Influence of Wetness Periods on Infection of Celery by *Septoria apiicola* and Use in Timing Sprays for Control

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ABSTRACT

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Conidia of *Septoria apiicola* collected from dried infected celery leaves began germinating 7–12 hr after plating or inoculation, depending on temperature and germination medium. Germination was 20% at 21 C and 10% at 25 C on water agar 8 hr after plating. Germination did not begin in distilled water or on celery leaves until 12 hr after plating or inoculation. Germination was complete (>95%) on water agar and reached 78–80% on leaves 36 hr after plating or inoculation at 21 or 25 C. Lesions formed on celery leaves in significant numbers (one or more lesions per leaflet) only after 24 hr of continuous or interrupted (12 hr wet/12 hr dry/12 hr wet) dew within 15 days after inoculation at 21 C. Lesions formed as early as 8 days after inoculation following wet periods of 36–48 hr and reached a maximum of 14 lesions per leaflet after 21 days at 21 C. On celery leaves exposed to 36–48 hr of dew at 25 C, a maximum of 2.5 lesions per leaflet formed after 21 days. With a wetness period of 12 hr (or longer) used as a conservative threshold value representing a risk of infection, chlorothalonil sprays were applied to inoculated field plots after ≥12 hr of wetness (provided that no sprays had been applied within the previous 7 days) or weekly. In 3 yr of trials, two fewer sprays were applied annually with the 12-hr wetness threshold than with the weekly schedule without any sacrifice in efficacy of disease control.

Additional keywords: celery late blight, disease forecasting

Septoria leaf blight (late blight), caused by *Septoria apiicola* Speg., of celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.) is the most important foliar disease of celery in Michigan (11). If this disease is not controlled, losses can exceed 70% (10).

Septoria spp. produce conidia within pycnidia embedded in infected leaf or petiole tissues (4). Each pycnidium is capable of releasing up to 5,400 conidia (12). The conidia within the pycnidia are surrounded by a mucilaginous matrix composed of proteins and sugars that swells when the mucilage imbibes free water or is exposed to humidities ≥90%, forcing conidia and mucilage out through the ostiole in cirrhi (5). Dilute cirrhus extract has been shown to stimulate germination of *S. nodorum* Berk. conidia (2). *Septoria* conidia are disseminated by splash droplets from rainfall or overhead irrigation (3,4). High amounts of precipitation promoted disease development in celery (1,18). A similar situation was observed on wheat where increases in Septoria blight, caused by *S. tritici* Roberge in Desmaz., were associated with rainfall events 14–16 days earlier (7).

Conidia of *S. apiicola* germinate within a film of water on celery leaf surfaces (17) and also have been reported to germinate on leaves and infect at relative humidities >95%, although whether leaves were actually wet at these humidities was not recorded (18). A change of only 1 C at humidities >90% will cool an atmosphere below its dew point, and condensation of water results (16). Recent work demonstrated that conidia of *Stemphylium vesicarium* (Wallr.) E. Simmons germinated at RH ≥98% where temperature was accurately controlled to ±0.2 C (14), although similar data are lacking for *S. apiicola.*

In field observations, infection of celery did not occur when mean RH was <90% during the 2 days following inoculation with *S. apiicola* (18). In growth chambers, numbers of lesions increased with increasing periods of leaf wetness up to 96 hr at temperatures of 10, 15, and 20 C but were lower at 25 or 30 C (13). Mudita and Kushalappa (15) attempted to time sprays for Septoria blight control using a threshold of disease incidence for initiation of fungicide applications to transplant seedlings that had 0, 2, 4, 8, or 16% infection incidence. Their model indicated that losses in excess of the cost of one fungicide application occurred even if healthy transplants (0% initial blight incidence) were used at the beginning of the season and protective fungicide sprays were applied every 7–10 days beginning 3 wk after transplanting. There was no indication of how favorable conditions were for blight development during the 2 yr of these experiments. Plots were irrigated "as needed."

Many celery growers have historically applied fungicides for control at regular 7- to 10-day intervals over the entire season (6,8). If length of leaf wetness periods required for significant infection could be defined and used to time protective fungicide sprays only when actually needed, it should be possible to reduce the number of sprays per season required for disease control.

The objectives of these experiments were to determine: 1) the effects of temperature and substrate on conidial germination, 2) the effect of duration of leaf wetness on infection of celery by germinating conidia, and 3) whether this information could be used to devise a model for scheduling application of protective fungicide sprays only as needed to prevent disease development.

MATERIALS AND METHODS

Inoculum preparation. Celery leaves with Septoria leaf blight lesions were collected from unsprayed field plot rows in September 1990–1993, air-dried at 22 ± 2 C for 48 hr, placed in plastic bags, and stored up to 6 mo in a refrigerator at 3 ± 1 C until used. Conidial suspensions were prepared for germination or infection studies by immersing 20 g of dried infected leaves in 400 ml of distilled water for 5 min, straining the suspension through four layers of cheesecloth, and adjusting it to 10^6 conidia per milliliter by dilution with distilled water (9).

Conidial germination. An aqueous conidial suspension prepared as described above was placed in a plastic trigger-type spray bottle and sprayed onto surfaces of water agar plates (15 g/L of Difco Bacto agar) and on plants of celery cv. Florida 683 (8 ml per plant) grown in the greenhouse for 16 wk. Drops of spore suspension were also placed in depressions on two glass hanging-drop slides resting on glass rod supports in petri dishes lined with moist filter paper. Petri dishes were placed in 21 or 25 C incubators in the dark. Celery plants were placed in a 21 or 25 C dew chamber (model I-35DL, Percival Manufacturing, Boone, IA) in the dark where leaves stayed continuously wet. Agar plates and slides were examined at intervals; at each interval, 100 conidia were examined under a microscope (×200) for germination and numbers were recorded. Leaflets from inoculated celery plants were detached at 2- to 4-hr intervals, and 2 × 2 cm^2 segments were cut from the leaves and placed on glass slides. Three drops of cotton blue stain in lactic acid (28 mg of cotton blue dissolved in 20 ml of distilled water, 10 ml of glycerol,

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and 10 ml of lactic acid) were placed on top of each leaf segment, and a 22 × 22 mm glass coverslip was affixed and weighted down with a 1.5 × 1.5 cm cylindrical lead weight for 15 min. Leaf segments were then examined for conidial germination under a microscope, and 100 conidia samples were evaluated for germination. A conidium was considered to have germinated if there was an identifiable germ tube at least as long as the width of the conidium.

Effect of dew period on infection. Eighteen 16-wk-old celery plants grown in the greenhouse in 15.3-cm-diameter clay pots were inoculated by spraying leaves with 8 ml each of a conidial suspension (10^6/ml), which was enough to wet the upper surfaces of all leaves. Two plants sprayed only with distilled water were used as noninoculated controls. At the same time, surfaces of two water agar plates were sprayed with conidial suspension, plate covers were replaced, and plates were incubated at 21 C as a determination of viability of conidia used in each inoculation. All plants receiving variable periods of leaf wetness were placed in the dew chamber in the dark at either 21 or 25 C, except for two inoculated plants that were immediately placed in the growth chamber (0 hr dew) along with two noninoculated controls. After periods of 8, 12, 24, 36, or 48 hr, two plants were removed from the dew chamber and placed in the growth chamber at the same temperature for symptom development. To determine whether interruption of dew periods would have an effect on lesion numbers, two treatments received interrupted dew periods. Two plants each were removed from the dew chamber after 12 or 24 hr, placed in the growth chamber for a dry period of 12 hr, replaced in the dew chamber for 12 or 24 hr, then placed in the growth chamber for symptom development.

Lights were left on continuously in the growth chamber for 60 hr following inoculation to ensure that leaves dried within 15 min regardless of the time of day they were moved into the growth chamber from the dew chamber. In preliminary experiments, some leaves stayed wet for several hours after movement from dew chamber to growth chamber because of the higher humidity during dark hours. After the 60-hr light period, a 14-hr photoperiod was initiated using mixed fluorescent and incandescent lights (230 $\mu E \cdot m^{-2} \cdot s^{-1}$). Leaves remained dry during the remainder of the incubation period. Water was supplied by filling saucers under the pots daily with distilled water; care was taken not to get any water on leaves. Temperature and humidity were monitored with a recording hygrothermograph. Temperatures were maintained within ±1 C, and RH varied from 40 to 50% during light periods and from 60 to 80% during dark periods. Leaves were examined daily until the first lesions appeared, at which time lesions were circled with a permanent-ink marking pen and counted. Every day or every other day thereafter, up to 21 days after inoculation, lesions were counted and circled and new lesions were recorded. Each experiment was repeated at least once, with similar results. Data were analyzed statistically (SigmaStat, Jandel Scientific, San Rafael, CA) with a two-way analysis of variance, and significance of differences was determined using the Student-Newman-Keuls multiple paired comparison test ($P = 0.05$).

Timing sprays with leaf wetness. Celery seedlings were transplanted into 15.25-m rows at the Michigan State University Muck Experimental Farm, Bath, on 1–3 June in 1991 (cv. Florida 683), 1992 (cv. A863), and 1993 (cv. Peto 285). Plants were spaced 16.5 cm apart within rows 0.8 m apart in experimental blocks 61 m long. Treatment replications consisted of single 15.25-m rows separated by single untreated guard rows. Three treatments replicated four times were set up in a complete randomized block design: nonsprayed, sprayed weekly, or sprayed after a continuous leaf wetness period of ≥12 hr (provided that a spray had not been applied within the previous 7 days), hereafter referred to as a timed spray. Temperature was ignored as a factor in the predictive model for the sake of simplicity, since temperatures high enough to inhibit infection rarely (if ever) occur in Michigan during hours of leaf wetness when infection occurs. A leaf wetness threshold of 12 hr was chosen because only trace amounts of leaf infection occurred after 12 hr of leaf wetness (0.3 lesion per leaflet) and because no infection occurred after 8 hr of leaf wetness 14 days after inoculation in dew chamber experiments. The fungicide chlorothalonil (Bravo 720, 720 g a.i./L) was applied for disease control at a rate of 1.75 L/ha. Leaf wetness episodes were measured with a DeWit recorder (Valley Stream Farm, Orono, Ont., Canada) placed within a celery row at a height of 0.3 m. To ensure disease development and to allow spread within and between rows, the southernmost 2 m of each plot row and of each guard row between treatment rows were inoculated with a conidial suspension (prepared as described previously) within 2 days after the first weekly fungicide spray. Inoculated plants were examined visually and percent diseased leaves was estimated a few days before harvest. Distance of spread within rows from inoculated areas was measured in 1993. Plots were harvested on 20–24 August each year, trimmed for packing, and weighed for yield estimates.

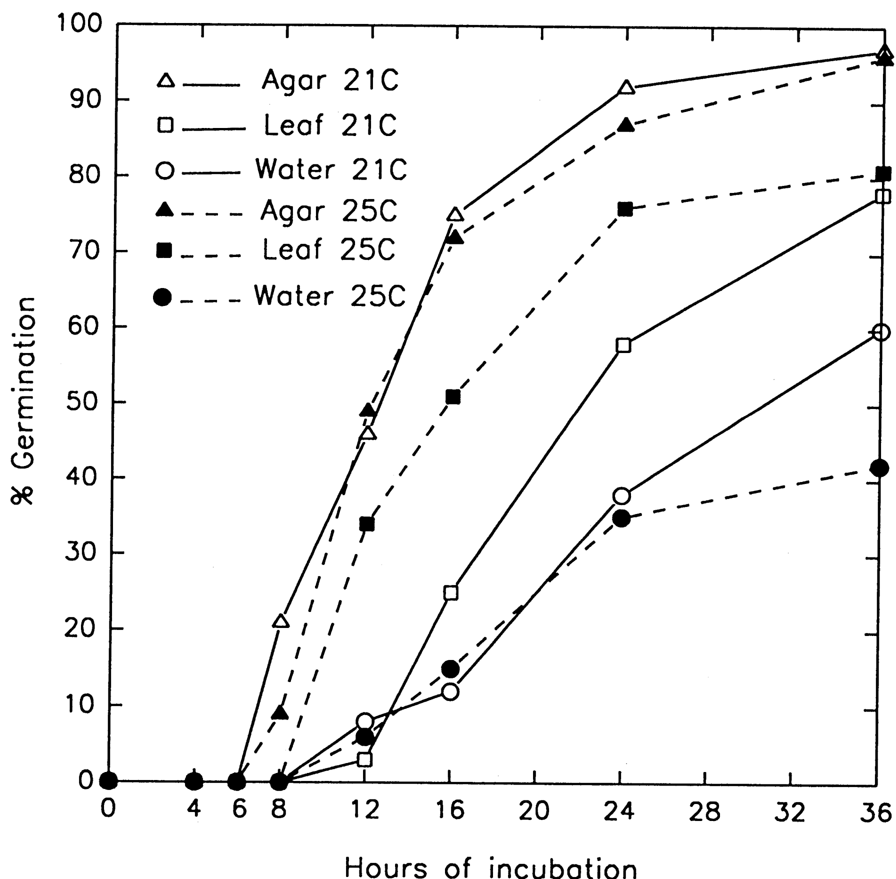

Fig. 1. Conidial germination of *Septoria apiicola* on water agar, on celery leaves, and in distilled water at 21 and 25 C.

RESULTS

Conidial germination. Conidia began to germinate 7–12 hr after plating or inoculation, depending on temperature and germination medium (Fig. 1). At 21 C, germination on water agar and on celery leaves did not begin until 8 and 12 hr, respectively, after inoculation. Germination rate on leaves was faster at 25 C than at 21 C for the first 24 hr but was similar at both temperatures (about 80%) after 36 hr. Germination occurred significantly earlier and faster on water agar than in distilled water at both temperatures. Differences in germination on water agar and on leaves were not significant. Germination of ≥95% was achieved on water agar 36 hr after plating, whereas germination was only 40–60% in distilled water.

Effect of dew period on infection. Numbers of lesions per leaflet on plants receiving 24 hr of continuous or interrupted wetness at 21 C were low (one or two lesions per leaflet) and not significantly different from plants receiving 12 hr or less of continuous dew (Fig. 2). However, with 36 hr of continuous dew or 48 hr of continuous or interrupted dew, lesion numbers were >13 per leaflet, which was significantly different from other treatments. First lesions were detected 10 days following inoculation. No lesions occurred on celery plants kept in the dew chamber <12 hr at 21 C, then incubated in a growth chamber at the same temperature for 21 days.

At 25 C, lesion numbers increased with increasing leaf wetness duration, but there was a maximum of 2.5 lesions per leaflet after exposure to 48 hr of continuous or interrupted leaf wetness after 21 days (Fig. 3), which was much lower than lesion numbers resulting at 21 C (Fig. 2). Dew periods of 48 hr (continuous or interrupted) or 36 hr were required to obtain one or more lesions per leaflet at 25 C. First lesions were detected after 9 days.

All batches of conidia prepared for inoculations germinated >90% after 36 hr of incubation at 21 C on water agar.

Timing sprays with leaf wetness. The fact that only very low numbers of lesions occurred on celery plants exposed to ≤12 hr of leaf wetness in dew chamber studies suggested that if celery in the field was exposed to ≤12 hr of leaf wetness, risk of infection would be very low. Field studies were carried out to test this hypothesis. Using 12 hr of leaf wetness as the threshold for spray application of fungicide resulted in two fewer sprays per season than when plots were sprayed weekly over 3 yr, without any loss in effectiveness of control (Table 1). Nonsprayed plots and guard rows between treatment rows were severely diseased in each year. In 1993, Septoria blight spread an average of 4.3 m along the unsprayed rows from the point of inoculation over 8 wk. There was essentially no spread along rows sprayed weekly or according to leaf wetness.

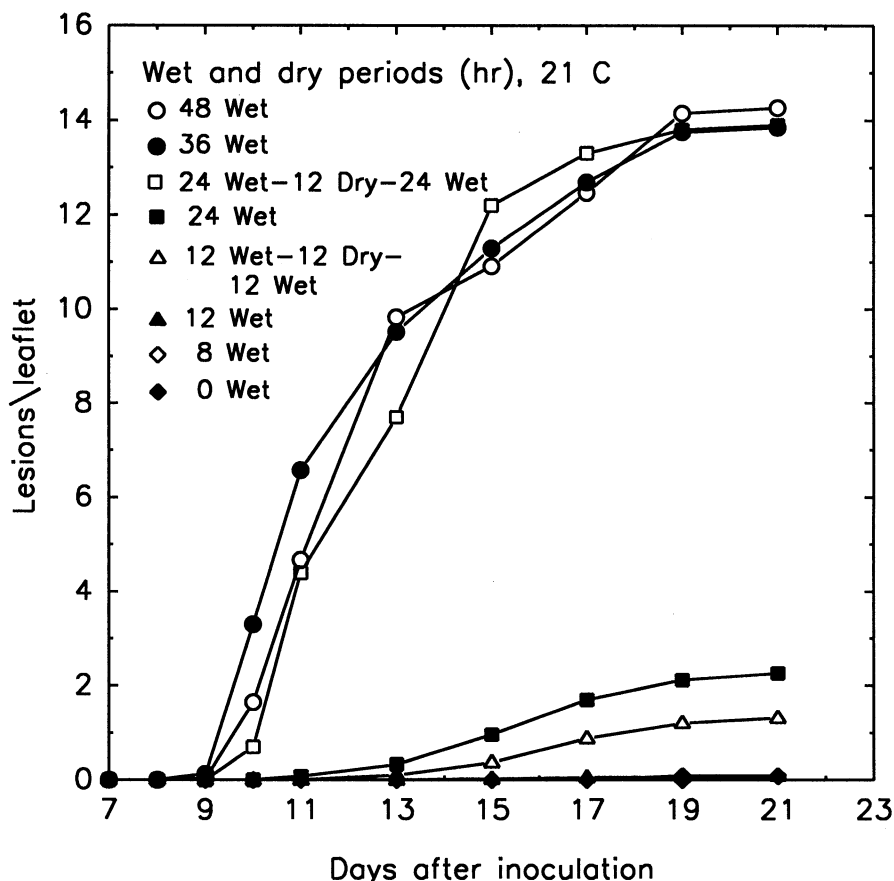

Fig. 2. Numbers of lesions per leaflet resulting from continuous leaf wetness periods of 0, 8, 12, 24, 36, or 48 hr and from two interrupted wet periods (12 hr wet/12 hr dry/12 hr wet and 24 hr wet/12 hr dry/24 hr wet) at 21 C followed by incubation in a growth chamber at 21 C with continuous light for 60 hr following inoculation and a 14-hr photoperiod thereafter.

DISCUSSION

In Sheridan's (17) work, conidial germination began sooner at similar temperatures (20% after 6 hr at 20 C) than in this work (0% after 6 hr and 10% after 8 hr at 21 C); however, >92% germination was achieved after 36 hr in both studies. The difference in onset of germination was probably due to conidial age and history, since Sheridan collected conidia from freshly gathered diseased leaves, and conidia in this study were obtained from pycnidia on diseased leaves collected in late summer and stored for up to 6 mo at 2 C until used. Sheridan (17) found that *S. apiicola* conidia collected from infected leaves germinated readily in distilled water but that germination of conidia produced on potato-dextrose agar was highly variable.

Previous studies (5,17,18) reported that conidial germination and infection occur at humidities ≥97%. Schein (16) pointed out that a decrease in temperature of 1 C (which commonly occurs in cyclic operation of refrigeration units in incubators) would lower temperature below the dew point and condensation would occur on surfaces (including fungal conidia). Unless ambient temperatures could be controlled within ±1 C in incubation chambers, condensation of water would occur at high humidities. Montesinos and Vilardell (14) observed that conidia of *Stemphylium vesicarium* germinated on glass surfaces only at RH ≥98%, and that a minute layer of condensation was observed on conidia-laden glass coverslips at these humidities but not at lower humidities where germination did not occur. All germination and infection studies carried out in this work were done with conidia in a film of free water in a dew chamber, on agar, or on glass slides within a petri dish moist chamber and provide unambiguous evidence of spore germination and infection in free water, whereas results of studies done at "high humidities" (17) or at various RH percentages (18) left doubt as to whether conidia were in contact with water.

In conidial suspensions in distilled water prepared from dried, field-collected celery leaves and incubated on hanging drop slides, motile bacteria and paramecia became visible within 12 hr and were quite prevalent within 24 hr. These organisms were very likely absorbing nutrients present in the cirrhi (2) and were serving as a nutrient sink, depriving

conidia of nutrients otherwise available to support germination. This may account for the lower level of germination in water than on celery leaves or on agar plates.

Sheridan (18) reported that no infections occurred on celery plants incubated for 24 hr in continuous high humidity following inoculation. In this study, a maximum of approximately two lesions per leaflet developed following a 24-hr wetness period, and numbers of lesions increased dramatically with increasing duration of wetness period (Figs. 2 and 3). Sheridan (18) did not make clear whether leaves in the humidity chamber remained wet.

In this study, fewer lesions occurred at 25 C than at 21 C, using the same conidial concentrations and with similar conidial viability, in contrast to the work of Mathieu and Kushalappa (13), who reported higher numbers of lesions at 25 C than at 20 C. However, they moved all plants incubated in mist chambers at 10, 15, 20, 25, or 30 C into growth chambers at 20 C for symptom development. In these experiments, plants were incubated at the same temperatures (21 or 25 C) in the dew chamber as in the growth chamber.

Disease was severe each year in inoculated celery field plots, with 45–50% of leaf area diseased in unsprayed plots. This level of disease in unsprayed controls led to severe petiole infections that made the crop unmarketable. The fact that only trace levels of disease were seen in plots sprayed when leaf wetness periods were ≥12 hr is indicative of the usefulness of leaf wetness in timing sprays for Septoria leaf blight control. Two new automated field weather instruments, Field Monitor (Sensor Instruments, Concord, NH) and Envirocaster (Neogen Food Tech, Lansing, MI), measure daily leaf wetness as well as other weather parameters. Envirocaster can be programmed to schedule sprays for several other diseases. These instruments should be useful to celery growers in timing sprays based on leaf wetness periods for maximum effectiveness.

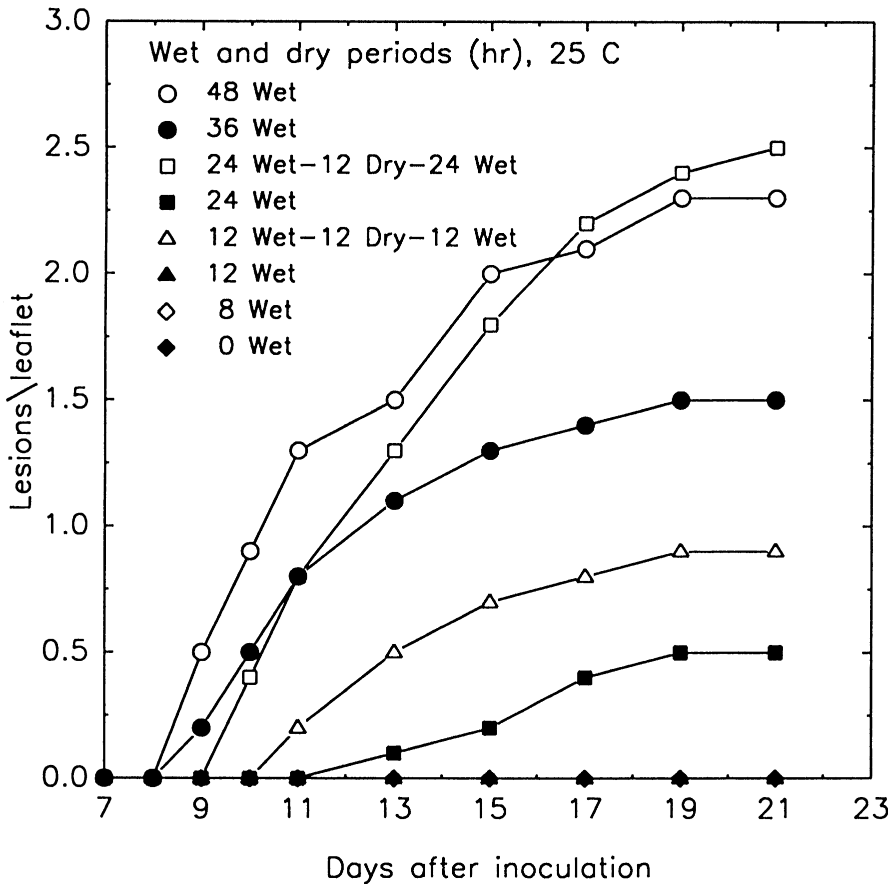

Fig. 3. Numbers of lesions per leaflet resulting from continuous leaf wetness periods of 0, 8, 12, 24, 36, or 48 hr and from two interrupted wet periods (12 hr wet/12 hr dry/12 hr wet and 24 hr wet/12 hr dry/24 hr wet) at 25 C followed by incubation in a growth chamber at 25 C with continuous light for 60 hr following inoculation and a 14-hr photoperiod thereafter.

Table 1. Disease severities and yields in celery plots sprayed according to leaf wetness periods or weekly with chlorothalonil

Year Spray[a]	No. of sprays	Disease[b] (%)	Yield[c] (kg/ha)
1991			
Timed	5	0.1	58,581
Weekly	7	0.1	68,868
Nonsprayed	0	45.0*[d]	17,864*
1992			
Timed	5	0.1	51,867
Weekly	7	0.0	50,204
Nonsprayed	0	52.5*	17,494*
1993			
Timed	5	0.0	61,600
Weekly	7	0.0	59,444
Nonsprayed	0	52.5*	17,864*

[a] Plots were sprayed with chlorothalonil (Bravo 720, 720 g a.i./L) at 1.75 L/ha (1.5 pt/acre). Timed plots were sprayed after leaf wetness periods reached or exceeded 12 hr, provided that no fungicide had been applied during the previous 7 days.

[b] Percentage of leaf area with Septoria leaf blight lesions was estimated visually.

[c] Weight of celery trimmed for packing.

[d] * = Significantly different from sprayed treatments ($P = 0.05$) in a given year. Sprayed treatments were not significantly different from each other in a given year.

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