Photomorphogenically Defined Light and Resistance of *Poa pratensis* to *Drechslera sorokiniana*¹

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ABSTRACT

Photomorphogenic light definitions were derived by mathematical determination of the estimated phytochrome photoequilibrium for each light treatment spectrum. A wide range of photomorphogenic light treatments represented by spectra with estimated phytochrome photoequilibria of 0.45, 0.54, 0.60, 0.67, and 0.71 was utilized to determine the influence of photomorphogenically defined light on resistance of *Poa pratensis* L. to pathogenesis by *Drechslera sorokiniana*. Accurate resolution of *D. sorokiniana* leaf spot development required evaluation of separate leaf ages due to the sequential appearance, development, and senescence of *P. pratensis* leaves. Disease development (all light treatments) was greatest on leaf 4 (oldest, postmature) followed by leaf 1 (youngest, premature). Low levels of disease occurred on leaves 2 and 3 (mature). Photomorphogenic light defined by estimated phytochrome photoequilibria greater than 0.60 (natural light = 0.60) was most disease promotive on leaf 1. Conversely, photomorphogenic light defined by estimated phytochrome photoequilibria of less than 0.60 was most disease promotive on leaf 4. These responses indicate that inherent resistance or susceptibility expressed by *P. pratensis* to pathogenesis by *D. sorokiniana* is regulated in part by leaf age (developmental senescent stage) and by photomorphogenically defined light quality. A hypothesis is presented and discussed which integrates and speculates on these observations with respect to the literature.

The fungal pathogen *Drechslera sorokiniana* (Sacc.) Subram. and Jain (*Helminthosporium sativum* P.K.&B.) infects the leaves, stems, and roots of numerous grass and cereal species (11, 28). The various diseases are chronic throughout the growing season on monocultures of *Poa pratensis* L. Leaf spot is the predominant disease on *P. pratensis*, and expression of leaf spot symptoms changes with seasonal environmental conditions (18, 19). In spring and early summer, lesions are small with faint chlorotic halos. Leaf spot symptoms of midfall to early winter generally are characterized by more prominent lesions surrounded by enlarged chlorotic halos, which may be interconnected by chlorotic streaks. The most severe symptoms may include complete chlorosis or straw-colored blighting of infected leaves, typical of premature senescence. This apparent premature leaf senescence is promoted by the combined presence of the pathogen and fall environmental conditions, particularly on older leaves positioned beneath the canopy of younger green leaves.

Recent studies have established that photoperiod and light quality can influence pathogenesis of *D. sorokiniana* leaf spot on *P. pratensis* (18, 19). Under low light level experimental conditions, leaves exposed to “balanced” or OR¹-biased light spectra show increased disease with short (10-h) photoperiods and decreased disease with longer (14-h) photoperiods (19). These responses are strongly influenced by leaf age. Disease is promoted by short photoperiods on both the youngest and oldest leaves (depending on light spectral treatment), but the decrease in disease evident with long photoperiods occurs exclusively on older leaves. These photoperiodic effects (in response to balanced and OR-biased light) are canceled under B and FR (B + FR)-biased light spectra where disease increase on older leaves regardless of photoperiod (18). The disease increase under B + FR-biased light spectra occurs independently of any direct effect on the pathogen (19) and, therefore, represents a predisposing or conditioning of host leaves. These observations suggest that disease expression may be regulated by photomorphogenically related processes, which differentially influence leaf susceptibility or resistance in response to light spectral and/or photoperiod conditions.

Photomorphogenic (nonphotosynthetic) light spectral distinctions can now be resolved by utilizing φₜ, a spectrum-derived value that facilitates qualitative description (20, 27). Each resultant φₜ value is spectrum-unique and obtained through mathematical analysis utilizing well established phytochrome isomer (Pr = Pfr) photoconversion action spectra (14) and the photon flux density spectrum (280–800 nm) of each light source or treatment. This technique of defining light quality reveals that the B + FR-biased light spectrum, which promotes pathogenesis regardless of photoperiod, has a lower φₜ (0.53) than that of the balanced (0.66) or OR-biased (0.73) light spectra, both of which are associated with an increase or decrease in disease, depending on photoperiod (19). FR-biased light spectra (low φₜ) are known to promote senescence, whereas R-biased spectra (high φₜ) retard senescence (1, 5, 7, 25). Within the natural or cultivated environments of *P. pratensis*, low level, low φₜ light conditions would be particularly prevalent under cloudy skies or within plant canopies (12). The inference that phytochrome is involved within these processes that respond to light quality and/or photoperiod by predisposing or conditioning *P. pratensis* susceptibility or resistance to *D. sorokiniana* leaf spot is justifiable and warrants further examination. The studies reported here were initiated to evaluate further this hypothesis of potential photochrome involvement by utilizing a broad spectrum light source and an irradiance-filtering system arranged so as to establish a range of photomorphogenic light treatments encompassing an expanded series of φₜ treatment values.

MATERIALS AND METHODS

Plant Materials. All studies were conducted with *P. pratensis* L. Newport, vegetatively propagated in a steamed 2:1 loam-peat soil mix.

¹ Abbreviations: OR: orange-red; B: blue; FR: far red; φₜ: estimated phytochrome photoequilibrium; PPFD: photosynthetic photon flux density; PFD: photon flux density; H₄: helminthosporal.
mix in 7.6-cm square plastic pots. Plants were grown in a glasshouse for a minimum of 60 days under a 16-h daylength maintained with supplemental incandescent lighting. Cultures of *D. sorokiniana* (sacc.) Subram. and Jain were grown at 22 C on 20 ml of 1.0% Czapek Dox Broth (10 g/l) in 3.0% (w/v) Bacto-agar in sterile, plastic Petri dishes (15 x 150 mm). Uniform virulence of *D. sorokiniana* was maintained by continuously cycling the pathogen on *P. pratensis* and preparing cultures from hyphal tip isolations from diseased tissue; only conidia from 20-day-old cultures were used for inoculations (10). Conidia harvested for inoculations were washed from the surface of cultures with distilled H2O and passed through a 90-μm microsieve to remove hyphal fragments. Suspensions of 500 conidia (±5.0%) per ml of distilled H2O were prepared with an automatic particle counter (High Accuracy Products Corp., Montclair, Calif.) for all inoculations.

**Physical Facilities and Light Treatments.** All studies were conducted in growth chambers (Sherr model CEL 25-7HL). A spectroradiometer (International Light 680, Newburyport, Mass.) was used to determine the appropriate combination of lamps and filtering materials needed to produce the five irradiance spectra (Fig. 1, A and B) from which the five corresponding ϕ0net were estimated (20). The resultant range of ϕ0net values was 0.45, 0.54, 0.60, 0.67, and 0.71. Previous observations established that an ϕ0net value of 0.60 approximates natural light (19). Therefore, 0.60 is used as a point of reference in describing ϕ0net values as high (Fig. 1A) or low (Fig. 1B), respectively. The five values of ϕ0net, which individually describe respective irradiance spectra (Fig. 1, A and B), represent the ϕ0net treatments against which the pathogenesis of *D. sorokiniana* leaf spot was evaluated on the leaves of *P. pratensis*. The specific lamp types and corresponding wattage, filters, lamp to upper leaf surface distance, PPFD, irradiance, and PFD associated with each ϕ0net treatment are shown in Table 1. All studies were conducted with a 12-h photoperiod, and all lamps were controlled by a 24-h timer adjusted to turn all lamps on or off simultaneously (nonsequencing). Temperature within the inoculation apparatus was maintained at 20 C (±1 C) for all studies.

**Inoculations.** Inoculations with *D. sorokiniana* were conducted on the four youngest, visible leaf blades of one shoot of each plant. The four leaves were individually inserted into four separate Pyrex glass inoculating tubes of a specially designed inoculation apparatus (23). The inoculation apparatus also functioned to hold each leaf in precisely the same orientation and distance from the light source. Plant and inoculating apparatus were placed within a plastic refrigerator crisper (9.5 height x 27 length x 19.5 depth cm). Each inoculating tube had five inoculating ports (about 2.0-mm diameter) spaced 1 cm apart over the upper epidermis of the leaf. Each leaf blade was inoculated by placing 10 conidia in a 0.02-ml droplet (original suspension 500 conidia per ml) on the surface of the leaf through each of the five inoculation ports. The crispers were then placed at the appropriate distance from the lamps and covered with the proper filter and/or shading material (Table 1) to provide a common PPFD for each spectral regime from which each ϕ0net treatment value was derived. Inoculated plants were incubated 6 days under each spectral regime before being evaluated for disease severity. Each ϕ0net treatment consisted of two plants (eight leaves) and was replicated five times (40 leaves evaluated per treatment) for evaluation of disease on progressively older leaves. Each ϕ0net treatment consisted of 10 replicate shoots (four leaves per shoot) for evaluations of disease on whole shoots.

**Disease Evaluation.** Disease was determined on inoculated leaf blades by harvesting 10-cm lengths of leaf blades from the inoculating tubes and estimating the total leaf area of the specimen and expressing the estimated diseased area as a percentage of the cambial total area of the leaf specimen. Total leaf blade area was estimated to the nearest whole number by multiplying the 10-cm length of the leaf by its width (determined with an ocular micrometer) at the midpoint of its length. The area of diseased tissue on leaf specimens was estimated by multiplying the estimated length and width (longest chords of the lesions) of each lesion. Lesion measurements included necrotic and chlorotic zones associated with lesions. Lesions with an area of less than 0.4 mm² were not included in the area estimation of diseased tissue.

![Fig. 1. Light spectra utilized to generate various ϕ0net. High (A) and low (B) ϕ0net values generated from lamp and filter combinations shown in Table 1. The ϕ0net value of 0.60 most closely approximates natural light and is presented with both high (A) and low (B) ϕ0net spectra for direct comparison.](image_url)

**RESULTS**

**Disease Severity on Whole Shoots.** The mean percentage of diseased tissue per leaf in response to infection by *D. sorokiniana* on whole shoots (consisting of the four youngest, visible leaves) of *P. pratensis* declined under light spectral conditions described by progressively higher values of ϕ0net (Fig. 2). The mean percentage of diseased tissue per leaf was greatest in response to light treatments with an ϕ0net of 0.45 and was significantly greater than that produced in response to all other ϕ0net treatments. Disease in response to an ϕ0net treatment of 0.54 was significantly less than that produced in response to 0.45 and significantly greater than that at 0.71 (Fig. 2). The decrease in disease from the lowest to the highest ϕ0net treatments was significant, but not every ϕ0net treatment between the extremes contributed to significantly different levels of disease (Fig. 2).

**Disease and Leaf Age.** The mean percentage of diseased tissue per leaf on whole shoots (Fig. 2) was not evenly distributed across the leaves of different ages on the shoot. Disease was greatest on leaf 4 (oldest) in response to all ϕ0net treatments (Fig. 3D). Leaf 1 (youngest) showed the second highest level of disease in response to most ϕ0net treatments (Fig. 3A); disease was minimal on leaves...
Table 1. Physical Arrangements and Spectral Characteristics for each Light Treatment

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Lamps</th>
<th>Filters</th>
<th>PPFD</th>
<th>PFD Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type a</td>
<td>Watts per lamp</td>
<td>No.</td>
<td>Distance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>φₑₜᵣ</td>
<td></td>
<td>25.4</td>
<td>6</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>0.45 Cool-white</td>
<td>110</td>
<td>110</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>GRO-LUX WS</td>
<td>110</td>
<td>4</td>
<td>4</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Far Red</td>
<td>40</td>
<td>4</td>
<td>4</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>0.60 Cool-white</td>
<td>110</td>
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<td>110</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>GRO-LUX WS</td>
<td>110</td>
<td>4</td>
<td>4</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Far Red</td>
<td>40</td>
<td>4</td>
<td>4</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>0.67 Cool-white</td>
<td>110</td>
<td>110</td>
<td>110</td>
<td>100 ± 5</td>
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<tr>
<td>GRO-LUX WS</td>
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<td>4</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Far Red</td>
<td>40</td>
<td>4</td>
<td>4</td>
<td>100 ± 5</td>
</tr>
</tbody>
</table>


*b* Distance from lamp to upper surface of leaves.

'Blue cellophane film, 0.025 mm (10 mil), stock no. 89-024, Dennison Manufacturing Co., Framingham, Mass. 01701; Clear vinyl film, 0.02 mm (0.8 mil). Saran Wrap, Dow Chemical Co., Indianapolis, Ind. 46268.

An inverse relationship was established between treatments with increasing values of φₑₜᵣ and corresponding disease on leaf 1 (youngest) versus leaf 4 (oldest). Disease on leaf 1 subjected to an φₑₜᵣ treatment of 0.45 was significantly lower than that produced in response to all other φₑₜᵣ treatments (Fig. 3A). Disease on leaf 1 in response to φₑₜᵣ treatments of 0.54 and 0.67 was not significantly different, but disease in response to both treatments was significantly greater than that produced in response to 0.45 and significantly lower than that produced in response to 0.67 and 0.71 (Fig. 3A). These φₑₜᵣ treatment disease responses were reversed on leaf 4, i.e. disease on leaf 4 in response to light treatment described by a φₑₜᵣ value of 0.45 was significantly greater than that produced in response to most light treatments with higher values of φₑₜᵣ (Fig. 3D). A series of light treatments described by progressively increasing φₑₜᵣ values produced a corresponding decrease in disease on leaf 4 (Fig. 3D). Disease in response to treatments with the various values of φₑₜᵣ was minimal on leaves 2 and 3, except for a significant increase in disease in response to the 0.45 φₑₜᵣ treatment (Fig. 3, B and C).

**DISCUSSION**

This study demonstrates that photomorphogenic light of specific φₑₜᵣ values interacts with sequentially developing and senescing leaves of *P. pratensis* to influence pathogenesis by *D. sorokiniana* in a manner suggestive of phytochrome involvement. Previous studies (18, 19) have stimulated the formulation of a three-part hypothesis relative to light-mediated interactions of *P. pratensis* and *D. sorokiniana*. (a) The interrelationship between pathogenesis and the inherent susceptibility or resistance of *P. pratensis* to *D. sorokiniana* is influenced by photoperiod and light quality (18, 19). (b) The influence of photoperiod and light quality on pathogenesis differs with the physiological age (developmental senescent stage) of the infected leaf (Fig. 3) (18). (c) The mode of action by light during pathogenesis is implemented by photomorphogenically related processes within which phytochrome may function as a receptor responder predisposing or conditioning host resistance (in conjunction with developmental and senescent processes) by mediating the membrane disruptive vulnerability to phytoxins. The formulation of this hypothesis necessitates integration of the results of this study with observations describing
stages of leaf development, the mode of action of the phytotoxin, H-a1, and the physiology of phytochrome.

Leaf development and senescence on P. pratensis is a continuously sequential process that maintains an average of four visible leaves on a mature shoot at any given time (8). In this respect, P. pratensis provides a model system for research involving sequential leaf development and senescence. The sequential development of P. pratensis leaves, together with the experimental techniques employed in this study, provide an assessment of leaf age-light interactions as factors influencing pathogenesis and provide part of the interpretive basis for the hypothesis presented.

The expression of pathogenesis on sequentially developing and senescing leaves of P. pratensis infected with D. sorokiniana (Fig. 3) reflects a potential phytotoxin-leaf age interaction. H-a1 is the primary determinant of pathogenesis in grasses infected by D. sorokiniana (16, 24). The chemical nature and mode of action of H-a1 provides considerable insight into host susceptibility or resistance. H-a1 is a low mol-wt sesquiterpenoid dialdehyde of nonspecific action (6, 26) with distinctive lipophilic and hydrophilic solubility characteristics (9). A primary mode of action involving carbonyl and/or sulfhydryl sites could account for the membrane integrity modifications and essential control of permeability, which is critical to cellular compartmentation (21, 32).

Such potential responses to H-a1 provide an additional part of the interpretive basis for the hypothesis presented.

Phytochrome is a probable receptor responder for the morphogenetic light-mediated changes in the pathogenesis observed in this and previous studies (18, 19). The \( \phi_{\text{int}} \) utilized in this study provides a means of defining light quality in terms of its predominant nonphotosynthetic photophysiological function. The correlation of disease response with the three different means of expressing light quality (Table I) is facilitated by utilizing \( \phi_{\text{int}} \). Therefore, the \( \phi_{\text{int}} \)-evaluated light spectra utilized in this study improve the clarity of the data and provide insight into potential phytochrome involvement. The resultant phytochrome considerations contribute to the interpretive basis for the final part of the hypothesis.

An integration of recognized leaf development characteristics, H-a1 action, and phytochrome phenomenon provides the comprehensive interpretive basis for D. sorokiniana pathogenesis on sequentially senescent leaves of P. pratensis in response to the various \( \phi_{\text{int}} \) light treatment spectra utilized in this study. The moderate disease observed on leaf 1 in response to the various light treatments is believed due to the premature leaf’s vulnerability to membrane attack by H-a1. This leaf stage (Table II, leaf 1) is dependent on optimal functioning of a diverse array of

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**Fig. 3.** Influence of light spectral regimes with progressively higher \( \phi_{\text{int}} \) on pathogenesis by D. sorokiniana on sequentially developing and senescing leaves of P. pratensis. Means within each leaf age group in response to progressively higher \( \phi_{\text{int}} \) (a/ ) and means between progressively older leaves at specific \( \phi_{\text{int}} \) ( /a) followed by the same letter are not significantly different. Duncan’s multiple range test (\( P = 0.05 \)).
enzymes plus strict metabolic compartmentation. Any H-al-induced membrane disturbance would have critical consequences. High \( \Delta \phi_{ext} \) light treatments would be anticipated to promote high relative Pfr levels, which would promote extensive phytohormonal and anabolic activity (1, 25, 29, 31) sustaining leaf growth and prolonging this premature stage period of vulnerability to pathogenesis (Fig. 3A). By contrast, low \( \Delta \phi_{ext} \) light treatment would likely promote an earlier attainment of early maturity (Table II), which would predispose toward enhanced resistance to pathogenesis (Fig. 3A). (5, 7, 25, 29). There is growing evidence that phytohormonal and enzymatic interactions on or within membranes may be related to the action of phytoxins (4, 15, 22).

The mature stage of leaf development (Table II, leaves 2 and 3) shows minimal disease in response to the various light treatment spectra (Fig. 3, B and C). This leaf stage represents a period wherein photosynthesis and leaf partitioning of photosynthetic are principal functions. Consequently, much of a mature leaf’s metabolic activity is sited within chloroplasts and, compared with the other cellular organelles, chloroplasts retain greater resistance to early H-al action (21). These characteristics, in combination with sustained membrane integrity, may account for the reduced vulnerability. Of the two mature leaves, only leaf 3 (Fig. 3C) reveals an \( \phi_{ext} \) treatment response. Low \( \phi_{ext} \) spectral conditions (0.45) promote senescence, and the increase in disease on leaf 3 (Fig. 3C) in response to low \( \phi_{ext} \) suggests that this treatment is advancing leaf development toward postmaturity and, concomitantly, enhanced disease susceptibility.

The greatest expression of pathogenesis in response to the various \( \phi_{ext} \) light treatments occurs on leaf 4 (Fig. 3D). This leaf is approaching senescence (Table II) and undergoing degenerative changes characterized by a decline in phytohormonal activity, a rise in respiration-related catabolism, declining membrane integrity, and increased hydrolytic enzyme activity (2, 17, 33). Coupled with these advancing leaf conditions, the presence of H-al and enhanced ethylene levels could synergistically promote extensive chlorosis (18, 19). Under such circumstances, high \( \Delta \phi_{ext} \) light treatment could retard pathogenesis by retarding senescence, whereas low \( \phi_{ext} \) treatment could enhance pathogenesis by promoting senescence (Fig. 3D). Low \( \phi_{ext} \) light treatments will generate lower relative Pfr levels (27), limit the synthesis of gibberellins and cytokinins, and increase the synthesis of ethylene while promoting general catabolism and the export of transportable metabolites (5, 7, 25, 29, 31). Such light quality effects would serve to enhance the conditions already initiated within older leaves in response to declining Pfr levels, a consequence of increased rate of Pfr loss during dark periods (34, 35). Low relative Pfr levels, acting in a manner similar to H-al and ethylene, are believed to enhance senescence via membrane-sited regulatory action that mediates permeability and metabolic compartmentation (3, 30). This interpretation implies that the predisposition or conditioning of \( P. pratensis \) resistance to \( D. sorokiniana \) by light is implemented via both static and dynamic mode phytochrome action (13), which accounts for the observed photoperiodic and light quality effects (18, 19).

**Table II. Sequential Appearance of \( P. pratensis \) Leaves as Related to Their Development-Senescence Stage**

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Developmental Stage</th>
<th>Expansion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (youngest)</td>
<td>Premature</td>
<td>Incomplete</td>
</tr>
<tr>
<td>2</td>
<td>Early mature</td>
<td>Near complete</td>
</tr>
<tr>
<td>3</td>
<td>Late mature</td>
<td>Complete</td>
</tr>
<tr>
<td>4 (oldest)</td>
<td>Postmature (near senescent)</td>
<td>Complete</td>
</tr>
</tbody>
</table>

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