Infection Periods of *Gymnosporangium juniperi-virginianae* on Apple

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ABSTRACT

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Duration of leaf wetness period (LWP) required for light and severe infection of apple (*Malus pumila* 'Rome Beauty') seedlings by basidiospores of *Gymnosporangium juniperi-virginianae* were determined experimentally at 2–32 C. Infection occurred from 2 C (light infection, LWP 24 hr) to 24 C (light infection, LWP 2 hr). The optimum temperatures for infection were 10–24 C (light infection, LWP 2–5 hr; severe infection, LWP 4–6 hr). Leaves 4, 6, and 8 days old generally bore more lesions than did 10- and 12-day-old leaves, which bore very few lesions at low temperatures. Models developed to predict light and severe infection accounted for 98 and 97%, respectively, of the observed variation. The models were tested with data from LWP's monitored in the field for two yr and successfully predicted infection or no infection in 23 of 24 LWP's. The models, together with available information on requirements for teliospore germination and basidiospore formation, enable prediction of infection periods in the field, thus allowing efficient use of eradicant fungicides for the control of cedar apple rust.

Additional key words: epidemiology, disease forecast.

Gymnosporangium juniperi-virginianae Schw., the heteroecious, demicyclic incitant of cedar apple rust develops through several phases during transfer from Eastern red cedar (*Juniperuis virginiana* L.) to apple (*Malus pumila* Miller). The time-temperature relationships for the processes of teliospore germination, basidiospore formation, and basidiospore germination have been determined experimentally and modeled mathematically ([9] and R. C. Seem, *unpublished*). We now report the time-temperature relationships for the final phase of the disease; the infection of apple leaves by basidiospores.

MATERIALS AND METHODS

Basidiospores. Galls of *G. juniperi-virginianae* were collected and stored frozen as described previously (9). Frozen galls were immersed in tap water for 20 min, incubated on moist paper towels in a sealed plastic box at 20 C for 10–12 hr, and rinsed in cold (~ 5 C) sterile distilled water to obtain a suspension of basidiospores, which then was filtered through two layers of cheesecloth. The basidiospore concentration was determined with a hemacytometer and adjusted to 1×10^5 spores per milliliter with sterile distilled water.

Apple plants. Seedlings of open-pollinated *M. pumila* 'Rome Beauty' were grown in 200-ml plastic cups in a greenhouse at ~ 23 C. They were inoculated when they were 3 wk old and had five to seven leaves.

Inoculation. Plants were preconditioned for 8 hr in incubators maintained at temperatures ranging from 2 to 32 with 2 C increments. The spore suspension was atomized and deposited uniformly as small droplets on the upper surfaces of all the leaves. Each plant and its pot were immediately put into a polyethylene bag just large enough to contain them without disturbing the leaves. A little water was added to the bag which then was sealed with a rubber band. The inoculated plants were returned to the same controlled-temperature incubators for the prescribed leaf wetness period (LWP). Plants were removed from the bags 1 hr before the end of the prescribed LWP so that leaves would be dry at the end of it and then transferred to a controlled environment chamber at 21 C until symptoms were recorded.

Symptoms. Pycnial lesions were counted and measured on all leaves 2 wk after inoculation (Fig. 1). Light infection was defined as > 0 lesions on any leaf. Light infection became severe infection at $\log_{10}(10\ nd^2) \geq 2.5$, in which n is the highest number of lesions on any leaf of a seedling and d is the mean diameter (in millimeters) of the largest lesion on that seedling (2). The severe level of infection, which corresponds with at least eight 2-mm-diameter lesions per leaf, resulted in substantial premature leaf fall on naturally infected York Imperial apple (4,5).

Experimental design. A treatment consisted of duration of LWP following inoculation at a given temperature and there was a minimum of five replicate plants per treatment. At each temperature, treatments close to the minimum LWP for infection were emphasized. Thus, those treatment groups contained more replicates than did treatment groups at the same temperature, but with greater LWP's. It was considered unnecessary to complete the temperature-LWP matrix since treatments far removed from the infection/non-infection interface either had very large numbers of lesions or were uninfected.

Statistical analysis. Regression lines were developed to enable prediction of the minimum LWP's (hours) necessary for light and severe infections. Mean temperature during the LWP was used as the predictor. Temperature was transformed to its inverse. A polynomial regression equation was used if required for a good fit. The 90% confidence band was calculated for each regression line.

Tests of infection models in the field. In 1976 and 1977 at Highland, NY, branches of *J. virginiana* bearing numerous cedar apple rust galls were placed in buckets of water and arranged in a 4-m-diameter ring around a Burkard 7-day volumetric spore trap to monitor airborne *G. juniperi-virginianae* basidiospores (11). Environmental parameters, including temperature, rainfall, and leaf wetness period, were recorded continuously within 6 m of the spore trap as described previously (11). Potted open-pollinated seedlings of Golden Delicious and of Rome Beauty apples were arranged in a ring 1 m within the ring of *J. virginiana* during each LWP. The seedlings were exposed to spore deposition only for the duration of a specific LWP so that subsequent cedar apple rust infections could be related unequivocally to that LWP. After exposure all seedlings were maintained in a greenhouse at 18–29 C unit pycnial lesions were recorded (approximately 3 wk after initial exposure). For each LWP, temperature and time coordinates were compared with confidence bands of models for light and severe infection (Fig. 2) in order to give a prediction of no, light, or severe infection.

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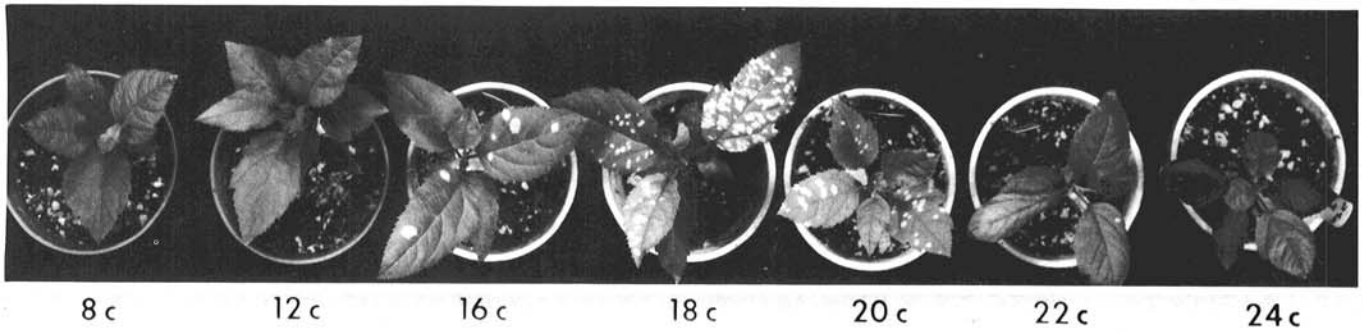

Fig. 1. Cedar apple rust lesions on leaves of open-pollinated Rome Beauty apple seedlings 3 wk after inoculation with basidiospores of *Gymnosporangium juniperi-virginianae* and exposure to a 4-hr leaf wetness period at the indicated temperatures.

RESULTS

Lesion size. Among all treatments the mean diameter of the largest lesion on any infected leaf was 2 mm.

Effect of leaf age on infection period. In general, more lesions were formed on 4-, 6-, and 8-day-old leaves than on 10- and 12-day-old leaves following inoculation at 4, 10, and 20 C (Table 1). The 6-day-old leaves most frequently bore the highest number of lesions, but in certain temperature-LWP treatments 4- or 8-day old leaves had more lesions than did the 6-day-old leaves.

The age of leaf favoring production of lesions under the shortest LWP was inconsistent (Table 1). However, 6- or 8-day-old leaves produced lesions under the shortest LWP except at 20 C, at which temperature 10- and 12-day-old leaves each bore an average of 0.1 lesions per leaf after a 2-hr LWP.

Effects of temperature and LWP on numbers of lesions. Because lower numbers of lesions generally formed and longer LWP's usually were needed for lesion formation on 4-, 10-, and 12-day-old leaves, data are reported for 6- and 8-day-old leaves only (Table 2). Footnotes to the table record where lesions were formed under shorter LWP's on 4-, 10-, and 12-day-old leaves than on 6- and 8-day-old leaves.

The lowest temperature at which infection took place was 2 C under a 24- to 25-hr LWP, although numbers of lesions never reached an average of one lesion per leaf. At 4 C, very few lesions were formed (0.03 lesions per leaf) under an 11-hr LWP but there were 8.0 and 54.3 lesions per leaf under 24- to 25- and 48- to 49-hr LWP's, respectively. At higher temperatures infection took place during shorter LWP's; at 20, 22, and 24 C a few lesions were observed after 2-hr LWP's. Severe infections were observed on leaves kept wet for 5 hr or longer at 16–22 C. No lesions were

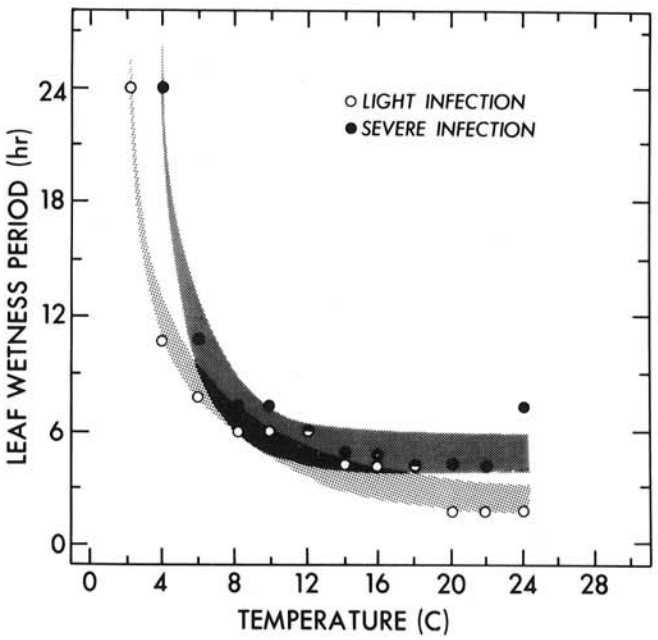

Fig. 2. Minimum leaf wetness periods (LWP) for development of light and severe infections by *Gymnosporangium juniperi-virginianae* on apple leaves maintained at different temperatures during the LWP. Light infection was defined as > 0 lesions per leaf. Light infection became severe infection at $\log_{10}(10\ nd^2) \geq 2.5$, in which n is the highest number of lesions on any leaf of a seedling and d is the mean diameter (in millimeters) of the largest lesion on that seedling. Shaded bands are 90% confidence intervals of equations for minimum LWP's for light and severe infections. Circles are experimental data points. (LWP's greater than the minimum level at a particular temperature also resulted in infection.)

TABLE 1. Effect of leaf age on numbers of lesions that developed on leaves of open-pollinated Rome Beauty apple seedlings that were inoculated with *Gymnosporangium juniperi-virginianae* basidiospores and subjected to a range of leaf wetness periods at different temperatures[a]

Temperature	LWP[b] (hr)	Mean number[c] of lesions per leaf of indicated age (days) at inoculation: 4	6	8	10	12
4 C	6	0	0	0	0	0
	7	0	0	0	0	0
	9	0	0	0	0	0
	11	0	0.1	0	0	0
	13	0	0.2	0	0	0
	15	0	0	0	0	0
	16	0	0	0.1	0.1	0
	17	0	0	0.2	0	0
	24	0	0.2	0.4	0.4	0.2
	25	14.3	16.3	7.5	0.2	0.1
	49	99.0	91.2	17.5	0	0
10 C	2	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0.1	0.5	1.1	0
	7	24.7	23.8	24.7	0	0
	13	56.8	141.6	84.6	21.6	3.0
	25	13.2	240.4	246.6	21.0	4.0
	49	205.6	252.4	216.0	75.6	20.8
20 C	2	0	0	0	0.1	0.1
	3	-	0	0.2	0.2	0
	4	0	0.4	2.9	8.2	5.6
	5	0.5	0.1	1.9	3.2	1.1
	6	-	0.2	8.6	6.0	1.4
	7	195.5	180.8	164.6	44.6	9.0
	13	388.3	602.6	368.8	158.1	66.4
	25	354.7	747.3	548.7	135.1	59.0
	49	273.3	224.5	177.9	42.9	5.0
	73	282.6	496.0	322.3	214.9	118.1

[a] Leaves of 3-wk-old seedlings were sprayed with a suspension of *G. juniperi-virginianae* basidiospores, and were then maintained wet by holding the plants in plastic bags at the indicated temperatures until the end of the leaf wetness period. The plants were then grown at 20 C in a controlled environment chamber until symptoms were recorded after 2 wk.

[b] Leaf wetness period.

[c] Means of five to 24 leaves.

observed on leaves kept wet for up to 72 hr at temperatures above 24 C.

Infection models. The following equations were derived for the temperature effect on time required for infection:

$$Y_L = 0.65 + 45.91\ X^{-1}$$

$$Y_s = 6.33 - 52.11\ X^{-1} + 490.09\ X^{-2}$$

in which Y_L and Y_S are minimum LWP's (in hours) required for light and severe infection, respectively, and X is the mean temperature (C) during the LWP when $0 < X \leq 24$ C. The equations accounted for 98 and 97%, respectively, of the data variation when corrected for degrees of freedom. The 90% confidence bands are presented in Fig. 2. It is important to note that the confidence bands are for the minimum LWP's for light and severe infection. LWP's greater than the minimum level would, of course, also result in infection.

Tests of infection models in the field. Numbers of plants infected during each LWP were recorded in 1976 and 1977. In addition, numbers of lesions/leaf were recorded in 1977 (Table 3). In all cases when an infection was predicted by the models, an infection did in fact occur. Because of the limited observations made, differentiation of light and severe infections could not be made precisely. However, three LWP's (5 C/32 hr in 1976, and 7 C/22 hr and 9 C/12 hr in 1977) were identified with low levels of infection, although severe infections were predicted. During those LWP's the aerial concentration of basidiospores was relatively low (Table 3).

In only one case (16 C for 2 hr in 1976) did infection occur during an LWP in which no infection was predicted. However, only one plant of 24 became infected. The duration of this LWP was within 1 hr of the 90% confidence band for a light infection (Fig. 2).

DISCUSSION

The effect of leaf age on susceptibility of open-pollinated Rome Beauty apple seedlings was similar to that for clonal Rome Beauty apple plants (1); leaves 4–8 days of age usually were more susceptible than older leaves. The results also agree, in general, with observations of natural infections of York Imperial apple leaves of different ages (5). The 10- and 12-day-old leaves of the seedlings required a longer LWP at a given temperature to sustain the same level of infection as the 4- to 8-day-old leaves. At 4 C very little infection occurred on 10- and 12-day-old leaves even after 49-hr LWP's, although up to 99 lesions per leaf were observed on younger leaves.

Our data indicate that the optimum range for infection of apple leaves by *G. juniperi-virginianae* is 10–24 C. At those temperatures, and with the inoculum concentrations that we used, a light infection occurs during a 2- to 5-hr LWP and a severe infection during a 4- to 6-hr LWP. Infection can occur at 3–24 C but at the low end of the range, particularly at 3–6 C, progressively longer LWP's are required. These findings contrast with some reports on infection periods for cedar apple rust in the literature. Optima have been reported as 13–16 C (7,8) and around 18 C (4). Palmiter (8) reported little basidiospore germination below 8 C, and Giddings (4) that there was little infection at 13 C, although our data show that severe infections can be obtained with 7-hr LWP's in that temperature range.

Teliospores do not germinate and form basidiospores below 8 C (9), although basidiospores formed at higher temperatures will germinate at temperatures as low as 2 C (R. C. Seem, *unpublished*). Our data show that such basidiospores also are able to infect at these low temperatures.

Correct predictions of infection or no infection from 23 of 24 LWP's in field tests over 2 yr indicates that the models are functional under field conditions. Although the regression lines for light and severe infections do not overlap, there is not much difference in LWP between light and severe infection at temperatures above 4 C. Thus, a practical forecasting system probably would not distinguish between the two.

Rome Beauty and Golden Delicious are both very susceptible to *G. juniperi-virginianae* (2). Less susceptible apple cultivars sustain less severe infections from similar LWP's (2).

The data reported here relate to infection of leaves, but with caution they may be extrapolated to infection of fruits. Limited

TABLE 2. Effect of temperature and duration of leaf wetness period (LWP) on numbers of pycnial lesions on 6- to 8-day-old leaves of open-pollinated Rome Beauty apple seedlings inoculated with *Gymnosporangium juniperi-virginianae* basidiospores[a]

	Mean numbers[b] of lesions per leaf resulting from inoculations at the indicated temperatures (C):															
LWP (hr)	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
2				0	0	0	0	0	0	0[c]	0	0[d]				
3								0	0	0.1		0[e]				
4				0	0	0	0.3	0.1	3.8	1.6	3.7	0.4				
5			0	0	0	0	0.7	32.7	22.9	1.0		0[f]				
6		0	0	0.6	0.4	2.3	14.2	108.5	46.0	4.7		0.2				
7	0	0	0	1.9	17.6	10.2	75.3	64.5	18.5	172.2	220.6	40.2	0	0		
8			0[g]	1.4												
9		0		3.3												
11		0.03		0.9												
13	0	0.08	8.7	97.4	113.1					485.7	481.0	50.4	0	0		
15		0														
16		0.06														
17		0.1														
20–21	0															
24–25	0[h]	8.0	58.2	120.9	243.5					648.0	680.5	37.5	0	0		
28–29	0[i]															
48–49	0.6	54.3	72.8	250.0	234.2					201.2		32.3	0[j]	0	0	0
72–73	0.5									409.2			0	0	0	0

[a] Three-week-old seedlings were sprayed with a suspension of *G. juniperi-virginianae* basidiospores and kept wet until the end of the LWP at the indicated temperatures. The plants were then grown at 21 C until symptoms were recorded after 2 wk.

[b] Means of 10 to 60 leaves.

[c] 0.1 lesions per leaf on 10-day-old leaves, and 0.01 lesions per leaf on 12-day-old leaves.

[d] 3.3 lesions per leaf on 10-day-old leaves.

[e] 0.08 lesions per leaf on 10-day-old leaves.

[f] 0.1 lesions per leaf on 12-day-old leaves.

[g] 0.2 lesions per leaf on 4-day-old leaves, and 0.08 lesions per leaf on 10-day-old leaves.

[h] 0.3 lesions per leaf on 4-day-old leaves.

[i] 0.2 lesions per leaf on 10-day-old leaves, and 0.2 lesions per leaf on 12-day-old leaves.

[j] One 4-day-old leaf bore 25 lesions; thirty other 4-day-old leaves and all 6-, 8-, and 12-day-old leaves were lesion-free.

TABLE 3. Comparison of *Gymnosporangium juniperi-virginianae* infections of apple seedlings that were observed under monitored conditions in the field with predictions generated by model equations that were developed from experimental inoculations under controlled conditions

Year and temp[a] (C)	LWP[b] (hr)	Basidiospores[c] (m^{-3} air)	Golden Delicious seedlings[d]				Rome Beauty seedlings[e]				Infection predicted[h]
			Number of plants		Lesions/leaf		Number of plants		Lesions/leaf		
			Total	Infected	Mean[f]	Max[g]	Total	Infected	Mean[f]	Max[g]	
1976											
3.5	4	0	3	0	–	–	3	0	–	–	0
5[i]	32	86	5	1	–	–	0	–	–	–	S
9	1	33	6	0	–	–	3	0	–	–	0
11[j]	23	1,899	5	4	–	–	12	9	–	–	S
12	2	0	12	0	–	–	6	0	–	–	0
12	17	8,250	11	9	–	–	11	6	–	–	S
13	4	–	6	0	–	–	6	0	–	–	0
13	13	6,134	8	7	–	–	4	3	–	–	S
13	18	12,844	13	13	–	–	12	12	–	–	S
15	2	–	6	0	–	–	0	–	–	–	0
15.5	2	13	6	0	–	–	4	0	–	–	0
16	2	0	12	1	–	–	12	0	–	–	0
16	13	1,242	6	3	–	–	6	3	–	–	S
19	11.5	1,955	6	3	–	–	18	7	–	–	S
20	1	0	9	0	–	–	4	0	–	–	0
1977											
7	22	384	5	0	–	–	7	2	2.0	2	S
9	12	63	6	1	1.0	1	6	0	–	–	S
11	20	4,272	6	5	25.1	270	6	5	13.8	53	S
11	23	4,037	6	4	25.5	99	6	6	36.5	187	S
13.5[k]	10	1,458	6	5	5.0	12	7	3	6.0	13	S
15	12	–	5	5	6.8	23	6	6	6.4	18	S
18	2.5	0[l]	6	3	1.6	2	7	5	3.4	14	S
20	11	3,806	6	5	23.9	126	6	2	12.3	37	S
22	1	33	6	0	–	–	6	0	–	–	0

[a] Means of hourly temperatures during leaf wetness period (LWP). During all LWP's temperature varied from mean by ± 2 C except as noted.
[b] Leaf wetness period.
[c] Mean of hourly counts of basidiospores trapped per cubic meter of air during LWP.
[d] Open-pollinated seedlings of Golden Delicious.
[e] Open-pollinated seedlings of Rome Beauty.
[f] Mean number of lesions on all infected leaves of all seedlings within treatment.
[g] Greatest number of lesions on any leaf within treatment.
[h] 0 = no infection, L = light infection, S = severe infection. Predictions were made by comparing observed temperature and LWP with the 90% confidence interval bands shown in Fig. 2.
[i] Temperature range during LWP: 3–8 C.
[j] Temperature range during LWP: 9–18 C.
[k] Temperature range during LWP: 12–18 C.
[l] Basidiospores were trapped at a mean concentration of 100 m^{-3} air during a 17-hr period that ended 5 hr prior to beginning of the LWP.

information (6,10) indicates that fruits, like leaves, rapidly become less susceptible as they mature, and that the period of maximum susceptibility is from early pink through full bloom (3). At this age fruits may be as susceptible as 6- and 8-day-old leaves and may have similar infection periods.

Together with data we have developed previously on the time-temperature relationships of teliospore germination and basidiospore formation (9), the information reported here will allow prediction of whether and to what extent infection of apple trees has taken place during a particular natural rain episode. Combining these predictions and the use of the eradicant fungicides that are expected to become available (12), it will be possible to control cedar apple rust more efficiently and economically.

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