Effects of Duration, Frequency, and Temperature of Leaf Wetness Periods on Soybean Rust

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ABSTRACT

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On soybean leaves at 20 C in the dark, uredospores of *Phakopsora pachyrhizi* began germinating 1.5 hr after dew was provided and reached a maximum level after 6–7 hr. Susceptible soybeans inoculated with viable uredospores developed no rust at dew periods less than 6 hr. At 6 hr, trace levels of primary rust lesions developed at 18, 20, 23, and 26.5 C. After 8 hr of dew at 18–26.5 C, lesion intensities were 10-fold higher than those at 6 hr at the corresponding temperatures. Increasing dew duration from 12 to 16 hr resulted in no significant increase in rust intensity, even at the most favorable temperatures (18–26.5 C). No lesions developed at 9 and 28.5 C, even with dew periods as long as 20 hr. Uredospores on unwetted soybean leaves progressively lost infectivity during sunshine conditions, but exhibited enhanced infectivity during 1 or 2 days on dry foliage under cloudy conditions. After 8 days on dry foliage, no uredospores caused lesions following a 12-hr dew period at 18 C. Spores on leaves exposed to 4 or 6 hr of dew followed by drying for up to 4 days were able to infect when a 12-hr dew period was provided, but were 50% or less as infectious as similar spores that had not been exposed to brief initial wetting. Uniformly inoculated soybean plants given initial dew and then receiving dew every third, sixth, or ninth day after inoculation until maturity developed fewer lesions per square centimeter and higher yields with decreasing frequency of dews. Rust reduced numbers of filled pods, numbers of seeds per pod, and mean seed weight.

Additional keywords: *Glycine max*

Soybean rust, caused by the fungus *Phakopsora pachyrhizi* Sydow, has been known since the early 1900s in the Eastern Hemisphere, where it causes serious yield reductions (3,9,10). The pathogen was first reported on soybean (*Glycine max* (L.) Merr.) in the Western Hemisphere in 1976 when a weakly aggressive strain was found in Puerto Rico (23). It has since been reported in Brazil (5), Colombia (7), and Costa Rica (3). No confirmed reports of soybean rust within the continental United States or Canada are known. Commercial soybean cultivars in the United States are susceptible to Eastern Hemisphere isolates of the pathogen (8,18).

This research is part of our program to assess the potential threat of foreign pathogens to major U.S. crops. The objectives of this study were: 1) to determine the influence of temperature and duration of leaf wetness on infection and rust development, 2) to compare continuous with interrupted periods of wetness in relation to subsequent disease development, 3) to determine the effect of varying the time interval between inoculation and wetness period on infection and lesion development, and 4) to compare effects of different frequencies of dew periods on rust buildup and yield. The study was conducted within the pathogen containment facilities of the Foreign Disease-Weed Science Research Laboratory. Preliminary results were reported earlier (17).

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MATERIALS AND METHODS

Test plants. Seeds of soybean cultivar Wayne were planted in 10-cm-diameter clay pots containing a steam-pasteurized potting mixture of loam soil, sand, peat, and vermiculite (2:1:1:1, v/v). Plants were thinned to one per pot and selected for uniformity before inoculation. In studies requiring mature plants, wooden boxes 1.23 m wide × 2.46 m long filled to a depth of 0.26 m with potting mixture were planted with seeds in rows 0.46 m apart. Emerging plants were thinned at the 2–3 leaf stage to 20 plants/m within rows. Plants were grown in a glasshouse at day/night temperatures and relative humidities of 25–30/20–24 C and 43–55/52–68%, respectively.

Inoculum. The pathogen, from Taiwan (19), was maintained on soybean cultivar Wayne. Inocula used in these studies were uredospores either freshly harvested from sporulating pustules or from liquid nitrogen storage. Spores from storage were heat shocked at 41 C for 6 min and hydrated for 8–16 hr before use.

Inoculation. When the fourth trifoliolate leaves were fully expanded, plants in pots were inoculated in a turntable settling tower (16). Plants grown in planting boxes were covered with a plastic tent and a cloud of spores was produced within the tent from a disseminator attached to a CO_2 pistol (16).

Dew period. After inoculation, plants in pots were placed in dew chambers (11) at selected temperatures for selected periods of time. Plants in planting boxes were enclosed by polyethylene plastic film held by a supporting framework of tubular plastic pipe, and were atomized intermittently with water to maintain leaf wetness throughout the specified "dew period." All dew periods took place during continuous darkness.

In this paper, the term "dew" is used to describe liquid water on leaf surfaces regardless of the manner in which the water was deposited on the surfaces.

Spore germination and estimates of spore deposition density. Uredospores were seeded on 1.25% water agar in 5-cm-diameter culture plates and on soybean plants in a turntable settling tower (16). Seeded plates and plants were immediately placed in dew chambers at 20 ± 0.4 C for 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, or 16 hr. Four plates and four sample leaves were removed at each specified time. Plates were placed into desiccators containing formaldehyde to stop biological activity. Leaves were placed on filter paper saturated with Carnoy's solution (absolute ethyl alcohol, glacial acetic acid, chloroform, 6:1:3, v/v) in covered 9-cm-diameter glass dishes. A minimum of 250 spores per plate were examined to determine germination percentages. Twenty microscopic fields (100×) on one leaflet from each sample leaf were examined to determine spore germination percentages. A spore was considered germinated if the length of the germ tube equalled or exceeded the minor diameter of the spore. Higher magnification using a calibrated ocular micrometer was used for measuring germ tube length.

In all other studies, 1.25% water agar

Table 1. Soybean rust lesions on Wayne soybeans 14 days after inoculation with uredospores of *Phakopsora pachyrhizi* and placement in dew chambers at 10 different temperatures for 6, 8, 12, or 16 hr[y]

Dew period temperature (C)	Dew duration (hr)			
	6	8	12	16
9	0.00[z]	0.00	0.00	0.00
10	0.00	0.00	0.02 ± 0.0075	0.05 ± 0.0086
12	0.00	0.01 ± 0.0048	0.04 ± 0.0083	0.22 ± 0.0253
14	0.00	0.01 ± 0.0052	0.16 ± 0.0191	1.12 ± 0.1200
16	0.00	0.03 ± 0.0075	4.06 ± 0.3221	4.50 ± 0.5218
18	0.03 ± 0.0083	0.61 ± 0.0571	8.20 ± 0.8457	8.70 ± 1.0009
20	0.04 ± 0.0075	0.48 ± 0.0484	6.80 ± 0.7800	7.51 ± 0.8909
23	0.03 ± 0.0065	0.41 ± 0.0412	7.25 ± 0.5549	8.09 ± 0.8970
26.5	0.03 ± 0.0059	0.35 ± 0.0384	6.70 ± 0.5531	6.95 ± 0.9226
28.5	0.00	0.00	0.00	0.0

[y]Spore viability was 51.7% and infection efficiency at highest disease level (8.70 lesions/cm^2) was 2.03%.

[z]Each value is the mean number of lesions per square centimeter of leaf tissue examined. There is a total of 36 leaflets for each temperature-time combination (the first, second, and third trifoliolate leaves on each of four replicate plants). Each mean is followed by the confidence level at $P = 0.05$.

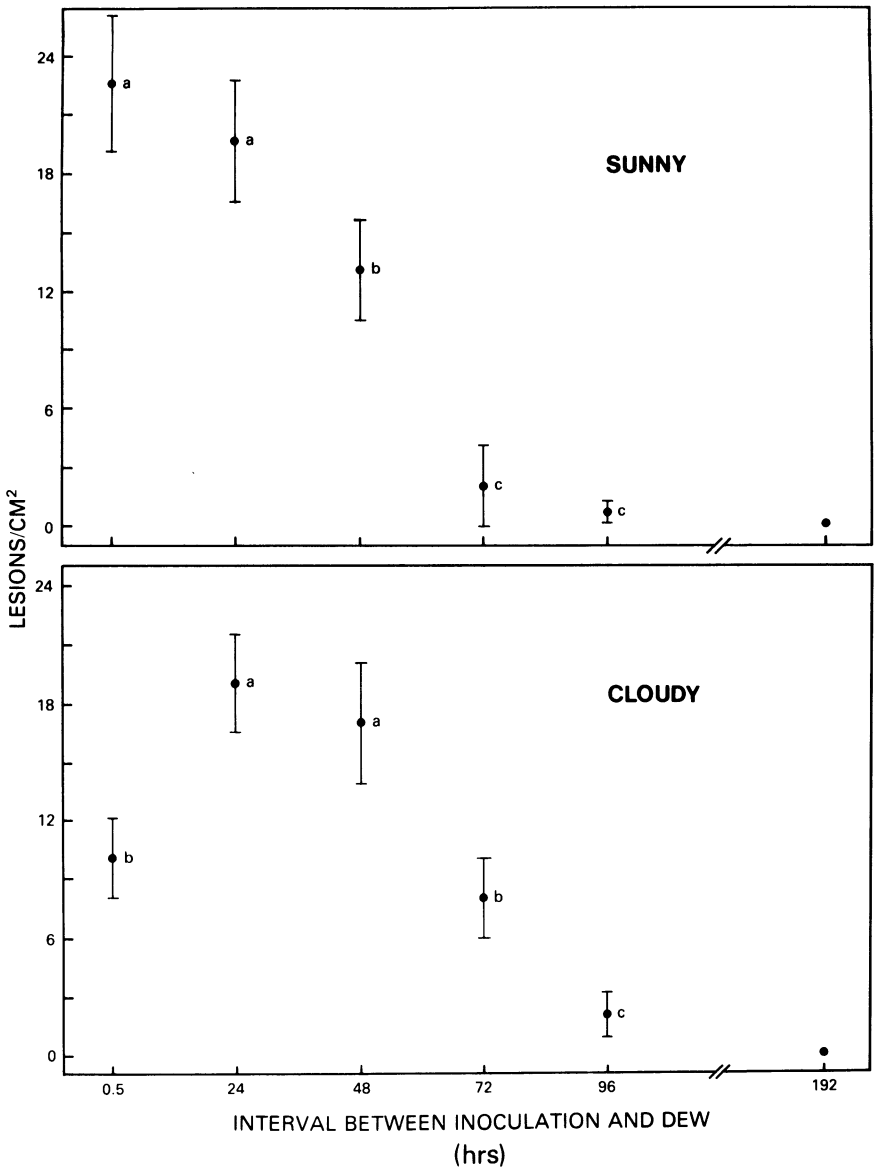

Fig. 1. Soybean rust lesion intensity on soybean cultivar Wayne inoculated with uredospores of *Phakopsora pachyrhizi* and provided with a single, 12-hr dew period at various times after inoculation. Dew temperature was 18 C. The curve labeled "sunny" indicates that during the 4 days following inoculation, the plants were exposed to bright sunshine in the glasshouse; "cloudy" refers to overcast conditions during the 4-day period following inoculation. Data points are mean values of four replicates, vertical bars represent standard deviation, and different lowercase letters indicate that means were significantly different at $P = 0.05$ using Duncan's multiple range test.

plates were seeded during the inoculation of plants to provide estimates of inoculum viability and density of spore deposition. Four replicate plates were used per inoculation. Estimates of spore deposition density were made by counting spores in 20 microscopic fields (100×) on each plate. The mean value was later used to calculate the infection efficiency, based on the highest observed lesion density on plants from the inoculation.

Postdew period. Plants in pots were removed from dew chambers and held in glasshouses under environmental conditions described previously. Some of these plants were returned to dew chambers for additional wetness exposures, as described later for specific experiments. At the end of the required dew periods, the polyethylene tents were removed from the large planting boxes, exposing the plants to the normal glasshouse environment.

Temperature and length of single immediate dew period. Soybeans were inoculated and then placed immediately in dew chambers at temperatures of 9, 10, 12, 14, 16, 18, 20, 23, 26.5, or 28.5 C (± 0.4 C) for selected periods ranging from 4.5 to 20 hr. Four replicate plants were used for each temperature-time combination. Lesions were counted on the first, second, and third trifoliolate leaves of all plants 14 days after inoculation. All leaflets were measured and areas were estimated by use of the formula: A = length × width × 0.76. This nondestructive method was derived from comparisons of leaflet areas calculated by formula with areas of the same leaflets measured by photocell area meter; in a comparison of 100 leaflets, the areas by the two methods were within ± 5% of each other. Lesion counts were converted to a single value for each treatment—the mean number of lesions per square centimeter of suscept tissue evaluated. Lesion intensity was so expressed in all studies.

Delayed dew period following inoculation. Inoculated plants were held in a glasshouse for 1, 2, 3, 4, 5, 6, 7, 8, or 9 days before being placed in dew chambers for 12 hr at 18 C. Four replicate plants were used for each treatment within each experiment. Four inoculated plants were placed in dew chambers immediately after inoculation, then were held in the glasshouse to provide a nondelay comparison.

Delayed dew studies were done during periods of cloudy weather and during periods of sunny weather extending 4 days following inoculation. Light measurements at plant level in the glasshouse were taken at 1200–1215 hr during both cloudy and sunny days using an IL 150 plant growth photometer (International Light, Inc., Newburyport, MA). Readings were in μw/cm^2/nm taken at three spectral bandwidths: blue, 400–500 nm; red, 600–700 nm; far red,

700–800 nm. Average readings for the sunny day study were 40, 19, and 20 for the blue, far red, and red regions, respectively. Corresponding readings for the cloudy day study were 9.8, 4.5, and 4.8. The glasshouses were air conditioned. Air temperatures and relative humidities were similar during the two studies (day and night averages were within 3 C and 8% of each other).

Interrupted dew periods. Immediately following inoculation, plants were placed in dew chambers at 18 C for 4, 6, or 12 hr, then were held in the glasshouse for 1, 2, 3, or 4 days before a 12-hr dew period at 18 C was provided. Four replicate plants were used for each treatment within each experiment.

Different frequencies of dew periods. Four groups of 60 soybean plants (three rows of 20 plants each) were grown in spatially separated sections of large planting boxes as described previously. Three of these groups were inoculated at the same time (as the fifth trifoliolate leaves were expanding) and then were immediately provided with 12 continuous hours of dew at 20 ± 1.5 C. The fourth group was not inoculated but was subjected to the dew treatment. Following this initial dew period, one inoculated section was provided dew every third day thereafter, another every sixth day, and the remaining section every ninth day. The uninoculated section was given dew every sixth day. All dew periods were from 2000 hr until 0800 hr at 20–24 C. Before each dew period, the plants within each plastic-enclosed section were agitated by air from a low-speed blower for 1 min to distribute the spores.

Three plants within each row (excluding the plants at each end) were randomly selected and marked with stakes before inoculation. At 20, 36, and 54 days after inoculation, these selected plants were assessed for 1) number of leaves, 2) leaf areas, and 3) number of lesions.

At maturity, all pods on all plants were hand-harvested and the numbers of abortive pods, pods with seeds, and numbers of seeds were determined. The shelled beans were air-dried to 16% moisture content and the mean seed weights were recorded.

Data analysis. Data were analyzed by analysis of variance and means were separated using confidence levels or Duncan's multiple range test. Disease severity was plotted as a function of time (dates of reading) and analyzed by linear regression in the frequency of dew study.

RESULTS

Uredospore germination. Germ tubes averaged 4 and 8 μm in length after 0.5 and 1 hr, respectively, on agar. On leaves, after 1 hr, germ tubes averaged 6 μm. These spores were, by definition, not germinated because the germ tubes were less than the minor diameter of the spore. After 1.5 hr, germination was 21% on agar and 12% on leaves. By 3 hr, germination on agar had attained 91% of its maximum (58% after 5 hr), but on leaves by 3 hr, spore germination had reached only 63% of its maximum (55% after 6 hr). No increase in germination percentages occurred with incubation periods longer than 6 hr on either agar or leaves.

Temperature and length of single, immediate dew period. No lesions developed at 9 or 28.5 C, even with a 16-hr dew period (Table 1). With a 6-hr dew, a few rust lesions (0.03–0.04/cm^2) appeared only on plants from the 18, 20, 23, and 26.5 C treatments. The 8-hr dew period resulted in lesion development over a broader temperature range of 12–26.5 C, with only trace amounts at 16 C and below and 0.35–0.61 lesions/cm^2 at 18 C and above. At 12- and 16-hr wetness periods, a few lesions developed on plants from the 10 C temperature. From 10 to 14 C, a significant ($P = 0.05$) increase in rust occurred with increased duration of dew period up through 16 hr. From 16 to 26.5 C, a significant increase in rust occurred with increased dew duration up through 12 hr, with no significant increase between 12- and 16-hr values. No significant differences in rust development occurred among plants from the most favorable dew temperatures (18, 20, 23, and 26.5 C).

Studies similar to the one above were done using dew periods of 4.5, 5, 5.5, 6, 6.5, 7, 8, 12, 16, and 20 hr at temperatures of 18, 20, 23, and 26.5 C. Rust lesions never developed with periods shorter than 6 hr, and in some studies 7 hr was the shortest wetness period that resulted in lesion development. No increase in lesion intensity occurred when dew periods increased from 16 to 20 hr.

Delayed dew period. When inoculated plants were held in the glasshouse during periods of bright sunshine before dew was provided, there was a consistent decrease in lesion intensity as the time between inoculation and dew increased (Fig. 1). Compared with rust on plants that received dew immediately after inoculation (0.5 hr), delays of 24, 48, 72, and 96 hr before dew resulted in 86, 57, 11, and 1.3% as much disease, respectively.

When cloudy, overcast days followed inoculation, delays of 24 and 48 hr before dew resulted in higher lesion intensities than did the immediate dew treatment (Fig. 1). A 3-day delay showed a marked decrease in disease, and after 4 days lesion intensity was only 9.8% of the maximum observed. Regardless of radiation patterns beyond 3 or 4 days after inoculation, only an occasional lesion developed on plants receiving dew 7 days after inoculation, and no rust occurred when dew was delayed for 8 days or longer.

For the cloudy and sunny day studies, the inoculum spore viabilities were 46.5 and 42.4%, respectively, and the corresponding infection efficiencies (for the highest disease levels attained in each study) were 3.12 and 3.44%, respectively.

Interrupted dew periods. A single, 12-hr dew period immediately after inoculation resulted in 28 lesions/cm^2 (Table 2), whereas a single, immediate 6-hr dew period produced only 0.1 lesions/cm^2. When a second dew period of 12 hr was provided after dry period interruption intervals of 24 to 96 hr, lesions per square centimeter were increased but remained about half the number of lesions that occurred when single, 12-hr dew periods were provided after dry period delays.

Different frequencies of dew periods. Nine to 10 days after inoculation, incipient uredia in rust lesions were detected on plants within the three inoculated sections. By 10–11 days, 50% or more of the uredia were sporulating. By 20 days after inoculation, lesions were present on the first six trifoliolate leaves of inoculated plants from all three dew treatments (Fig. 2). By 36 days, the first leaf had abscised, and rust occurred on

Table 2. Soybean rust lesions on Wayne soybeans after inoculation with uredospores of *Phakopsora pachyrhizi* and placement in dew chambers at 18 C for one or two dew periods of selected duration[x]

Interval between inoculation and first dew (hr)	First dew period (hr)	Interval between first and second dew (hr)	Second dew period (hr)	Rust lesions[y] (av. no./cm^2)
0.5[z]	6	...	...	0.1 f
0.5	12	...	...	28 a
24	12	...	...	23 a
48	12	...	...	15 b
72	12	...	...	5 c
96	12	...	...	2 d
0.5	6	24	12	11 b
0.5	6	48	12	7 c
0.5	6	72	12	2 d
0.5	6	96	12	0.4 e

[x]Spore viability was 67.3% and infection efficiency at highest disease level (28 lesions/cm^2) was 3.76%.

[y]Rust lesions were counted on all plants 14 days after inoculation. Mean values followed by different lowercase letters are significantly different from each other as determined by Duncan's multiple range test ($P = 0.05$).

[z]When no intentional delay was desired between inoculation and dew, the plants were placed within dew chambers by 15–30 min after inoculation.

all but the topmost one or two expanded leaves in all the inoculated treatments. At this time, there were large differences in disease intensities between the oldest and youngest leaves present, with a generally consistent gradient between the two extremes. About 1.5–3 times as many lesions occurred on leaves at any selected position on plants receiving dew every third day compared with lesions on corresponding leaves of plants receiving dew every sixth or ninth day.

By 54 days after inoculation, disease gradients between the younger and older leaves on plants within each dew treatment had diminished. The first four trifoliolate leaves had abscised on all plants receiving dew every third day, but only the first three leaves were completely missing on plants from the other two treatments. The uninoculated control plants had about 50% of their third leaves remaining.

The linear regression coefficients for average rust intensities per plant on time (three dates of evaluation) were 0.97, 1.34, and 1.77 for the inoculated plants from the ninth, sixth, and third day dew treatments, respectively (Fig. 3). By the last date of evaluation (54 days after the primary inoculation), plants from the third, sixth, and ninth day dew treatments averaged 65, 54, and 40 lesions/cm^2, respectively.

Desiccation of diseased tissue and premature defoliation of plants became severe by 60 days after inoculation, ranging from 60% in the third day dew treatment to about 40% in the ninth day treatment. Plants in the uninoculated control group had lost about 20% of their leaves.

Numbers of pods containing seed, numbers of seed, and mean seed weight were significantly reduced on rusted compared with nonrusted plants (Table 3). More abortive pods were produced on rusted than on nonrusted plants. Plants from the third, sixth, and ninth day dew treatments yielded 19, 34, and 45%, respectively, as much as the control plants (extrapolated yield 3,834 kg/ha).

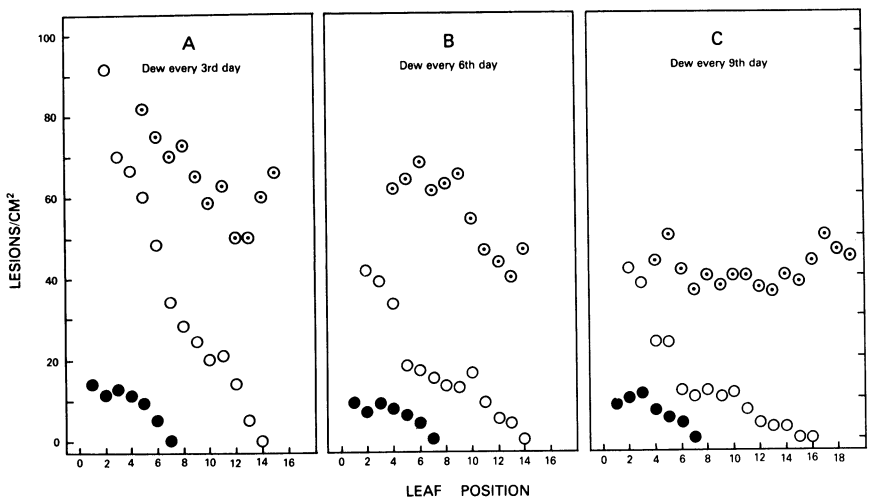

Fig. 2. Comparison of the soybean rust intensities among three groups of Wayne soybean plants all initially inoculated with uredospores of *Phakopsora pachyrhizi* and provided immediately with a 12-hr dew period, then provided with different numbers of dew periods. Lesion intensities are shown by leaf position along the main stem at 20, 36, and 54 days after the initial inoculation for **(A)** plants receiving a 12-hr dew every third day following the initial dew period, **(B)** plants receiving a dew period every sixth day, and **(C)** plants receiving dew every ninth day. Disease readings at 20 days (closed circle), 36 days (open circle), and 54 days (circle with dot) after initial inoculation.

Table 3. Effect of frequency of dew periods on yield of Wayne soybeans inoculated with uredospores of *Phakopsora pachyrhizi*

Treatment	Mean number per plant of:[y] Pods with seeds	Abortive pods	Seeds	Mean seed weight (g)	Extrapolated yield (kg/ha)[z]
		Inoculated			
Dew every third day	12.4 a	3.1 a	20.1 a	0.087 a	729 a
Dew every sixth day	15.8 b	2.9 ab	32.5 b	0.096 ab	1,301 b
Dew every ninth day	16.7 b	2.3 b	36.2 b	0.115 b	1,736 b
		Uninoculated			
Dew every sixth day	23.8 c	0.3 c	50.5 c	0.182 c	3,834 c

[y] Means were from 60 plants in each treatment. Data were processed by analysis of variance and Duncan's multiple range test. Means within each column followed by a different lowercase letter are significantly different from each other ($P = 0.05$).

[z] Extrapolation to kilograms per hectare, assuming 4.3×10^5 plants per hectare and adjusting to 13% moisture content.

DISCUSSION

A minimum of 6–7 hr of continuous leaf wetness was required for soybean rust lesions to develop at favorable dew temperatures (18–26.5 C) when viable uredospores were present on susceptible soybean. These results are similar to those reported from Japan (10), Australia (9), and preliminary work in our laboratory (14,17). The minimum dew temperature that permitted lesion development was 10 C. At minimum dew temperature-time combinations, the number of lesions that developed appears negligible compared with the numbers produced at the optimal dew periods—on the order of 0.1–0.5% as much rust at the minimum compared with optimum dew period conditions. The significance of these limits is that, in a temperate climate, the lower the temperature and the shorter the dew period required to enable infection by a rust pathogen, the more frequently the permissible environmental periods will likely occur during early development of the crop. Throughout the major soybean-producing areas of the United States, late spring and early summer dew conditions would permit rust initiation and maintenance if viable uredospores were present on the crop.

The upper dew temperature that permitted infection from uredospores was 26.5 C. Dew temperature above 26.5 C drastically limits infection. No lesions developed at 28.5 C with 16 hr of dew, indicating a very sharp decrease from the near-optimum disease occurrence at the just slightly (2.0 C) cooler dew period. This upper temperature limit is probably of little practical importance, as wetness periods (especially dew) on soybean foliage during the growing season would rarely occur when air temperatures were averaging 27 C or higher.

Dew conditions permitting nearly optimal penetration and lesion development (12 hr of dew from 16–26.5 C) occur frequently in the United States from mid-May throughout the season. These dew requirements are similar to those for optimum pustule formation on oats inoculated with uredospores of *Puccinia coronata* f. sp. *avenae*, the cause of crown rust on oats (22) and for stem rust on wheat (11).

Uredospores of *P. pachyrhizi* on field-collected leaves lost their germinability after 30–40 days of laboratory storage (10). Spores of an Australian isolate caused infection when, 2 days after being deposited on unwetted leaves, they were provided with a 24-hr dew period (9). In

the present studies, an immediate, progressive decline in infectivity was found in some tests when dry uredospores were held on unwetted leaf surfaces on plants in the glasshouse. In other tests, an initial increase in infectivity of spores held on unwetted foliage was observed, then a progressive decline began. Whether an initial increase or decrease occurred depended on the solar radiation intensity and duration during the several days following inoculation. Elevated leaf surface temperature and/or radiation per se may have caused the immediate decline noted when sunny days followed inoculation. Conidia of *Entomosporium mespili* on photinia leaves exposed to bright sunshine outdoors for 24 or 36 hr before dew caused only 2–3% as many leaf spots as conidia on leaves held in dew for 24 hr immediately following inoculation; short exposures (5 or 12 hr) on partly sunny days sometimes resulted in more leaf spotting than on the control plants (1). A study (13) in which spore temperature was controlled during exposure to direct sunlight or sunlight through various filters (including glass) indicated that a full day's exposure during sunshine reduced the germinability of uredospores of *Puccinia striiformis* Westend. to less than 0.1% of the shaded control spores. Spores exposed beneath a glass filter (which cut off virtually all radiation below 310 nm) were reduced in germinability compared with shaded control spores, 95 vs. 36% and 90 vs. 0.5% for 9 hr and 3 consecutive days' exposures, respectively. Compared with full sunlight, the glass filter provided some protection. However, visible radiation significantly reduced germinability of exposed spores even where temperature was removed as an influencing factor.

The increased infectious efficiency of uredospores of *P. pachyrhizi* during 1 or 2 days on dry foliage during cloudy weather may have been due to a hydration effect. Lower temperature and higher vapor pressure within the boundary layer near the leaf surface during cloudy conditions (compared with sunny) may have permitted hydration of the spores before the dew period. Spores of several fungi exhibited increased germinability following hydration (24). Once the decline in infectivity began in the cloudy day conditions, the extinction curves were similar for both sunny and cloudy situations. These tests were performed in the glasshouse, and spores exposed to direct sunlight on dry foliage in the field would probably lose infectivity much more rapidly. On shaded leaves or during extended cloudy weather, however, some of the uredospores would be capable of infecting after a week on dry foliage if an adequate dew period occurred.

Brief wetting, insufficient or barely minimal for infection, then drying of uredospores on soybean foliage diminished rust development when adequate dew periods were provided later. Some of the germ tubes and/or appressoria present after the brief wetting are not killed during 1–3 days on "dry" foliage and can proceed to penetrate when an adequate dew period is provided. On soybean leaves, nearly all viable uredospores of *P. pachyrhizi* germinate at favorable temperatures within a 6-hr dew period, and 40–50% of the germinated spores will form appressoria; penetration begins at about 6 hr after inoculation (2). Reduced infectivity caused by the brief dew period might be due to the death primarily of germinated spores that had not formed appressoria before drying occurred. The critical factor in survival might be the formation of the septum separating the germ tube from the appressorium. It has been suggested (6) that appressoria might play an auxiliary role as short-term survival structures, having the capacity to endure adverse conditions that are potentially lethal to unprotected germ tubes. In some rust-causing fungi, ultrastructural studies have shown that the germ tube wall is a single layer, continuous with the inner wall of the parent spore, but the appressorium has a bilayered wall and is separated at maturity from the germ tube by a nonperforate septum (12). In *P. pachyrhizi*, the formation of the septum between germ tube and appressorium is well documented by light microscopy (9,10,15).

The time required to effect leaf penetration from most of the appressoria

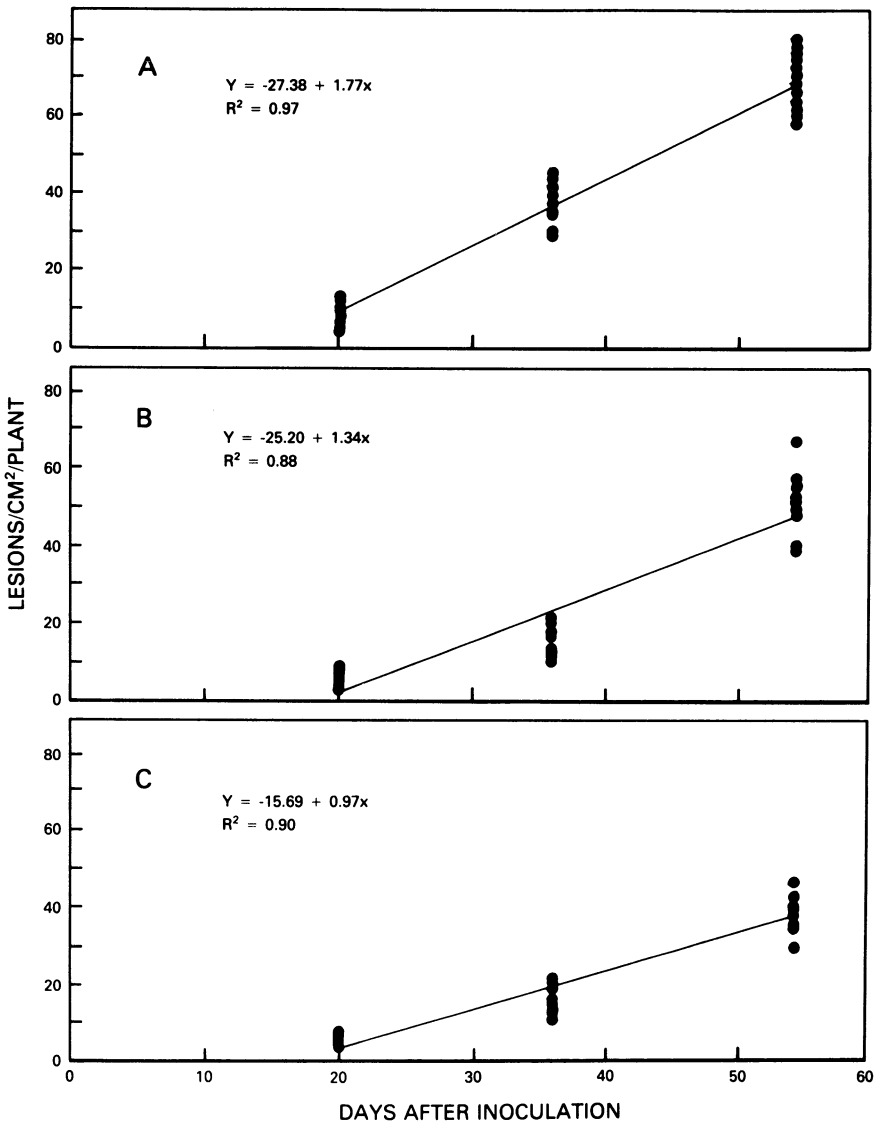

Fig. 3. Increase in average soybean rust intensity levels during 20–54 days after primary inoculation among three groups of Wayne soybean plants all initially inoculated with uredospores of *Phakopsora pachyrhizi* and provided immediately with a 12-hr dew period, then provided with **(A)** dew every third day after the initial dew period, **(B)** dew every sixth day, and **(C)** dew every ninth day. Means based on nine replicates. Linear regression coefficients were significantly different ($P = 0.05$ between third and sixth day and between sixth and ninth day dew treatments, and $P = 0.01$ between the third and ninth day dew treatments, as determined by t test). Viability of spores comprising initial inoculum was 36.1%, and infection efficiency (based on primary disease level at 20 days after primary inoculation in plants in the sixth day dew treatment) was 1.46%.

at favorable moisture and temperature was reported to exceed 24 hr for an Australian isolate of *P. pachyrhizi* (15). However, earlier work with an isolate from Taiwan showed no increase in penetrations after about 9 hr following inoculation (2). The present study also indicates that nearly all penetrations had occurred by 12 hr. The earlier work (2) also demonstrated that, whereas no further increase in penetrations occurred after 9 hr of dew, there was a large increase in number of lesions as the dew period was increased from 9 to 12 hr and there was a smaller increase between 12 and 16 hr. Although the penetrations were present by 9–10 hr, additional dew exposure apparently enhanced colonization leading to lesion formation. Significant increases in penetrations beyond 24 hr (15) conflict with results from other studies where lesion numbers did not increase as dew periods were lengthened beyond 12 (9) or 16 hr (2,14).

Soybean rust in the large planting boxes significantly reduced yield in proportion to the levels of rust intensities caused by variations in dew frequency. The environmental conditions for all groups of plants were nearly identical except for the experimental manipulation of dew periods. After the initial inoculation, subsequent natural inoculation of each group of plants was by uredospores produced on primary and secondary uredia within each group. The time from inoculation until incipient sporulation was 9–10 days. Rust intensity at 20 days after inoculation essentially reflected visible primary disease and, in fact, at this time no significant differences were evident among inoculated plants from the different dew treatments. Disease levels 36 days after inoculation reflected primary as well as secondary infections, the latter initiated between 12 and 27 days after primary inoculation. By 54 days after the original inoculation, the effects of the different dew frequencies on rust development were clearly evident, and differences in disease levels were significant among all groups.

At the onset of sporulation, soybean rust lesions are necrotic areas about 0.5 mm^2, within which are one to three uredia. The lesions enlarge to about 1.5 mm^2 at 4–5 wk after first sporulation (4,19). When lesion densities approach 35/cm^2, as occurred by 36 days after inoculation on many of the lower leaves in the dew frequency study, 18–53% of the total leaf area is composed of necrotic lesions, depending on age of lesions. Such leaves contribute little to the rest of the plant, and at 40 or more lesions per square centimeter the leaves rapidly desiccate and drop prematurely.

In agreement with field observations (10,21) and earlier work in our laboratory (18), yield reduction was characterized by fewer filled pods, fewer seeds, and lower mean seed weight. Decreased oil content has also been noted in beans from rusted plants (21).

This and previous studies (3,9,10,17) indicate that temperature and moisture conditions favorable to the soybean rust pathogen would occur in most "normal" growing seasons throughout much of the major U.S. soybean production area. Soybean rust persists as a serious disease in portions of Japan and China that are agro-climatic analogues to sections of the United States as far north as southern Kentucky (3,20). Considering infection requirements only, it seems highly probable that, in the presence of viable inoculum, rust establishment and epidemic buildup could occur during a growing season in much of the United States.

A critical question would be the source of the initial inoculum each new growing season. *P. pachyrhizi* produces abundant teliospores, but no aecial host is known (3). In the light of present knowledge, the pathogen appears totally dependent upon uredospores for initiating colonization of new suscepts. The chances of uredospores of *P. pachyrhizi* surviving the winter in the absence of living host tissue is probably negligible. Possible sources of required inocula each spring to initiate primary disease in U.S. soybean fields would be perennial weed legumes in the southern United States and airborne uredospores from rusted plantings in the Caribbean area and Mexico.

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