Discontinuous Wetting and Survival of Conidia of *Venturia inaequalis* on Apple Leaves

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ABSTRACT

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The viability of *Venturia inaequalis* conidia on apple leaves was determined after exposing them to wet and dry intervals at 10, 15, 20, and 25 C. Relative humidities (RH) during dry intervals were 60% or >90%. Viability of ungerminated conidia was not affected by exposure to dry intervals of ≥24 h regardless of temperature or RH and decreased only slightly after dry intervals of 96 h. Viability of germinated conidia (germlings) decreased by 20% during the first 15 min of drying and decreased an additional 10–30% after 96 h. Viability of germlings that had formed appressoria significantly decreased following drying intervals of 24 and 96 h but was greater (10–20%) than viability of germlings without appressoria. After the different dry intervals, either the spore or the germ tube in up to 19% of the germlings had dried. However, after a second 24-h wetting interval, as many as 85% of the viable cells or conidia continued growing and formed appressoria. When RH during the dry interval was >90%, the viability of conidia or germlings was similar to or less than that at 60% RH.

Additional keywords: apple scab, epidemiology, split-wetting periods.

The Mills table (16) lists the durations of continuous leaf wetness at different temperatures that constitute infection periods for ascospores and conidia of *Venturia inaequalis* (Cooke) G. Wint. Short, intermittent wet and dry periods, however, can make calculations of infection periods difficult. Individual wet intervals may not constitute an infection period, and the effect of the ensuing dry interval on the viability of dormant and germinated spores (germlings) of *V. inaequalis* is poorly understood. Infection by conidia after discontinuous wetting of potted apple trees in growth chambers was not affected by dry intervals of 4–15.5 h (21) and of 2–32 h (23). The incidence of foliar scab caused by ascospores was suppressed after dry intervals of 32 h (18) and 96 h (23). In orchards, "a half a day or more of dry, sunny weather" was sufficient to stop the scab infection process (17). The relative humidity (RH) during dry intervals also may affect survival of the pathogen (10,12,20); therefore, precise calculations of scab infection periods is often difficult.

We report the effect of discontinuous wetting intervals on the mortality of ungerminated conidia and germlings on apple leaves. The effects of wet and dry intervals, temperature, and RH were evaluated.

MATERIALS AND METHODS

Inoculations and treatments. Three-week-old apple (*Malus domestica* Borkh. 'McIntosh') seedlings were grown from pregerminated open-pollinated seeds. Apple scab-infected leaves were collected from unsprayed McIntosh trees during June 1988 and stored between sheets of paper towels and waxed paper at 4 C. Conidia were harvested by washing spores from scab lesions with a model 163 atomizing sprayer (DeVilbiss, Inc., Sommerset, PA) at 138 kPa. The suspensions were adjusted to approximately 5×10^4 conidia per milliliter with a hemacytometer. Conidia were then applied in four drops (7 μl each) to the adaxial surface of each of the two youngest leaves of the seedlings. The seedlings were subjected to consecutive intervals of wetting, drying, and wetting at 10, 15, 20, or 25 C within a factorial experiment. The ability of propagules to form appressoria after discontinuous wetting, the ability of conidia to survive dry intervals, and the survivability of germlings with or without appressoria were evaluated with fluorescent vital-staining techniques.

Effects of discontinuous wetting and drying on the formation of appressoria. The ability of appressoria to remain viable after drying was evaluated in an experiment with a split-split plot, factorial design. The main-plot treatments were temperature and RH during the dry interval. The first subplot treatment was the duration of initial wetting, and the second subplot treatment was the duration of the dry interval. For each temperature × RH combination, three initial wet intervals were tested: 15 min, the time at each temperature that was required for ~50% germination, and the time required for ~20% of the conidia to form appressoria. Initial wet intervals will subsequently be referred to as short, intermediate, or long. The times required for 50% germination were 7, 5, 4, or 5 h at 10, 15, 20, or 25 C, respectively. For 20% appressoria formation, intervals of 12, 8, 7, or 8 h at 10, 15, 20, or 25 C, respectively, were required. The initial wetting events were produced in temperature-controlled, walk-in mist chambers (27). Dry intervals were initiated by removing seedlings from the mist chambers and drying the water droplets with a hair dryer that was positioned ~1.3 m from the seedlings. The temperature of the air stream created by the dryer was <29 C, according to an FWTC-3 thermocouple that was connected to a CR21 datalogger (Campbell Scientific, Logan, UT). The drops dried within 15 min. Dry intervals were 0, 0.25, 6, 12, 24, or 96 h in duration, and the RH was 60% or >90%. For the dry interval, seedlings were placed in a growth chamber (I-600L dew chamber, Percival Equipment Co., Boone, IA) at the same temperature as that used for the initial wetting. The RH in the chamber was monitored with a set of Amprobe FLRBT-1 wet and dry bulb thermometers and FT8101 recorders (Amprobe Instrument Co., Lynbrook, NY). For the second wet interval, leaves were detached from seedlings, placed in petri dishes containing moistened filter paper, and rewetted by atomization with distilled

water. The petri dishes were placed in the mist chambers (at the same temperature as that used for initial wetting) for 24 h. Seedlings were subjected to eight separate blocks of treatments (four temperatures × two RHs) that were conducted three times. Within each run (block) of these experiments, the observed percentage of propagules with appressoria was divided by the percentage of conidia that germinated during a continuous 24-h wet interval. This process adjusted data for the initial proportion of the propagules that were viable.

The proportion of propagules with viable appressoria was determined immediately after the 24-h second wet interval (leaves still wet) by epi-UV fluorescent microscopy. Three leaf pieces (~1 cm^2 each) were cut from the leaves for each treatment. Pieces were placed on a microscope slide, covered with ~50 μl of a solution of calcofluor (CCF) and fluorescein diacetate (FDA) (Sigma Chemical Co., St. Louis, MO), and covered with a coverslip. CCF was used for viewing all *V. inaequalis* propagules on the leaf surface; appressoria that were FDA fluorescent were considered to be viable (1). FDA was prepared as a stock solution (2 mg/ml), stored at −20 C, and used at a concentration of 10 μl/ml in a 100 mM K_2HPO_4 buffer (pH 7.3) (2,25). Calcofluor White M2R (Sigma) was added to the FDA solution at 1 mg/ml. A fresh solution was prepared every 30 min. One hundred propagules per leaf piece were examined by epi-UV illumination with a Zeiss Photomicroscope III equipped with a 50W mercury lamp. CCF and FDA fluorescence was observed with excitation at 365 and 440–490 nm, dichroic reflectance at 395 and 460 nm, and emission at 420 and 520 nm, respectively.

Analysis of variance (ANOVA) was conducted with the SAS computer program (SAS Institute, Cary, NC) on the viable proportion of propagules with appressoria to evaluate the effects of temperature, RH during the dry interval, duration of drying, and all combinations of interactions. After the resultant significant treatments and interactions from ANOVA were determined, regression analysis (SAS) was conducted to determine the influence of the length of the dry interval on survival by each group of propagules. Regression equations were selected according to maximum values for R^2 and minimum values for the variance. Analysis of arcsine (square root)-transformed data failed to increase the level of significances due to treatments or interactions within ANOVA or to decrease variances or increase R^2 values for regression equations. All analyses, therefore, were conducted on raw proportional data. For ANOVA, data were pooled by replicate because, despite a significant effect of replicate (block), treatment × replicate interactions were not significant.

Ability of conidia or germlings with and without appressoria to survive dry intervals on leaves. The ability of propagules of *V. inaequalis* at different stages of growth to survive the dry intervals was quantified by recording the viable proportion of 1) conidia from the short initial wetting interval, 2) germlings from the intermediate initial wetting interval, and 3) germlings with appressoria from the long initial wetting interval that were viable after the 15-min second wetting interval. For germlings, the viability of the germ tube and/or the conidium was also recorded. These experiments were conducted as a split-plot factorial experiment. The design was as described previously, except that the subplot with wetting interval was omitted. The viability of conidia or germlings with or without appressoria was determined as described above. ANOVA and regression analysis (SAS) were conducted with raw proportional data as described above.

RESULTS

Effects of discontinuous wetting and drying on the formation of appressoria. The viability of appressoria was influenced by a significant interaction among temperature × RH × wet interval length × dry interval length (Table 1). Therefore, the influence of initial wet and dry intervals on the ability of appressoria to maintain viability was plotted within temperature × RH treatments (Fig 1). In addition, regression equations for predicting viability of appressoria after a 24-h second wet interval were generated from those data (Table 2 and Fig. 1). When propagules were subjected to dry intervals of ≤24 h, 60–95% had formed viable appressoria after a 24-h second wet interval, regardless of temperature or RH during the dry interval (Fig. 1). After 96 h of drying at 60% RH, 45–80% of the propagules had viable appressoria, compared with drying at >90% RH, for which 30–75% of the propagules had viable appressoria.

Ability of conidia or germlings with and without appressoria to survive dry intervals on leaves. After the propagules on leaves were dried, viability of conidia or germlings with appressoria was influenced by significant interaction between temperature × dry interval (Table 3). The viability of germlings without appressoria was influenced by a significant interaction between temperature × RH × dry interval. On the basis of those significant interactions, data were plotted (Fig. 2), and regression equations were selected for predicting proportional survival of the dry interval by the respective groups (Table 4).

For all drying treatments that lasted ≤24 h, the viability of conidia on apple foliage was reduced by <15% (Fig. 2A and B). During 96-h dry intervals, viability decreased to ~80%. Viability of germlings was reduced by ~20% during the initial 15-min dry interval, regardless of temperature and RH (Fig. 2C–F). During dry intervals to 24 h, germling viability decreased substantially only at 20 and 25 C at >90% RH. After dry intervals of 96 h, 60–75% of the germlings remained viable for most treatments; however, only 40% remained viable at temperatures of 15, 20, and 25 C at >90% RH. Only the spore or germ tube remained viable on some germlings after dry intervals. Approximately 85% of these had dead germ tubes, although the conidia were still viable. After a second 24-h wet interval, the viable portion frequently regerminated. The viability of germlings that had formed appressoria was substantially less affected by a 15-min dry interval than was the viability of germlings without appressoria (Fig. 2G and H). Viability was 82–97% and 60–85% when the dry intervals were 24 and 96 h in length, respectively.

DISCUSSION

The majority of the propagules of *V. inaequalis* remained viable after the discontinuous wetting regimes used in this study. More conidia and more germlings with appressoria than germlings without appressoria survived dry intervals, but at least 40% of the germlings still remained viable after 96-h drying intervals. Such high percentages of survival by propagules of *V. inaequalis* may explain the development of scab after wet intervals separated

TABLE 1. Analysis of variance for effects of temperature (T), relative humidity (RH), and duration of initial wetting (W) and drying (D) on the proportion of appressoria of the anamorph of *Venturia inaequalis* that were viable after a second 24-h wetting interval

Source of variation	df	Sum of squares	*F*	*P*
Replicate	2	3.7594	138.40	0.0001
T	3	0.6016	1.17	0.3567
RH	1	0.1307	0.76	0.3975
T × RH	3	0.0176	0.03	0.9911
Error for main plot	14	2.4020		
Length of initial wet period	2	2.9426	27.52	0.0001
T × W	6	0.7314	2.28	0.0605
RH × W	2	0.0567	0.53	0.5920
T × RH × W	6	0.7201	2.25	0.0640
Error for first subplot	32	1.7101		
Dry period length	5	7.6924	113.28	0.0001
T × D	15	1.2621	6.20	0.0001
RH × D	5	0.2078	3.06	0.0095
W × D	10	0.6301	4.64	0.0001
T × RH × D	15	0.0990	0.49	0.9484
RH × W × D	10	0.4665	3.43	0.0002
T × W × D	30	0.7649	1.88	0.0030
T × RH × W × D	30	7.6125	1.87	0.0032
Error	1,104	14.9938		
Corrected total	1,295	39.9511		

by 4- to 32-h dry intervals that individually did not constitute infection periods (8,11,18,21,23,28). In this study, the RH during intervals of drying as long as 96 h had no effect on survival of conidia and little effect on appressoria. After exposure to intervals of drying to 92 days at 10 C, detached conidia of *V. inaequalis* germinated independently of RH (24). Germination was at or near 0% when the RH was 75, 79, and 98%, but germination was 51, 30, and 57% when the RH was 78, 88, and 100%, respectively. These observations are not surprising given that the effect of RH on the ability of conidia to survive dry intervals was not significant in this study. In other experiments, however, RH during an interval of drying can affect the survival of conidia in foliar lesions (12). The germination of conidia after 163 or 196 days at 0% RH was 22 or 2%, respectively. No conidia remained viable after 33 days at 0% RH, however, when followed by at least 17 days at 100% RH. In those previous studies, the evaluation of survival by conidia was evaluated under laboratory conditions that may have seemed extreme; however, a recent study

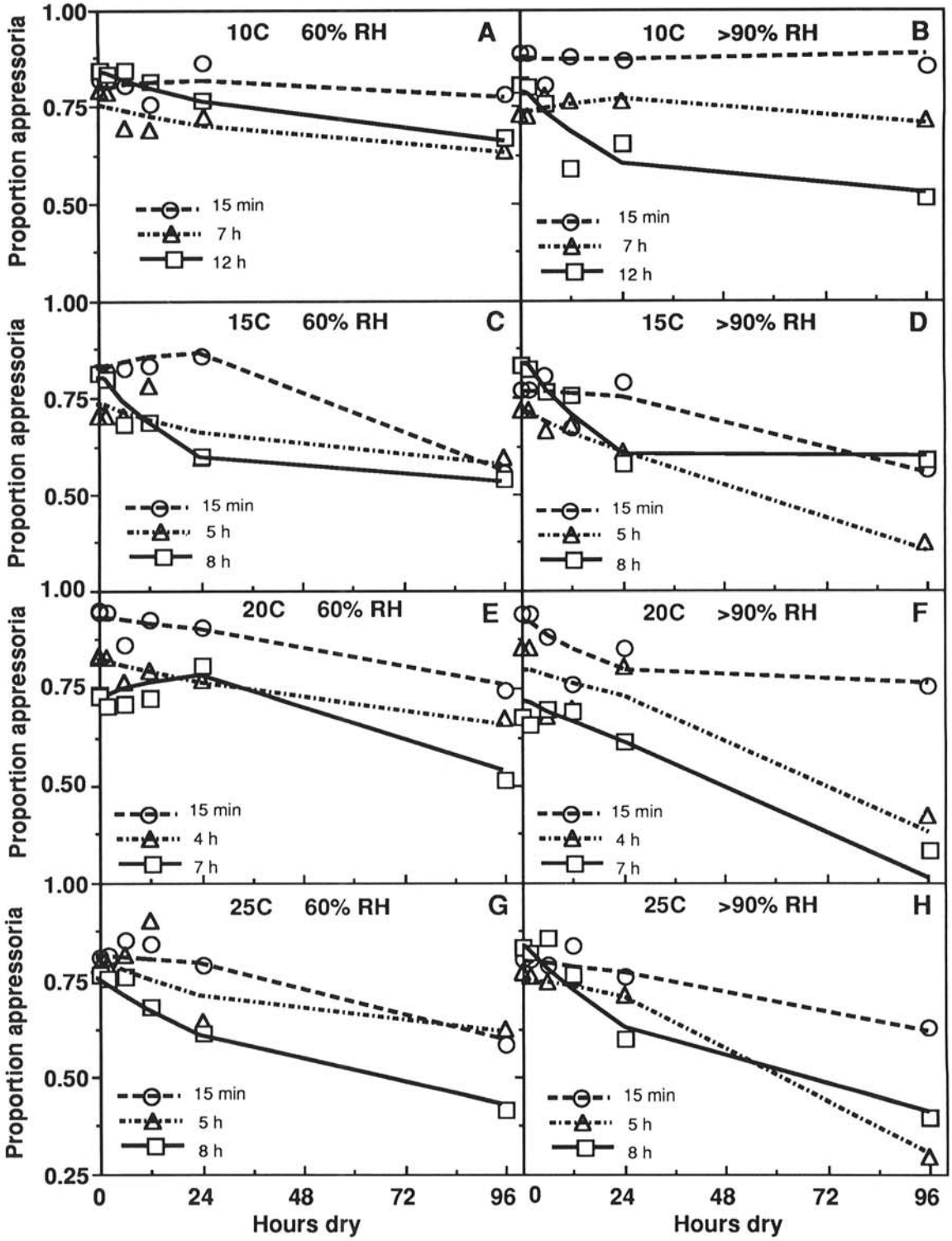

Fig. 1. Proportion of *Venturia inaequalis* propagules with viable appressoria after combinations of wet and dry intervals and a second 24-h wet interval. Points represent actual means from experiments conducted at 10, 15, 20, or 25 C with relative humidities of 60% or >90%. Lines represent values that are predicted from the same data and that are based on regression equations in Table 2. The curves within each figure represent different lengths of initial wetting (15 min and lengths that correspond to the time necessary for 50% germination or 20% appressoria formation). The estimate for the error associated with each regression line is presented with specific equations in Table 2.

indicated that ascospores of *V. inaequalis* remained sufficiently viable within lesions on leaves that had been in storage for 2 yr (26). On glass slides, germlings, but not conidia, were completely killed by drying in one study (6); however, the temperature and RH during the dry intervals were not reported.

When leaves were inoculated with conidia and exposed to direct sun, the number of lesions formed on leaves was not related to the duration of exposure to sun (29). In that study, up to 6 h of drying had no apparent effect on the formation of scab lesions. Ungerminated conidia of other ascomycetes have been better able to survive intervals of drying at lower RHs (4,15,19); this is consistent with our observations that germlings of *V. inaequalis* were better able to survive drying intervals at 60% than at >90% RH. If we had used longer intervals of drying, different levels of survival might have been observed for conidia. Effects of the dry interval on the structure and metabolism of conidia or germlings of *V. inaequalis* are not known. Viability was reduced more frequently when the RH during the dry interval was >90% compared with 60%, so the propagules appeared to enter a physiological state that is tolerant to drying at a lower RH. Furthermore, other microorganisms may have parasitized *V. inaequalis* germlings or utilized nutrients that were required for survival at higher RHs.

TABLE 2. Quadratic regression equations for predicting the proportion of propagules of *Venturia inaequalis* that formed appressoria during a 24-h second wet interval after various combinations of three initial wet intervals and six dry intervals to 96 h

Temperature[a] (C)	RH[b] (%)	Length of initial wetting	Equation[c]	R^2	S[d]
10	60	15 min	$Y = 0.800 + 0.00116X - 0.000015X^2$	9.7	0.1085
		7 h	$Y = 0.753 - 0.0026X + 0.000014X^2$	13.6	0.1106
		12 h	$Y = 0.841 - 0.0035X + 0.000017X^2$	26.2	0.1061
10	>90	15 min	$Y = 0.877 - 0.00037X - 0.000005X^2$	9.1	0.0953
		7 h	$Y = 0.733 + 0.0022X - 0.000026X^2$	12.7	0.1200
		12 h	$Y = 0.788 - 0.0094X + 0.00007X^2$	38.9	0.1265
15	60	15 min	$Y = 0.815 + 0.0030X - 0.00006X^2$	23.9	0.1904
		5 h	$Y = 0.723 - 0.0036X + 0.00002X^2$	15.1	0.1150
		8 h	$Y = 0.791 - 0.0105X + 0.00008X^2$	42.4	0.1152
15	>90	15 min	$Y = 0.767 - 0.0003X - 0.00002X^2$	11.2	0.2059
		5 h	$Y = 0.714 - 0.0047X + 0.00001X^2$	28.8	0.1902
		8 h	$Y = 0.838 - 0.0121X + 0.00001X^2$	30.1	0.1666
20	60	15 min	$Y = 0.932 - 0.0011X - 0.000007X^2$	22.9	0.1206
		4 h	$Y = 0.825 - 0.0027X + 0.00001X^2$	20.0	0.1069
		7 h	$Y = 0.729 + 0.0038X - 0.00006X^2$	39.9	0.1113
20	>90	15 min	$Y = 0.913 - 0.00643X + 0.00005X^2$	22.6	0.1180
		4 h	$Y = 0.788 - 0.0024X - 0.00002X^2$	31.9	0.2040
		7 h	$Y = 0.709 - 0.00418X - 0.00001X^2$	22.2	0.2016
25	60	15 min	$Y = 0.827 - 0.0003X - 0.00002X^2$	26.1	0.1541
		5 h	$Y = 0.824 - 0.0049X + 0.00003X^2$	28.0	0.1165
		8 h	$Y = 0.771 - 0.0072X + 0.00004X^2$	42.2	0.1490
25	>90	15 min	$Y = 0.808 - 0.0009X - 0.00001X^2$	28.9	0.1047
		5 h	$Y = 0.760 - 0.0008X - 0.00004X^2$	66.9	0.1220
		8 h	$Y = 0.850 - 0.0102X + 0.00006X^2$	62.9	0.1251

[a] Temperature for the combinations of wet, dry, and wet intervals.
[b] Relative humidity during the dry interval.
[c] Y = the proportion of propagules that formed viable appressoria. X and X^2 = hours and hours squared, respectively, for dry intervals.
[d] Standard deviation of the Y about the regression line using data pooled by replicate (block).

TABLE 3. Analysis of variance[a] for effects of temperature (T), relative humidity (RH), duration of dry interval (D), and interactions on the viability of *Venturia inaequalis* propagules on apple seedling leaves

	Nongerminated conidia[b]				Germlings[c]			Germlings with appressoria[d]		
Source	df	Sum of squares	F	P	Sum of squares	F	P	Sum of squares	F	P
Block	2	0.5357	41.43	0.0001	0.7287	17.25	0.0001	1.1533	22.14	0.0001
T	3	0.0988	1.21	0.3437	0.5286	1.63	0.2277	0.7604	1.74	0.2041
RH	1	0.0202	0.74	0.4042	0.0605	0.56	0.4669	0.0561	0.39	0.5445
T × RH	3	0.0209	0.26	0.8560	0.0642	0.20	0.8961	0.0272	0.06	0.9789
Error for main plot	14	0.3821			1.5145			2.0352		
D	5	2.9014	89.75	0.0001	9.7049	79.83	0.0001	4.2535	32.66	0.0001
T × D	15	0.2456	2.53	0.0014	0.6915	2.18	0.0066	0.7767	1.99	0.0153
RH × D	5	0.0606	1.88	0.1406	0.4261	4.03	0.0014	0.2025	1.55	0.1722
T × RH × D	15	0.1302	1.34	0.1735	0.8305	2.62	0.0009	0.3788	0.97	0.4872
Residual error	368	2.3793			7.7729			9.6130		
Corrected total	431	6.8073			22.3223			19.230		

[a] From each experiment data were analyzed as raw proportions.
[b] Viability was assessed on conidia that had been subjected to an initial wet interval of 15 min on apple seedling leaves followed by a dry interval to 96 h.
[c] Viability was assessed on germlings that had formed during an initial wet interval of sufficient length to allow 50% of the conidia to germinate on apple seedling leaves and that was followed by a dry interval to 96 h.
[d] Viability was assessed only on germlings with appressoria that formed during an initial wet interval of sufficient length to allow at least 20% of the conidia to form appressoria on apple seedling leaves and that was followed by a dry interval to 96 h.

Our results show that the apical cell of a germ tube may die during drying, whereas the subtending hyphae or the conidium remains viable and germinates upon rewetting. A germling may have three or more hyphal cells with septa between the cells and between the spore and the appressorium (14). The septa within

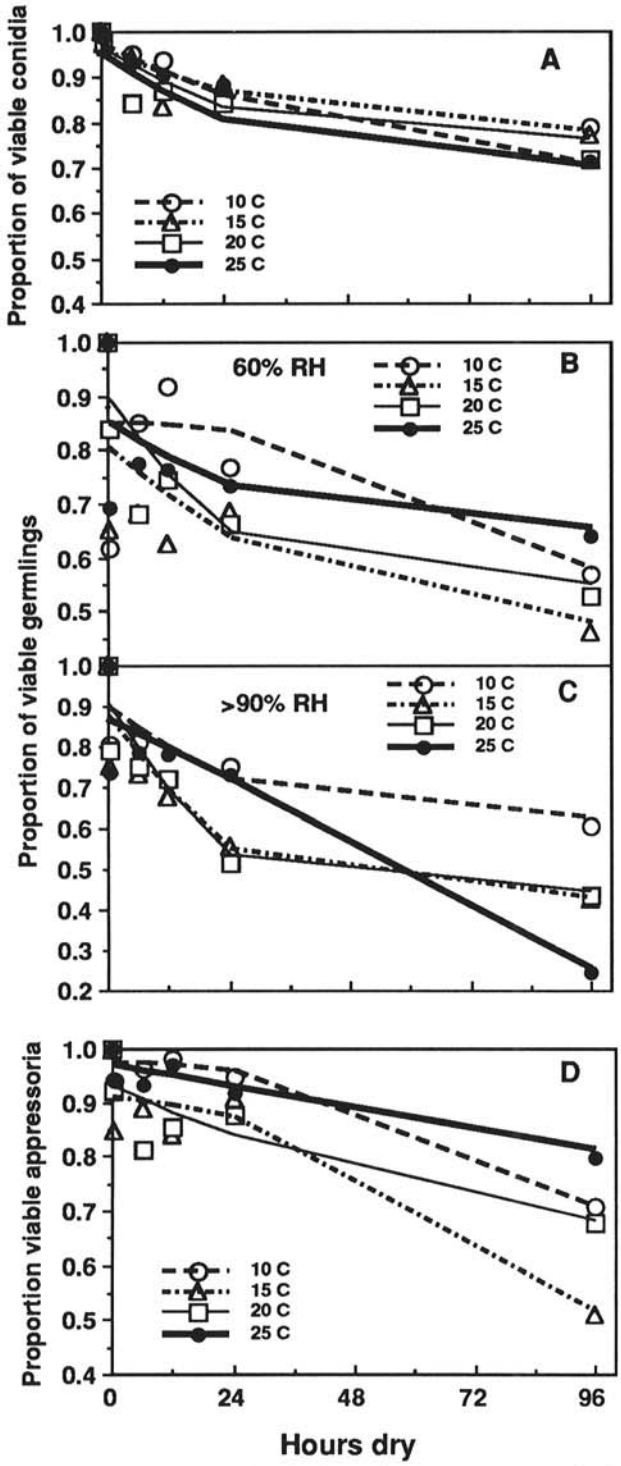

Fig. 2. Effect of the length of the drying period, temperature, and relative humidity (RH) on viability of *Venturia inaequalis* **A,** conidia, **B and C,** germlings with no appressoria, and **D,** germlings with appressoria. Points represent actual mean proportions, whereas lines represent values predicted from data based on regression equations in Table 4. Data for conidia **(A)** and germlings with appressoria **(D)** are the means of combinations of wet and dry intervals at 10, 15, 20, and 25 C averaged over both levels of RH. Data for germlings with appressoria were separated into survival of dry intervals at 60% RH **(B)** or at >90% RH **(C).** The estimate for the error associated with each regression line is presented with specific equations in Table 4.

V. inaequalis hyphae are typical of ascomycetes, each having a single, central pore and associated Woronin bodies (9,14,31). When a hyphal cell is disrupted in *Penicillium chrysogenum* Thom, Woronin bodies effectively seal the septal pores, and the adjacent cell or spore remains intact (5). Such a system may explain how spores or hyphal fragments of *V. inaequalis* survive prolonged drying. The time for germination or regrowth during the second wetting interval was not compared with the time required for infection according to the Mill's table (16). However, since high proportions of the propagules formed appressoria during the 24-h second wetting interval, at least viability appears to have been maintained.

Propagules of *V. inaequalis* remained viable during intervals of drying in an orchard, but "half a day or more of dry, sunny weather" was sufficient to inhibit a scab infection period (17). In that study, foliage became relatively resistant to infection during dry periods. The highest number of lesions per leaf formed on leaves that had unfolded 2 and 4 days prior to inoculation with conidia and ascospores, respectively (when observed after 14 and 28 days) (22). In the same study, leaves that had unfolded 2 days sooner or later than the leaves that developed the highest number of lesions formed significantly fewer lesions after inoculation with conidia or ascospores. In addition, on leaves that had unfolded 3 or 4 days prior to inoculation, the number of lesions per leaf was significantly lower than on leaves that had unfolded 2 days prior to inoculation.

Fifteen days after inoculation of leaves with conidia, observations subjacent to the appressoria revealed that formation of the subcuticular stroma was delayed as the age of the leaf increased and was almost or completely inhibited on the fourth-oldest leaf (7). When entire potted trees were inoculated with conidia (11), however, diffuse lesions formed on the abaxial surfaces of the oldest leaves but not until 39 or 55 days after inoculation. Therefore, older leaves can be infected by *V. inaequalis,* but a longer latent period occurs. Lesions probably would have formed on the older leaves in other studies (7,22) after longer intervals of time. The coupling of the observations on reduced or delayed susceptibility of the older leaves to scab (7,11,22) with observations from this study or others (17) in which propagules of scab remained viable after intervals of drying indicates that over time, the susceptibility of the host to scab development decreases more rapidly than the viability of conidia of *V. inaequalis* is reduced by drying.

The data generated by this study describe the proportion of *V. inaequalis* propagules that are viable at the end of a dry interval and clarify a portion of the understanding of the biology of the scab fungus. Effective use of these data requires information on duration of the wet and dry intervals and an indication of the abundance of initial inoculum. If inoculum is not abundant, then the likelihood of economic loss due to scab should be low (13). When inoculum is present, however, the proportion of the population that is viable at the end of the initial wet interval that is likely to be conidia, germlings, or germlings with appressoria can be determined from equations generated from growth chamber studies (30). Then at the end of a drying interval, the viable proportion of each of these groups (conidia and germlings with or without appressoria) can be determined by incorporating length of dry intervals into the appropriate regression equations (Table 4). For each of the three groups within the population (conidia and germlings with and without appressoria), the product of the relative proportion that is expected at the end of the initial wetting interval with its relative proportion that remains viable at the end of the ensuing dry interval yields a numerical value. The sum of those three numbers represents the proportion of the population remaining viable at the end of a dry interval. These calculated values will be most important in orchards that may have conidia overwintering in buds (3) or have an abundance of scab lesions on early developed tissues or in orchards that use over-tree irrigation. These data also may be useful for growers who wish to minimize fungicide use.

When wetting intervals are discontinuous, we propose that the following rules should be incorporated to calculate infection periods. If the interval of drying is less than 48 h in length, the

TABLE 4. Quadratic regression equations for predicting the proportion of nongerminated conidia and germlings with and without appressoria of *Venturia inaequalis* that were viable after dry periods to 96 h on apple leaves

	RH[a] (%)	Temperature[b] (C)	Equation[c]	R^2	S^d
Nongerminated conidia	...	10	$Y = 0.970 - 0.00436X + 0.000026X^2$	34.2	0.0894
	...	15	$Y = 0.975 - 0.00652X + 0.000046X^2$	35.1	0.0995
	...	20	$Y = 0.961 - 0.00727X + 0.000049X^2$	38.1	0.1106
	...	25	$Y = 0.984 - 0.00546X + 0.000028X^2$	43.0	0.1093
	60	...	$Y = 0.975 - 0.00536X + 0.000032X^2$	40.9	0.1158
	>90	...	$Y = 0.970 - 0.00646X + 0.000043X^2$	33.6	0.1119
Germlings	60	10	$Y = 0.836 - 0.00003X - 0.000029X^2$	17.9	0.2164
		15	$Y = 0.790 - 0.00820X + 0.000050X^2$	25.0	0.2041
		20	$Y = 0.879 - 0.0128X + 0.000096X^2$	36.9	0.1727
		25	$Y = 0.836 - 0.00589X + 0.000040X^2$	19.6	0.1433
	>90	10	$Y = 0.887 - 0.00887X + 0.000063X^2$	30.1	0.1533
		15	$Y = 0.864 - 0.01660X + 0.000125X^2$	45.5	0.1825
		20	$Y = 0.893 - 0.01890X + 0.000147X^2$	51.2	0.1738
		25	$Y = 0.856 - 0.00590X - 0.000005X^2$	58.7	0.1847
Germlings with appressoria	...	10	$Y = 0.970 + 0.00003X - 0.000029X^2$	32.9	0.1408
	...	15	$Y = 0.906 - 0.00074X - 0.000035X^2$	28.9	0.2293
	...	20	$Y = 0.925 - 0.00411X + 0.000016X^2$	12.5	0.2263
	...	25	$Y = 0.967 - 0.00175X + 0.000001X^2$	23.0	0.1022

[a]Relative humidity during dry intervals.
[b]Temperature for the combinations of wet, dry, and wet intervals.
[c] Y = the proportion of propagules that were fluorescein diacetate-fluorescent and considered to be viable. X and X^2 = the hours and hours squared, respectively, for the drying periods.
[d]Standard deviation of the Y about the regression line using data pooled by replicate (block).

initial and subsequent intervals of wetting should be summed to calculate Mills infection periods, but the regression equations (Table 4) should be used to correct for the proportion of the initial inoculum that remains viable at the beginning of the second wet interval. When the interval of drying exceeds 2 days in length, previous data indicate that the foliage on which the initial conidia began the infection process has substantially reduced susceptibility to the development of scab lesions; thus, lesions will likely occur only after long second wetting intervals, and the lesions might not be visible until later than currently expected for newly expanded foliage.

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