Concurrent Spore Release and Infection of Lettuce by *Bremia lactucae* During Mornings with Prolonged Leaf Wetness

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ABSTRACT

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In a previous field study in California, infection of lettuce by *Bremia lactucae*, the downy mildew pathogen, occurred mainly on days on which leaves dried late in the morning. This observation led to the hypothesis that spore release and infection take place concurrently during mornings with prolonged leaf wetness. To test this hypothesis, spore dispersal experiments were carried out during 13 nights and mornings for which prolonged leaf wetness was expected. At sunset, diseased spore-source plants (infected with a metalaxyl-insensitive isolate of *B. lactucae*) and healthy spore-trap plants (grown in metalaxyl-amended nutrient solution) were placed next to each other outdoors. Lesions on the source plants sporulated at night, and spore release (measured with a volumetric spore sampler) began at sunrise. After leaf wetness had ended later in the day, the trap plants were moved to a growth chamber and incubated in conditions favorable for colonization by *B. lactucae*, but not for infection. They were inspected for disease 10 to 14 days after exposure. Trap plants developed signs and symptoms of downy mildew after experiments from five nights, all of which had leaf wetness persisting for at least 3 h after sunrise. Infection on these plants must have taken place during the morning of the exposure period, concurrently with spore release from source plants. Since a metalaxyl-insensitive isolate of *B. lactucae* was used in all experiments and trap plants were grown in metalaxyl-amended nutrient solution, the possibility of infection by inoculum occurring naturally at the experimental site could be excluded.

Additional keywords: *Lactuca sativa*, Peronosporales, spore survival.

Downy mildew (causal agent: *Bremia lactucae* Regel) is a major disease in field and glasshouse lettuce (*Lactuca sativa* L.) production systems worldwide. *Bremia lactucae* can infect its host by means of asexually produced sporangia, sexually produced oospores, or vegetative mycelium (4,14). While oospores serve as survival structures and as initial inoculum (11,27), rapid secondary spread of the disease results from infection by airborne sporangia (4,14,16). Sporangia of most temperate-zone downy mildews, including *B. lactucae*, are produced at night, mature early in the morning, and are released during midmorning (4,14, 15,16,23,24). Release of *B. lactucae* sporangia starts at sunrise and peaks between 1000 and 1200 h (4,25).

In a previous field study in coastal California, infection of lettuce by *B. lactucae* occurred mainly on days on which leaf wetness ended late in the morning (20). The median time at which leaves dried was 1000 h on days with infection by *B. lactucae* and 0800 h on days without infection (all times are Pacific Standard Time). Weather variables other than morning leaf wetness were not associated consistently with infection (20). A hypothesis explaining the importance of leaf wetness in the morning (as opposed to leaf wetness at night or total leaf wetness duration) is that sporangia released at sunrise cause infection later in the morning if leaf wetness continues for at least another 3 to 4 h, the minimum wet period required for infection by *B. lactucae* (19,26). Similar hypotheses have been proposed previously for other members of the Peronosporales—*Phytophthora infestans* (7), *Pseudoperonospora cubensis* (3), and *Pseudoperonospora humuli* (23)—but the phenomenon of concurrent spore release and infection has not been demonstrated for any of these pathogens.

In this paper, we report the results of spore dispersal experiments in semicontrolled conditions to determine whether spore release and infection of lettuce by *B. lactucae* can occur concurrently during mornings with prolonged leaf wetness. A preliminary report has been published (21).

MATERIALS AND METHODS

Spore-trap plants. Lettuce seeds cv. Buttercrunch were surface sterilized for 15 min in 0.5% NaOCl, rinsed in two changes of sterile distilled water, and planted in polycarbonate tissue culture vessels (Magenta GA7; Sigma Chemical Co., St. Louis, Mo.) on two layers of blotter paper that were moistened with 6 ml of sterile half-strength Hoagland's solution (6). The nutrient solution contained the fungicide metalaxyl (Ridomil 2E; Ciba Geigy Co., Greensboro, N.C.) at a concentration of 50 μg ml^{-1}. Metalaxyl concentrations of 1 to 25 μg ml^{-1} are sufficient to prevent infection of lettuce by metalaxyl-sensitive isolates of *B. lactucae* (22). The seeds (30 to 40 per vessel) were grown at a temperature of 20°C and a 14-h photoperiod with a photon flux density of 250 to 350 μmol m^{-2} s^{-1} during the light phase. After 7 days, the seedlings had well-developed cotyledons. They were used as spore-trap plants when they were 8 to 10 days old.

Spore-source plants. An isolate of *B. lactucae*, California pathotype III with insensitivity to metalaxyl (22) was maintained and inoculum was produced as previously reported (12). This isolate was able to infect lettuce growing in a nutrient solution with 100 μg ml^{-1} metalaxyl (22). Lettuce seedlings were grown in

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tissue culture vessels as described above and inoculated with a spore suspension (5×10^4 sporangia per ml in distilled water with 0.02% Tween 80) when they were 8 to 10 days old. Inoculations were carried out by opening the lid of the vessels in sterile conditions and placing one drop (50 µl) of the spore suspension onto the upper surface of each cotyledon with a transfer pipette. After inoculation, the vessels were closed and incubated in a growth chamber at 15°C. After 6 days, the lids were opened to subject the seedlings to ambient humidity in the growth chamber (between 50 and 65% during the day and 70 and 80% at night), which prevented sporulation by *B. lactucae*. Small volumes of sterile water were added to the vessels as needed to prevent the seedlings from desiccation. On the morning of the third day at ambient humidity, the lids were closed to increase humidity and to provide conditions favorable for sporulation of *B. lactucae* during the following night.

Spore release and infection experiments. The experiments were performed outdoors on the campus of the University of California at Davis (latitude 38°32′ N, longitude 121°46′ W) during 13 winter nights and mornings for which radiation fog was forecast (Table 1). Radiation fog was expected to lead to prolonged morning wet periods, similar to advection fog in the coastal regions of California where lettuce is grown commercially. (The experiments were not carried out in the coastal regions because of a high background of natural inoculum of *B. lactucae* there, including metalaxyl-insensitive isolates.) There was no measurable background of natural inoculum at the experimental site in Davis.

Air temperature and humidity were measured at the site with shielded thermistors and sulfonated polystyrene humidity transducers, respectively (Campbell Scientific Inc., Logan, Utah). Leaf wetness duration was monitored with an electronic leaf wetness sensing grid (Campbell Scientific Inc.). All sensor signals were sampled with a data logger at 5-min intervals.

A volumetric spore sampler (Burkard Mfg. Co. Ltd., Rickmansworth, Hertfordshire, U.K.) was operated at the site to monitor the release of sporangia from spore-source plants. The device was adjusted to sample 10 liters min^{-1} (= 14.4 m^3 day^{-1}) of air. Spore slides were prepared and examined according to the instructions of the manufacturer after they had been stained in 0.2 g $liter^{-1}$ aniline blue in lactic acid/glycerol (3:1) overnight.

At the start of each experiment, vessels with diseased source plants (without any visually apparent signs of sporulation) and healthy trap plants were opened and any excess nutrient solution was decanted. Forceps were used to transfer the blotter papers with the seedlings onto 10-cm petri plates containing soft water agar (5 g $liter^{-1}$) amended with 25 µg ml^{-1} of the fungicide maneb (Manex; Griffin Co., Valdosta, Ga.). Separate forceps were used to handle source and trap plants. The purpose of the water agar was to prevent the blotter papers from desiccation, and maneb was added to prevent spore germination on the agar surface. At sunset (around 1730 h), one petri plate with source plants was moved outdoors and placed on a wire support at a horizontal distance of 10 cm from the orifice of the spore sampler. Subsequently, four petri plates with trap plants were exposed on a wire support so that they surrounded the source plants at horizontal and vertical distances of 20 and 10 cm, respectively. Another group of trap plants was kept in the laboratory as a control. The experiment was left undisturbed overnight. After leaves had dried the next morning or early afternoon, according to visual observations and the readings of the leaf wetness sensor, the trap plants were moved to a growth chamber and incubated in conditions favorable for colonization by *B. lactucae*, but not for infection (15°C; humidity between 50 and 65% during the day and 70 and 80% at night). After 1 day in these conditions, the trap plants were transferred back into tissue culture vessels in which high humidity, but no leaf wetness, was maintained. They were inspected for signs and symptoms of downy mildew 10 to 14 days after the exposure period.

RESULTS

Sporulation on source plants occurred during all 13 nights, and spore release was recorded after ten nights (Table 1). Temperatures during the morning were generally low (mostly between 5 and 10°C) but within the range favorable for infection by *B. lactucae* (14,19,26).

Trap plants became diseased after experiments from five nights, all of which had leaf wetness persisting for at least 3 h after sunrise (Table 1). For example, on 25 December 1992 spore release began at 0800 h and leaves dried 5 h later (Fig. 1). The next day, spore release began at 0700 h and leaf wetness ended at 1400 h (Fig. 1). Spore release and leaf wetness overlapped for at least 3 to 4 h, the minimum wet period required for infection by *B. lactucae* (19,26), during four of the five exposure periods after which trap plants became diseased. An exception was 27 January 1993, when leaf wetness ended at 1400 h and some trap plants became diseased although no spore release was recorded (Table 1). The concentration of spores released during this dispersal event may have been below the detection threshold of the spore sampler (<10 spores m^{-3}) (8).

During the eight exposure periods after which trap plants remained healthy, leaf wetness ended before or within 2 h after the onset of spore release. For example, on 25 December 1993 leaves dried at 0700 h and spore release began 1 h later (Fig. 2). The next day, there was no leaf wetness and spore release began at 0800 h (Fig. 2). Total numbers of spores collected with the sampler were low on both days, probably because only a small fraction of the spores produced during a given night was released during the following day (2).

Unexposed trap plants kept in the laboratory in the absence of spore-source plants did not develop signs or symptoms of downy mildew.

DISCUSSION

Our hypothesis that sporangia released at sunrise cause infection during the same morning if leaf wetness continues long enough was based on an association between infection periods of *B. lactucae* and the length of the morning wet period in an earlier 2-year field study (20). Similar hypotheses have been proposed previously for other members of the Peronosporales—*Phytophthora infestans* (7), *Pseudoperonospora cubensis* (3), and *Pseudoperonospora humuli* (23)—also based on empirical results or field observations. However, these hypotheses have not been

TABLE 1. Aspects of 13 experiments performed to test for concurrent spore release and infection by *Bremia lactucae* during winter mornings with prolonged leaf wetness

Day	Sporulation on source plants[a]	Beginning of spore release (PST[b])	End of leaf wetness (PST)	Infection of trap plants[c]
24 Dec. 92	+	0400	All day[d]	+
25 Dec. 92	+	0800	1300	+
26 Dec. 92	+	0700	1400	+
27 Jan. 93	+	...[e]	1400	+
28 Jan. 93	+	1000	0900	–
4 Dec. 93	+	0800	1100	+
5 Dec. 93	+	0800	...[f]	–
6 Dec. 93	+	...[e]	0800	–
16 Dec. 93	+	0900	1000	–
17 Dec. 93	+	...[e]	0600	–
20 Dec. 93	+	0600	0700	–
25 Dec. 93	+	0800	0700	–
26 Dec. 93	+	0800	...[f]	–

[a] + = Sporulation occurred on source plants during the night.
[b] Pacific Standard Time.
[c] + = At least one trap plant became infected; - = no infection.
[d] Trap plants were moved back indoors at sunset (around 1730 PST).
[e] No measurable spore release from source plants.
[f] No leaf wetness observed.

tested for any of these pathogens. The present study shows, to our knowledge for the first time, that spore release and infection by a temperate-zone downy mildew can occur concurrently during mornings with prolonged leaf wetness. Since a metalaxyl-insensitive isolate of *B. lactucae* was used in all experiments and trap plants were grown in metalaxyl-amended nutrient solution, the possibility of infection by inoculum occurring naturally at the experimental site could be excluded. Insensitivity to metalaxyl had not been detected in isolates naturally occurring at Davis at the time when our experiments were carried out.

Additional support for the importance for infection of leaf wetness in the morning (as opposed to leaf wetness at night or total leaf wetness duration) comes from spore survival experiments showing that short periods of exposure to bright sunlight and/or low humidity (similar to those encountered during sunny afternoons in coastal California) are sufficient to kill detached downy mildew sporangia (1,5,17). Furthermore, even if spores survive during the day, they may be killed at night, due to alternate wetting and drying that occurs when dew deposition rates are slow (9). Lack of spore survival during the afternoon or early night

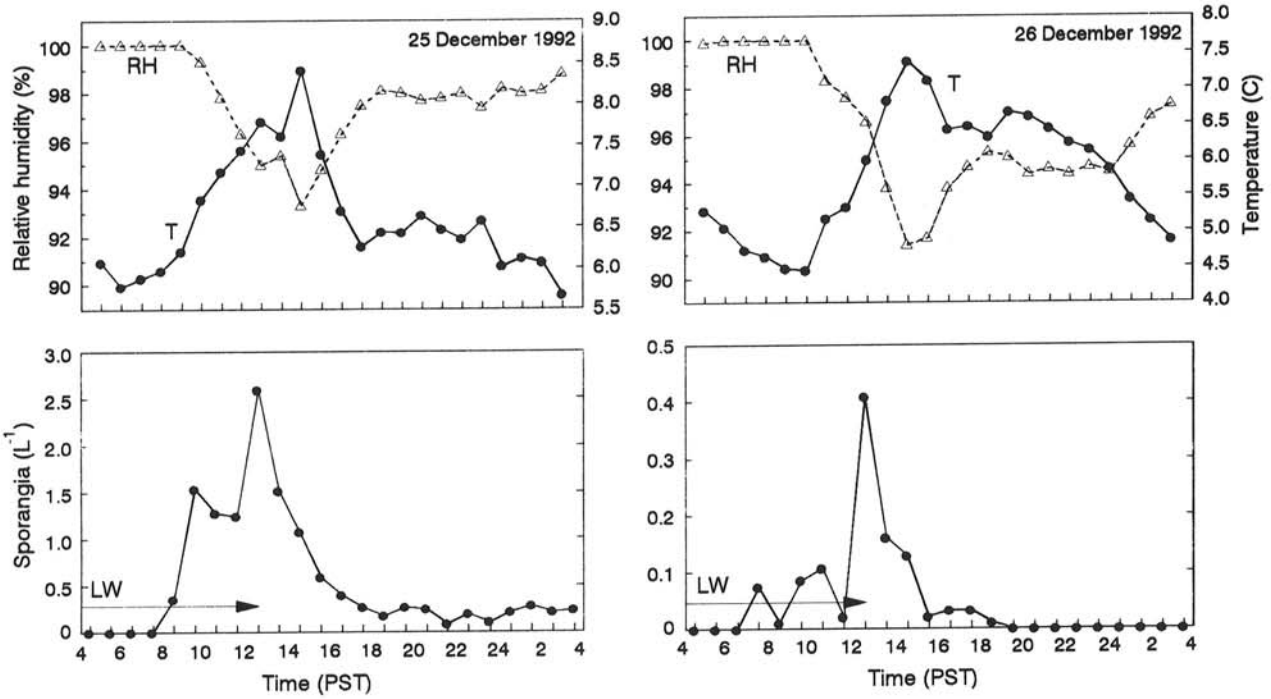

Fig. 1. Time sequence of air temperature (T), humidity (RH), and spore release by *Bremia lactucae* from infected lettuce plants during two consecutive winter mornings with prolonged leaf wetness (LW). Arrow head indicates end of the wet period. Spore-trap plants became infected during both exposure periods. PST = Pacific Standard Time.

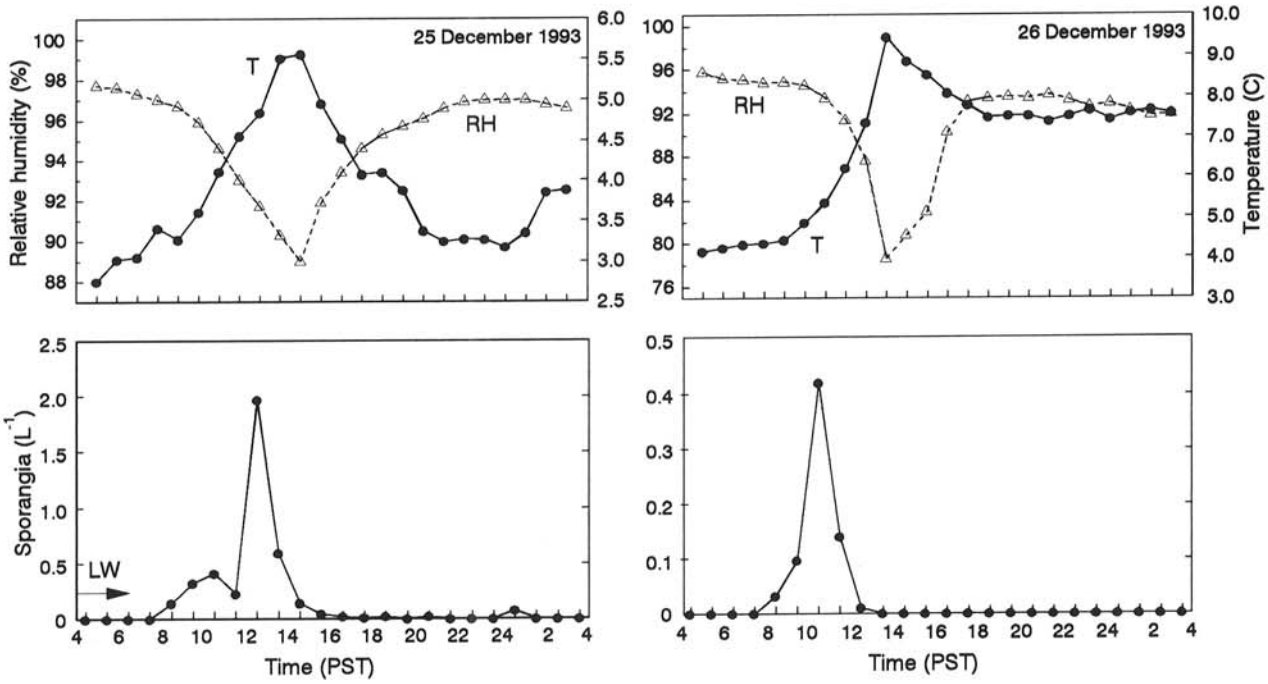

Fig. 2. Time sequence of air temperature (T), humidity (RH), and spore release by *Bremia lactucae* from infected lettuce plants during two consecutive winter mornings without prolonged leaf wetness (LW). Arrow head indicates end of the wet period. (There was no leaf wetness on 26 December.) Spore-trap plants did not become infected during the two exposure periods. PST = Pacific Standard Time.

may check infection during the dew period at night and increase the importance of morning wetness for infection.

The mechanism of spore release during mornings with prolonged wetness is not known. Spore release episodes of downy mildew pathogens have been associated with decreasing humidity, rising temperature, and evaporation of leaf wetness (15,16, 24). In our experiments, humidity did not decrease for several hours after sunrise during mornings with prolonged wetness (Fig. 1), suggesting that conditions may not have been favorable for spore release by *B. lactucae*. However, Populer (15), working with *Peronospora tabacina*, reported that spore release occurred after solar radiation had exceeded a threshold of about 0.5 cal min^{-1} cm^{-2} in the morning, independent of temperature or humidity. He concluded that radiation "seems to play an all-important part in the process of spore liberation." Similarly, red-infrared radiation was found to trigger spore release of another downy mildew pathogen, *Peronospora destructor*, in the laboratory, even in conditions of high humidity (13). These studies help explain why spores of *B. lactucae*, although in small numbers, are released during mornings with high humidity and prolonged leaf wetness.

Leaves in upper layers of a plant canopy may dry several hours earlier than leaves in lower canopy layers (10). Similarly, the tip of a leaf dries faster than its base. Spores from upper leaves may be released during the process of drying (24) and may infect lower leaves while those are still wet. In our experiments, only the upper leaf surfaces of spore-source and spore-trap plants were wetted by fog. Thus, spore release from lesions on the lower (dry) leaf surfaces of source plants may have occurred before wetness on the upper leaf surfaces of the trap plants had ended, leading to conditions favorable for concurrent spore release and infection.

Mornings with prolonged leaf wetness occurred on about 5% of the days in the coastal lettuce production areas of California during seven field trials (encompassing a total of 247 site-days) in 1993 to 1994 (18). This is consistent with the sporadic occurrence of downy mildew in these trials (18). An empirical infection model of lettuce downy mildew, based on the length of the morning wet period, has been developed as part of an ongoing project on the epidemiology of the disease in California (18,20). An understanding of the phenomenon of concurrent spore release and infection is of practical importance, because it provides a biological basis for disease prediction with this model.

In conclusion, spores of *B. lactucae*, some of which are released at sunrise even under conditions of high humidity, can cause infection during the same morning if leaf wetness continues long enough. This phenomenon may be an epidemiologically important trait in the life cycle of the pathogen: for a population of spores released on a given day, the risk of extinction due to adverse weather will be reduced if at least a small fraction of the spores can germinate and infect during or immediately after being released.

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