Impact of Diurnal Periodicity, Temperature and Light on Sporulation of *Bremia lactucae*

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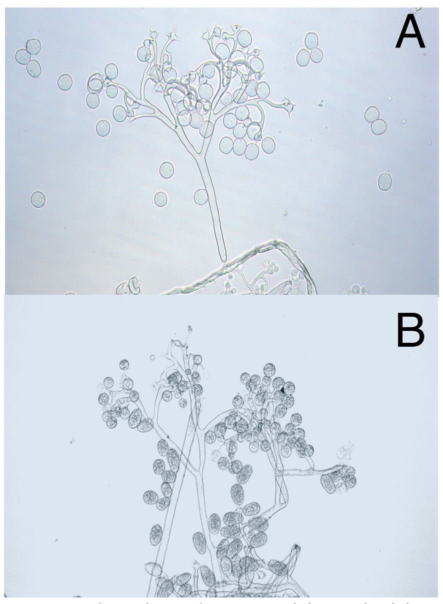


Figure 1. Sporulation of *Bremia lactucae* in (A) darkness and (B) light. Sporangia produced in light are smaller with darkened, granular cytoplasm, and germinate poorly.

INTRODUCTION

Lettuce downy mildew, caused by the oomycete *Bremia lactucae* (Fig. 1 and 2.), causes extensive losses in lettuce fields worldwide. The disease is controlled by timely applications of fungicides, and models that forecast infection are important tools for timing of sprays. The interactions among environmental factors (such as temperature, relative humidity, rainfall and light/dark cycles) required for sporulation and infection are complex and these factors are incorporated in many present forecasting models for lettuce downy mildew. In particular, daylight has been reported to suppress sporulation of *B. lactucae*. However, the suppressive effect of specific light intensities and qualities remains poorly understood. Similarly, how light interacts with other environmental factors to affect sporulation is not known. At Nordic latitudes, day length, light intensity and light quality differ substantially from most other lettuce producing regions and are therefore of great interest when evaluating forecasting models for sporulation and infection of *B. lactucae*.

OBJECTIVES

- Evaluate the effects of light intensity and light quality on sporulation.
- Elucidate how temperature modifies the impact of light on sporulation.
- Assess the impact of diurnal rhythm as a factor affecting sporulation.

Figure 2. Sporulation of *Bremia lactucae* on a young lettuce leaf.

INOCULATION PROCEDURES

We inoculated 7-day-old lettuce seedlings of the susceptible variety Black Seeded Simpson with a suspension of 3×10^5 spores per ml and incubated them until the 6th night whereupon intact seedlings or detached cotyledons were incubated at different light and temperature treatments overnight. The following morning sporangia were harvested and enumerated. Mean numbers of sporangia per cotyledon were calculated, and the response to treatments was indicated as percentage of sporulation relative to a dark control. All experiments had three replicates and were repeated three times. Isolates from Geneva, NY and Norway were included in the experiments.

INTERACTIONS OF LIGHT AND TEMPERATURE

Inoculated lettuce seedlings were incubated overnight in growth chambers in darkness and at a light intensity of 40 μmol m^{-2} s^{-1} and 100% RH at temperatures ranging from 5-25 ºC.

The suppressive effect of light was moderated by temperatures below 20 ºC. The optimal temperature range for sporulation of *B. lactucae* was 10 to 15 ºC (Fig 3). Within this range of temperature, light partially suppressed production of sporangia and the magnitude of the suppressive effect increased with increasing temperatures. (Fig 4). Cohen (1976) and Cohen and Eyal (1977) reported a similar interaction between temperature and suppression of sporulation for some downy mildews, but not for *B. lactucae*.

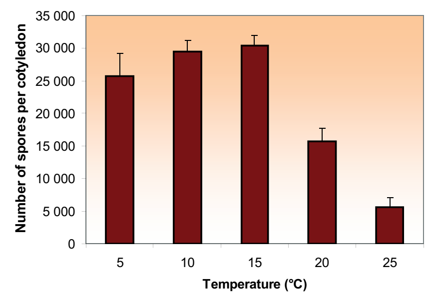


Figure 3. Number of spores produced per cotyledon at temperatures from 5 to 25 ºC when incubated for 10 hours in darkness.

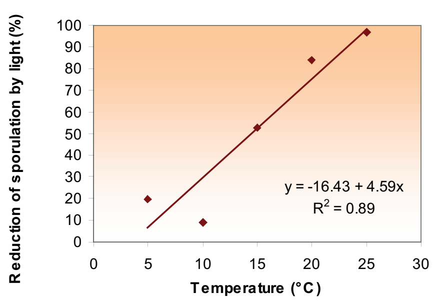


Figure 4. Reduction of sporulation by light at temperatures from 5 to 25 ºC.

EFFECTS OF LIGHT QUALITY ON SPORULATION

At low solar angles typical of spring and early summer at Nordic latitudes the proportion of total radiation composed of blue and infrared wavelengths is increased. This may be important in how light affects the sporulation of *B. lactucae*, as only certain wavelengths may be involved in inhibition of sporulation (Raffray and Sequiera 1971). We evaluated the suppressive effect of different wavebands of light as follows. Detached cotyledons were placed upside down on wet filter paper and incubated at 20 ºC and 100 % RH while exposed to different wavebands of light supplied through wide bandpass filters (Fig. 5, 6 and 7), including a dark control and a daylight-balanced light control for 10 hours over-night.

Light in the violet range supplied through a wide bandpass filter caused suppression of sporulation to nearly the same extent as in daylight-balanced light (Fig. 8). It is therefore possible that even during twilight conditions the light will have suppressive effects on sporulation. However, the suppressive effect of violet light was lost when the spectrum was further divided by using narrow bandpass filters of 50 nm steps within the violet range (Fig. 9), indicating that a threshold quantum of light within the violet range is required for suppression. Subsequent experiments at higher light intensities supplied the necessary suppressive quantity of light through the narrow bandpass filters, and indicated that light in the range of 400-450 nm was the most effective component in the violet range for reduction of sporulation (Fig. 10), although the additive effects of the wavebands from 450-500 nm and 500-550 nm could still be detected (Fig 10).

Figure 5. Incubators with filtered light applied from fiberoptic lamps.

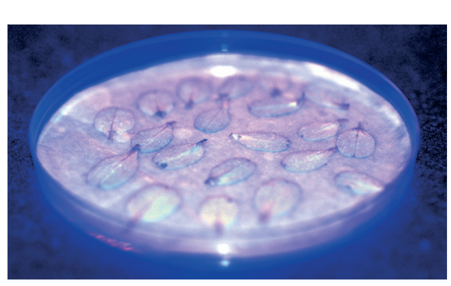


Figure 6. Detached cotyledons incubated in violet light.

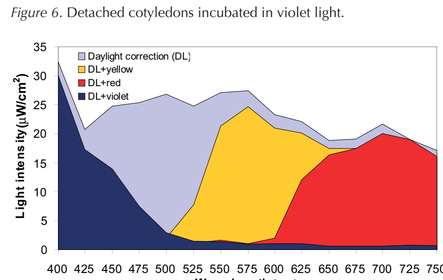
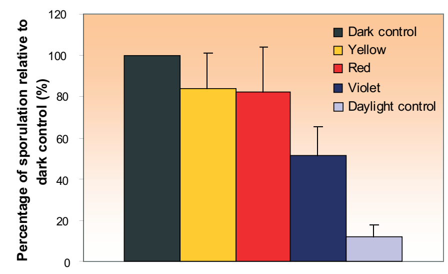


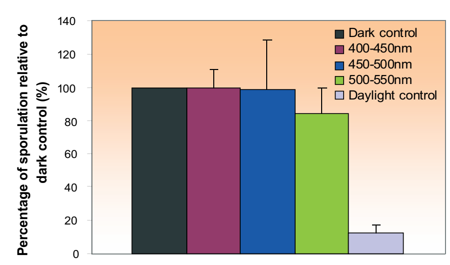
Figure 7. Intensity and quality of light provided by wide bandpass filters used in studies of sporulation at different light qualities.

Figure 8. Suppression of sporulation at different light qualities supplied



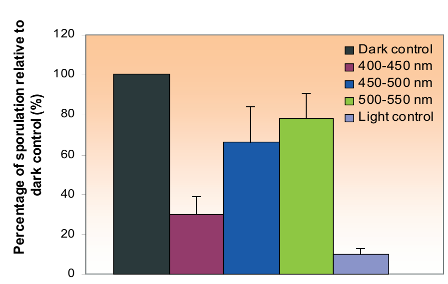
through wide bandpass filters shown in Fig. 7. Base intensity of the light source was 13 μmol m^{-2} s^{-1}.

Figure 9. Suppression of sporulation of Bremia lactucae at different light



qualities supplied through narrow bandpass filters. Experiments performed at Geneva, NY, USA. Base intensity of the light source was 13 μmol m^{-2} s^{-1}.

Figure 10. Suppression of sporulation of a Norwegian isolate at different



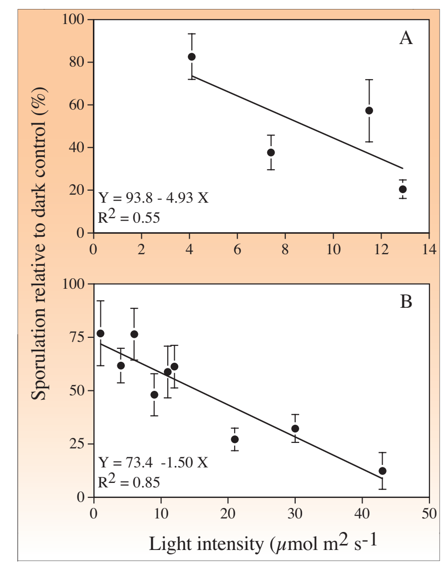
light qualities supplied through narrow bandpass filters. Base intensity of the light source was 90 μmol m^{-2} s^{-1}.

LIGHT INTENSITY

The continuous dusk during Nordic summer nights may influence the epidemiology of *B. lactucae* in these areas. A minimum radiation threshold of 3 W m^{-2} has been described for suppression of sporulation of *Plasmopara viticola* (Brook 1979). No similar threshold has been reported for *B. lactucae*, although release of sporangia of *B. lactucae* and their detection by volumetric traps (Wu et al. 2002) has been associated with a radiation threshold of 8 W m^{-2} (41 μmol m^{-2} s^{-1}).

The suppressive effect of light at different intensities was evaluated under controlled conditions for isolates of *B. lactucae* from Geneva, NY and Norway. Detached cotyledons were incubated overnight while continuously exposed to different intensities of daylight-balanced light.

Figure 11. Effect of light intensity on sporulation of an isolate of *Bremia*



lactucae from (A) New York and (B) Norway.

The response to light at low intensities was highly variable, but light intensities lower than 15 μmol m^{-2} s^{-1} did reduce sporulation for both isolates at 20 ºC (Fig. 11). However, some mature spores were produced at all light intensities tested.

EFFECT OF DIURNAL RHYTHM ON SPORULATION

We raised lettuce seedlings to an age of 1 wk in growth chambers at 15 ˚C in 12 hr of daylight-balanced light that began at either 0900 h (daytime light), or 2100 h (night-time light). Seedlings under each light regime were then inoculated and incubated an additional 7 days until the latent period had passed and the pathogen was able to sporulate. We then raised the relative humidity to 100 % to promote sporulation and exposed subsets of the seedlings to the following: (i) darkness during the expected "night" interval (e.g., darkness starting at 2100 for seedlings previously conditioned to darkness starting at 2100), (ii) light during the expected "night" interval, (iii) darkness during the expected day interval, and (iv) light during the expected day interval.

The results shown in Table 1 clearly demonstrate the presence of a diurnal rhythm that, at 15 ˚C, operates independently of a suppressive effect of light. When exposure to high RH coincided with an expected "night" interval (based upon the regime of the previous 14 days), sporulation occurred irrespective of the presence of light or darkness (Table 1). Conversely, when exposure to high RH did not coincide with the expected "night" interval, only traces of sporulation occurred, irrespective of light or darkness (Table 1).

Table 1. Sporulation of *Bremia lactucae* on seedlings during expected "light" or "dark" intervals following the setting of the diurnal rhythm by different cycles of illumination.

Day/night cycle during seedling growth and incubation			Starting time of and illumination	
0900-2100	2100-0900	*Expected* night interval begins	during interval of high RH	Sporulation* (mean ± SE)
Light	Dark	2100	Dark at 2100	28.83 ± 13.17
Light	Dark	2100	Light at 2100	27.97 ± 10.69
Light	Dark	2100	Dark at 0900	0.08 ± 0.06
Light	Dark	2100	Light at 0900	0.03 ± 0.03
Dark	Light	0900	Dark at 2100	0.22 ± 0.09
Dark	Light	0900	Light at 2100	0.05 ± 0.03
Dark	Light	0900	Dark at 0900	49.92 ± 9.66
Dark	Light	0900	Dark at 0900	32.00 ± 2.65

*Mean number of sporangia harvested per cotyledon

CONCLUSIONS

The suppressive effect of light on sporulation is temperature dependent and is greatly reduced at low temperatures (10-15 ºC) common in many lettuce-growing regions.

Light in the range of 400-450 nm is the component responsible for the greatest inhibition of sporulation. However, narrow bandpass filter results indicate a light quantum effect in which wavelengths from 450-500 nm may also affect sporulation.

At temperatures commonly seen in many lettuce growing areas, diurnal rhythm may be the principal factor controlling sporulation and may have confounded previous studies in which an association of daylight with suppressed sporulation was assumed to be causative.

ACKNOWLEDGEMENTS

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