The Relationship of Sporulation to Photosynthesis in Some Obligatory and Facultative Parasites

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ABSTRACT

Exposure of host plants to light conditions favoring photosynthesis, or blocking this process by chemical means, revealed a causal relation between photosynthesis and sporulation of fungal parasites.

Extending the photoperiod from 6 to 48 hr before exposing *Pseudoperonospora cubensis* on cucumbers to dark and moist conditions resulted in an increase in subsequent sporulation. A similar increase in sporulation was also associated with increases in light intensity and temp of incubation, as well as with illumination of the plants with the light spectra most effective for photosynthesis. In *Uromyces phaseoli* on beans and in *Phytophthora infestans* on potatoes, the highest sporulation followed the longest photoperiods to which the plants had been exposed.

When dichlorophenyl-dimethylurea (DCMU) was applied to suppress photosynthesis of tobacco and cucumbers infected with *Peronospora tabacina* and *P. cubensis,* respectively, its effect on the subsequent sporulation depended on whether it had been applied before or after exposure of the plants to a light period. In the first case, few or no spores were formed; in the second case there was normal sporulation. When DCMU was applied at various dates after inoculation to beans, potatoes, and tomatoes infected with *U. phaseoli, P. infestans,* and *Alternaria porri* f. sp. *solani,* respectively, the lowest sporulation of the first two parasites was associated with the earliest date of DCMU application, while the reverse was true for the last species. It was concluded that photosynthesis is an essential condition for sporulation in obligatory parasites, but varies in its effect in facultative parasites. Phytopathology 60:1600-1604.

Additional key words: reproduction, host-parasite relationships.

The relationship of sporulation to photoperiod was demonstrated in downy mildews of hop, onion, grape, lettuce (14), cucumber (7), tobacco (2, 12), and in *Phytophthora phaseoli* on lima beans (6). Since in most of these cases actual sporulation started after termination of the light period and occurred in darkness (2, 12), the previous effect of light seems to have been indirect; however, the relationship of sporulation to photosynthesis of the host plants has never been proved. There have been reports of a few cases in which light was not necessary for sporulation in vivo; e.g., for *Helminthosporium gramineum* on barley (5) and for *Alternaria porri* f. sp. *solani* on potatoes and tomatoes (10).

The aim of this study was to determine the relationship between photosynthesis and sporulation in some obligatory and facultative parasites. This was accomplished by exposing the infected hosts at the presporulation stage to light conditions known to affect photosynthesis, or by chemically blocking photosynthesis in plants exposed to light. In order to simulate natural conditions, the experimental plants were exposed to light under atmospheric conditions too dry for sporulation. Although the effect of these treatments was evaluated according to the level of the subsequent sporulation, this was induced by a standard method and was not an experimental variable.

MATERIALS AND METHODS.—Most of the work was done with *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow on cucumbers (*Cucumis sativus* L. 'Bet-Alpha'). Additional tests were made with *Peronospora tabacina* Adam on Michal tobacco (*Nicotiana tabacum* L.), *Uromyces phaseoli* (Reb.) Wint. var. *typica* Arthur on Bulgarian beans (*Phaseolus vulgaris* L.), *Phytophthora infestans* D By on Up-to-Date potatoes (*Solanum tuberosum* L.), and *Alternaria porri* (ELL.) Neerg. f. sp. *solani* (E. & M.) on Marmande tomatoes (*Lycopersicon esculentum* Mill.).

Prior to the experimental period, all plants had been grown in an air-conditioned greenhouse in 0.3-kg pots (tomatoes in 2-kg pots) filled with a 2:2:1:1 sterilized mixture of sandy loam, peat, sand, and vermiculite.

Beans, cucumbers, potatoes, tobacco, and tomatoes were inoculated after 2, 3, 6, 8, and 9 weeks of growth, respectively, at which time beans and cucumbers had two true leaves, tobacco, five to six leaves, and potatoes and tomatoes were well-developed plants on which only the 10 oldest leaves were infected.

Each plant species except tomatoes was inoculated by its respective pathogen with Schein's inoculator (11) with 800 ± 10% sporangia or conidia/4 cm^2 target on the lower side of each leaf. Inoculation of tomatoes with *A. porri* f. sp. *solani* was accomplished by spraying plants with 200 conidia/target.

The inoculated plants were kept for 24 hr in a dark, moist chamber at 20 C. They were then moved into growth chambers maintained at 20 ± 1 C, 50-70% relative humidity, and illuminated on a 12-hr photoperiod regime with Gro-Lux lamps supplying, according to Epply Perheliometer, 3100 μw/cm^2 (620 ft-c) at plant level. This stage of incubation varied from 1 to 8 days in accordance with the specific demands of a

given experiment. It ended with "starving" of the test plants in darkness for 12 hr (cucumbers, beans, and potatoes) or 24 hr (tobacco plants).

The next procedure consisted of the actual experimental treatments; viz., exposure of the plants to various photoperiods, light intensities, and spectra. In most cases, experimental treatments were carried out before plants were placed for 24 hr in 20-C moist chambers in darkness, during which time spores actually formed. In the case of bean rust, the continuous sporulation of *U. phaseoli* made the moisture treatment unnecessary. In a few cases, the light treatments were applied to plants already in moist chambers.

Inhibition of photosynthesis in the illuminated plants was accomplished by spraying with 3,3,4-dichlorophenyl-1,1-dimethylurea (DCMU), which blocks the Hill reaction in the photosynthetic process (13) without interfering with other biological processes (1). In cucumbers and beans, DCMU at a concn of 10^{-5} M was found to inhibit photosynthesis; in tobacco, potato, and tomato a concn of 10^{-4} M was needed.

In all cases, sporulation was evaluated by cutting a 4-cm-diam disc from each test leaf. The discs were shaken for 10 min in 2 ml FAA (Formalin, acetic acid, alcohol, 5:5:90, v/v), and the removed spores were counted with the help of a cytometer.

Analysis of results was done with the aid of a CDC 3600 computer using either the one-way multiple range test, the two-way analysis of variance for factorial design, or the stepwise regression test (3).

RESULTS.—*The effect of light during the moist period.*—The effects on sporulation of 24-hr constant light, constant darkness, and 12-hr photoperiods beginning with the light period were tested at 20 and 25 C with *P. cubensis* on cucumbers. Table 1 shows that little sporulation occurred under constant light, an intermediate amt in darkness, and abundant sporulation in photoperiods applied either at a constant temp of 20 C or at thermoperiods starting with the first 12 hr at 25 C. There were significant differences at the 95% level between these treatments.

In an additional test (8 replicates), 252×10^3 sporangia/leaf were produced on plants exposed to a 12-hr photoperiod starting with light, and significantly (99%) exceeded the number of sporangia produced on plants exposed to a photoperiod starting with darkness (103×10^3). In a similar test performed with *P. tabacina* on tobacco (6 replicates) and *P. infestans* on potato (8 replicates) exposed for 24 hr to continuous light, continuous darkness, and 12-hr light and dark periods, the number of spores or sporangia produced per leaf was 2.2×10^3, 75.2×10^3, 660.8×10^3 and 2.7×10^3, 35.3×10^3 and 115.4×10^3, respectively.

The effect of light applied before the moist period.—With *P. cubensis* on cucumbers, extending the light period from 6 to 48 hr before placing the plants in dark

TABLE 1. The effect of different combinations of two temp and three light conditions on sporulation of *Pseudoperonospora cubensis* in cucumbers enclosed in moist chambers for 24 hr

Temp (C)		Spores/lesion[a] (in thousands) Plants held in:		
First 12-hr period	Second 12-hr period	Constant light	Constant darkness	Light-dark photoperiod
20	20	8.90	255.30	546.66
25	25	9.40	250.92	328.26
20	25	5.92	131.76	280.00
25	20	62.60	255.64	542.46

[a] Sporangia were removed from plants exposed to two consecutive 12-hr temperature periods under light conditions of either constant light, constant darkness, or light-dark photoperiod. Average of eight plants/treatment.

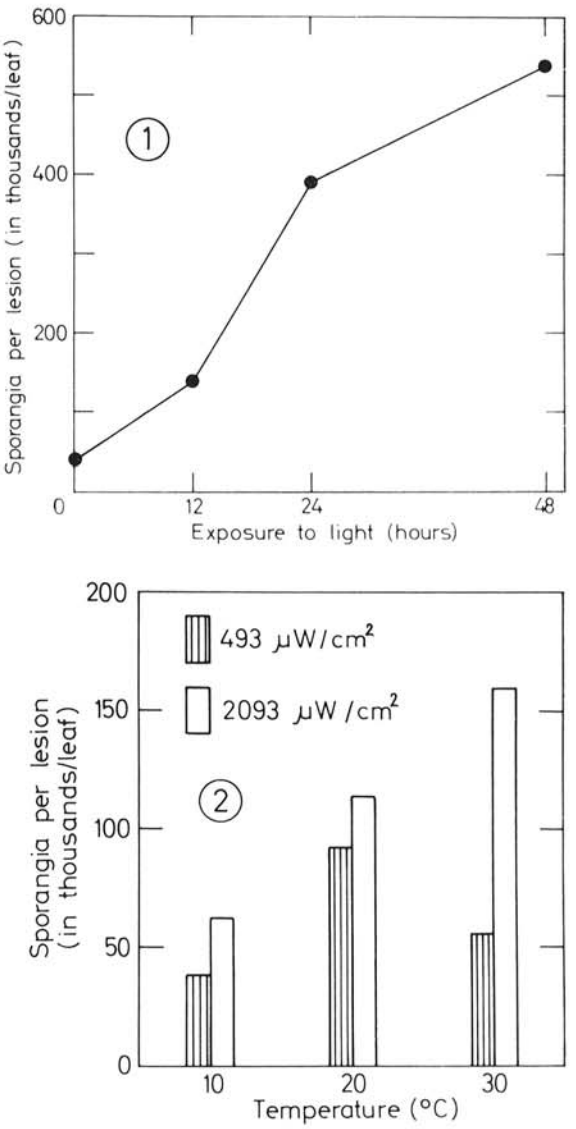

Fig. 1-2. **1)** The predisposing effect of the length of a single light period on the subsequent sporulating capacity of *Pseudoperonospora cubensis* on cucumbers (average of 8 plants/treatment). **2)** The predisposing effect of two light and three temp on the subsequent sporulating capacity of *Pseudoperonospora cubensis* on cucumbers (average of 8 plants/treatment).

moist chambers resulted in an increase of sporulation which was highly significant (99%) for all treatments (Fig. 1). In the following tests, all plants were exposed to a standard 12-hr photoperiod, but at light intensities of 493 μW/cm² and 2093 μw/cm² (110 and 620 ft-c, respectively), maintained at temp of either 10, 20, or 30 C. An increase in light intensity or in temp resulted in a significant (95%) increase in sporulation except on plants kept at the low light intensity at 30 C, where comparatively few sporangia were produced (Fig. 2).

The effect of light spectra on sporulation of *P. cubensis* on cucumbers was tested in two ways. In the first test (eight replicates), plants previously cultivated under standard conditions were exposed to a single 12-hr period of red, green, or blue light. The different colors of light were achieved by covering the light source with celluloid filters with transmission peaks of 400-480, 520, and 650 μM, respectively. According to Epply Perheliometer, a uniform light intensity of 775 μw/cm² of each light color was obtained at the plant level by proper adjustment of the distance between the plants and the light source.

The application of blue, green, and red light resulted in 112×10^3, 56×10^3, and 168×10^3 sporangia/leaf. No significant differences were found between the effects of blue and red light, both of which exceeded significantly (95%) the results obtained with green light.

In the second test, the infected plants were cultivated for 5 days under 12-hr blue, green, or red photoperiods. The light spectra were obtained from General Electric lamps and measured with an ISCO spectroradiograph. The highest and lowest sporulation followed the red and the green photoperiods, respectively. Significant differences in the 95% level were found between each treatment. In Fig. 3, data for sporulation, light spectra, and intensities are presented. This figure also presents the photosynthetic activity of wheat in different light spectra, as reported by Hoover (4). A similarity exists between his data on photosynthesis and our data on sporulation.

The effect on sporulation of different photoperiods starting 1 day after inoculation was tested for 8 days in *U. phaseoli* on beans and for 4 days in *P. infestans* on potatoes. In both cases, an increase in sporulation followed each prolongation of the daily light periods. Significant differences (95%) were found between all treatments with the exception of between the 12- and 24-hr light periods in the case of *U. phaseoli* on beans (Fig. 4).

Suppression of the photosynthetic effect of light by DCMU.—Infected cucumbers and tobacco were sprayed with DCMU either before or after their exposure to a 12-hr light period and then kept in a moist chamber for sporulation. Other plants were similarly treated while already in moist chambers maintained either in light or in darkness. In a 12-replicated test of *P. cubensis* on cucumbers, control plants and those sprayed with DCMU before exposure to light produced 248.7×10^3 and 52.5×10^3 sporangia/leaf, respectively; i.e., the DCMU treatment decreased the level of sporulation by 78.9%. In similarly treated tobacco plants infected with *P. tabacina* (six replicates), no spores were found on DCMU-treated plants, as opposed to abundant sporulation on the untreated plants. When both hosts were sprayed with DCMU, after previous exposure to light and just before exposure to dark and moist conditions, sporangia were formed on both treated and untreated plants. Application of DCMU was associated with an 11% decrease in sporu-

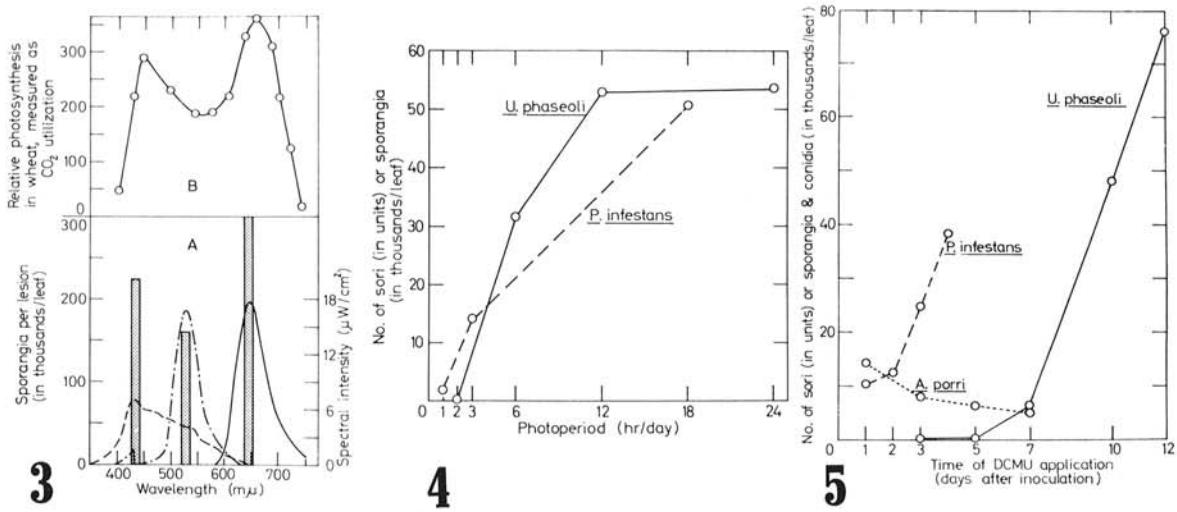

Fig. 3-5. **3)** Sporulation of *Pseudoperonospora cubensis* on cucumbers previously incubated for 5 days under 12-hr blue, green, or red photoperiods (average of 8 plants/treatment) (A); and photosynthetic activity of wheat cultivated under various light spectra, as presented by Hoover (4) (B). **4)** The effect of different daily photoperiods on the subsequent sporulation of *Uromyces phaseoli* on beans (evaluated after 8 days of incubation) and of *Phytophthora infestans* on potatoes (evaluated after 4 days of incubation) (average of 4 plants/treatment). **5)** The effect of dichlorophenyldimethylurea (DCMU) applied at various times after inoculation on the sporulation of *Phytophthora infestans* on potato, *Uromyces phaseoli* on bean, and *Alternaria porri* f. sp. *solani* on tomato (average of 4 plants/treatment).

lation, but this difference was not significant. Similarly treated plants exposed to moist conditions in light produced a negligible amount of spores whether or not they were sprayed with DCMU. Thus, application of DCMU to plants in moist chambers did not change the normal patterns of sporulation or that resulting from the inhibitory effect of light.

In experiments with *U. phaseoli* on beans, *P. infestans* on potatoes, and *A. porri* f. sp. *solani* on tomatoes, DCMU was applied at various times after inoculation. With *U. phaseoli* on beans, and to a lesser degree with *P. infestans* on potatoes, the greatest decrease in the subsequent sporulation was associated with the earliest application of DCMU. In contrast, early application of DCMU resulted in the highest amt of spores produced with *A. porri* f. sp. *solani* on tomatoes. In most cases, the difference between adjacent treatments was significant at the 95% level (Fig. 5).

DISCUSSION.—Constant light applied during the moist period inhibited sporulation of *P. cubensis* in cucumbers, *P. tabacina* in tobacco, and *P. infestans* in potato, while the same pathogens sporulated well in darkness (Table 1), as reported by Yarwood (14) and Cruickshank (2). This seems to be a direct effect of light and of darkness on sporulation, the mechanism of which was not investigated in this study. However, we would like to stress that the highest sporulation was associated with illumination of the plants kept in moist chambers for 12 hr, followed by a similar period of darkness. Since this result was obtained in spite of the inhibitory effect of light on sporulation, a second effect of light is involved in this case: it acts through the host or, as explained later, through photosynthesis.

With the same diseases, light applied before exposure of the infected plants to moist conditions was an essential condition for the subsequent sporulation in moisture. In these cases, light is therefore a prerequisite. As indirectly proved by the application of light treatments, the more a given variation suited the photosynthetic activity of the host plant, the higher was the subsequent level of sporulation (Fig. 1-4).

The association of photoperiods and light intensities with photosynthesis is well known. In all cases, light has the most marked effect under reasonable high temp conditions; however, in an experiment which proved a similar trend in relation to sporulation, only the high light intensity resulted in higher sporulation at 30 C than at 20 or 10 C, while the low light intensity was associated with the highest sporulation at 20 C (Fig. 2). It is assumed that the high rate of respiration at 30 C decreased the net level of assimilates produced at this temp under low light intensity, which in turn was reflected in sporulation. In another experiment, the levels of sporulation obtained after illumination of the infected plants with blue, green, or red lights matched the effects of these spectra on photosynthesis (Fig. 3).

The final proof that light does affect sporulation through photosynthesis was obtained by application of DCMU, which, by inhibiting the Hill reaction (13), stops the accumulation of assimilates. With the exception of *A. porri* f. sp. *solani* on tomatoes, DCMU treatment decreased the subsequent sporulation to a very low level, but usually did not inhibit it completely (Fig. 5). This lack of full inhibition may be attributed either to a somewhat delayed action of DCMU in stopping photosynthesis, or to the presence of previously accumulated assimilates.

It seems that the effect of photosynthesis on sporulation depends on the nutritional requirements of the parasite. In the case of obligatory parasites such as downy mildews and rust, inhibition of photosynthesis resulted in a most striking decrease in sporulation. In the case of a facultative parasite such as *P. infestans*, the effect was somewhat less pronounced (Fig. 5).

A completely opposite effect of photosynthesis on sporulation was found in the case of *A. porri* f. sp. *solani* in tomatoes. This is a rather weak and often a wound parasite (9) which sporulates after its vegetative development has ceased (10) and reaches its max sporulating potential on desiccated foliage (8). This characteristic of *A. porri* f. sp. *solani* explains why the retardation of normal development of tomatoes, which followed the inhibition of their photosynthetic activity, resulted in the highest level of sporulation.

The prerequisite of light for subsequent sporulation thus seems to be associated with the nutritional preference of the parasite. It is most prominent in obligatory parasites and loses its importance or may even reduce sporulation when a fungus with increasing saprophytic properties is able to utilize dead tissue for its nutrition.

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