Species of Leptosphaerulina on forage plants. Phytopathology 51:680-693.
4. Jones, F. R. 1916. Pleosphaerulina on alfalfa. Phytopathology 6:299-300.
5. Leach, C. M. 1963. The qualitative and quantitative relationship of monochromatic radiation to sexual and asexual reproduction of Pleospora herbarum. Mycologia 55:151-163.
6. Leach, C. M., & E. J. Trione. 1966. Action spectra for light-induced sporulation of the fungi Pleospora herbarum and Alternaria dauci. Photochem. Photobiol. 5:621-630.
7. Leath, K. T. 1969. Light requirement for sporulation in Leptosphaerulina spp. Phytopathology 59:400 (Abstr.).
8. Martinez, E. S., & E. W. Hanson. 1963. Factors affecting growth sporulation, pathogenicity, and dissemination of Leptosphaerulina briosiana. Phytopathology 53:938-945.
9. Miller, P. M. 1955. V-8 juice as a general purpose medium for fungi and bacteria. Phytopathology 45: 461-462.
10. Thomas, C. E. 1966. Studies on the effects of light and other factors on the growth and sporulation of selected Leptosphaerulina species. Ph.D. Thesis, Clemson Univ. 96 p.
11. Trione, E. J., C. M. Leach, & J. T. Mutch. 1966. Sporogenic substances isolated from fungi. Nature 212:163-164.