Influence of Temperature and Wetness Duration on Conidia and Appressoria of *Colletotrichum acutatum* on Symptomless Strawberry Leaves

L. F. S. Leandro, M. L. Gleason, F. W. Nutter, Jr., S. N. Wegulo, and P. M. Dixon

First, second, third, and fourth authors: Department of Plant Pathology; and fifth author: Department of Statistics, Iowa State University, Ames 50011.

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ABSTRACT

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Strawberry leaves (cv. Tristar) inoculated with *Colletotrichum acutatum* conidia were incubated at 10, 15, 20, 25, 30, and 35°C under continuous wetness, and at 25°C under six intermittent wetness regimes. The number of conidia and appressoria was quantified on excised leaf disks. In order to assess pathogen survival, inoculated leaves were frozen and incubated to induce acervular development. Germination, secondary conidiation, and appressorial development were significantly ($P \leq 0.05$) affected by temperature and wetness treatments. Under continuous wetness, the optimum temperature range for conidial germination was 23.0 to 27.7°C, whereas the optimum temperature for appressorial develop-

ment ranged from 17.6 to 26.5°C. Secondary conidiation showed an optimum temperature range of 21.3 to 32.7°C and was most abundant between 12 and 36 h after inoculation. Conidial germination, appressorial production, and secondary conidiation were favored by increasing wetness duration and more than 4 h of wetness were required for secondary conidiation. In a greenhouse, *C. acutatum* survived up to 8 weeks on leaves. The number of acervuli formed on leaves after freezing and incubation was closely ($r^2 \geq 0.95$) related to appressorial populations prior to this treatment and was greatest following periods of continuous wetness. Production of secondary conidia and appressoria of *C. acutatum* on symptomless strawberry leaves under a range of environmental conditions suggests that these processes also occur under field conditions and contribute to inoculum availability during the growing season.

Additional keywords: anthracnose fruit rot, phyllosphere.

Anthracnose fruit rot, caused by *Colletotrichum acutatum* J.H. Simmonds, presents a major challenge for strawberry production worldwide (4,16–18,36). Under favorable environmental conditions, this disease can devastate entire production fields (14,17) and cause significant losses in the nursery industry (7,8,37). Fruit rot is the most important symptom caused by *C. acutatum* on strawberries, but lesions can develop on all other plant parts (13,17,36). Stolon lesions reduce runner plant production, and crown rots kill plants in nurseries and production fields after transplanting (17,36,37). *C. acutatum* has also been implicated in recent disease outbreaks in greenhouse strawberry production (15).

Long-distance dissemination of the pathogen occurs on contaminated propagation material (7,8,13,14,22,36). Transplants may harbor inoculum in lesions (17,24), in infested soil attached to roots (7), or as conidia or quiescent infections on symptomless foliage (10,12,19,33). After sporulation occurs on lesions or senescent plant material, dissemination of the fungus occurs mainly by splash dispersal (43), but may also occur through handling of plants (31), especially during harvest (22).

The epidemiology of *C. acutatum* on strawberry has been well characterized with regard to splash dispersal of conidia (25–27,42,43), infection and sporulation on fruit (18,39), and survival in plant debris and soil (7,8,40). Little is known, however, about the behavior of *C. acutatum* on symptomless plants prior to the onset of an epidemic. *C. acutatum* conidia can germinate, form appressoria, and produce secondary conidia on symptomless strawberry

leaves when incubated at 26°C under continuous wetness (19). The fungus was also shown to survive under greenhouse conditions on asymptomatic leaves of several plant species (12). These studies suggested that symptomless foliage might play an important role in the anthracnose disease cycle both as a refuge for fungal survival and a source of inoculum for fruit and flower infections (12,19,33). Temperature and wetness have been reported to affect mycelial growth (1,7,11,21,37,39), germination and sporulation of *C. acutatum* on agar (11), and infection and sporulation on fruit (18,39), but similar work has not been done on strawberry foliage. Furthermore, production of secondary conidia was not addressed in these earlier studies.

In order to develop more effective management tactics, there is a need to understand the behavior of *C. acutatum* on strawberry leaves under a range of environmental conditions. The objectives of this study were to (i) determine the effects of temperature and intermittent wetness on germination and secondary conidiation of *C. acutatum* on strawberry leaves, and (ii) assess the potential for *C. acutatum* to survive on symptomless strawberry leaves. A preliminary report has been published (20).

MATERIALS AND METHODS

Plant production and maintenance. Day-neutral strawberry crowns (cv. Tristar) were planted in 15-cm-diameter plastic pots containing a 1:2:1 mixture of peat, perlite, and soil, and grown in a greenhouse at $25 \pm 10^\circ\text{C}$ with a 16-h photoperiod. The plants were drip-irrigated and fertilized with a solution of a 21-5-20 fertilizer (Miracle Gro Excel, 400 ppm N) as needed. For each experiment, newly emerged leaves were tagged 7 to 14 days before inoculation to ensure uniformity of leaf age.

Corresponding author: M. L. Gleason; E-mail address: mgleason@iastate.edu

Inoculum production. Isolate HF-ac-98 of *C. acutatum* was obtained from an infected strawberry fruit collected at the Iowa State University Horticulture Research Farm near Gilbert, IA, and transferred to silica gel for long-term storage. All cultures used for inoculum preparation were started by depositing infested silica gel crystals on potato dextrose agar (PDA) plates and culturing at 25°C in darkness until conidia developed. Conidia collected from PDA cultures were spread onto strawberry fruit agar (SFA) (19) and incubated for 7 days at 25°C in darkness. Conidial suspensions used for inoculations were prepared by flooding each SFA culture plate with 9 ml of sterile deionized water (SDW), scraping with a rubber policeman to dislodge conidia, filtering through two layers of cheesecloth into a flask, and adjusting the concentration to 5×10^5 conidia ml^{-1} .

Temperature experiments. Tagged strawberry leaves were excised at the petiole base and placed in moist chambers ($24 \times$

34×6 -cm plastic boxes containing 500 ml of SDW). Leaves were suspended above the water on a wire mesh and the excised ends of the petioles were inserted through the mesh into the water. For inoculation, the wire mesh was removed from each box and the leaves were sprayed with a conidial suspension with an atomizer (DeVilbiss Air Compressor, 571 Series, Somerset, PA). Approximately 5 ml of conidial suspension was sprayed over a 20×30 -cm area, resulting in an average application of 40 conidia mm^{-2} of leaf area. The leaves and wire mesh were returned to the boxes, which were then sealed in plastic bags to maintain approximately 100% relative humidity and incubated at 10, 15, 20, 25, 30, or 35°C ($\pm 1^\circ\text{C}$) in darkness. Temperature in the incubators (Model 6; Thelco, Chicago, IL / Model I37-L; Percival, Perry, IA) was monitored and recorded every 15 min using WatchDog Data Loggers (Model 150; Spectrum Technologies, Plainfield, IL).

Fig. 1. Conidial and appressorial populations of *Colletotrichum acutatum* on strawberry leaves incubated at 10 to 35°C under continuous wetness: **A to C**, Germinated conidia; **D to F**, conidia producing secondary conidia; **G to I**, total conidia; and **J to L**, total appressoria. Data shown are averaged over both runs of the experiment.

Four leaves were sampled arbitrarily from each incubator at 6, 12, 18, 24, 36, 60, 84, and 132 h after inoculation. Two leaf disks were excised from each leaf, one on either side of the central vein of the middle leaflet, with a 1-cm-diameter cork borer. The disks were mounted on a drop of Hoyer's medium (6), stained with a drop of 0.1% aniline blue in lactophenol, covered with a glass coverslip, and observed under a light microscope. The total number of germinated and ungerminated conidia was counted in each of 16 microscope fields ($\times 400$) per leaf disk. A conidium was considered germinated when it had formed either a germ tube at least as long as its width, an appressorium (a globose, septum-delimited structures formed on germ tubes or sessile to conidia), or a conidial phialide. Unmelanized and melanized appressoria were also counted. The number of conidia with a secondary conidium still attached to a conidial or hyphal phialide was quantified to estimate secondary conidiation (19). The number of total conidia (ungerminated + germinated) and total appressoria (unmelanized + melanized) was calculated. Data were expressed on an area basis (square millimeter) by dividing the total number of conidia or appressoria in each leaf disk by the area of 16 microscope fields observed per disk. Eight disks were counted at each sampling time (two disks $\times$ four leaves per sampling time), and the experiment was repeated once.

Intermittent wetness experiments. Germination and conidiation of *C. acutatum* was further investigated on the leaf surface of intact strawberry plants incubated at $25 \pm 2^\circ\text{C}$ under intermittent wetness. In order to establish six wet/dry treatment regimes, plants were alternated between dew chambers (Model I60DL; Percival) for the wet periods and a growth chamber (Model PGW36; Conviron, Winnipeg, Canada) for the dry periods. Treatments were defined by the ratio of dry to wet hours per day as

follows: 0/24, 4/20, 8/16, 12/12, 16/8, and 24/0. The 24-h cycles were repeated for 7 days. All plants were watered daily at the start of each cycle and kept under a 12-h dark/12-h light regime.

Ten plants arbitrarily assigned to each treatment were distributed within dew chambers and growth chambers according to a split-plot design with two blocks, using day of sampling as the whole-plot treatment and wet/dry regime as the sub-plot treatment. Each plant was spray-inoculated with a conidial suspension, using an atomizer as described previously, until all leaves were thoroughly covered with inoculum but no runoff had occurred. Following inoculation, plants were placed in dew chambers to start the wet incubation period, with the exception of plants in the continuous dry treatment, which were dried with fans for 10 to 15 min until no free water was observed on the leaves, then placed in the growth chamber. At the end of each wet period, plants were removed from the dew chamber, dried with fans (15 to 30 min), and placed in the growth chamber for the remaining dry period. Initial conidial populations on the leaves were estimated using an additional set of eight plants that were inoculated, dried, and sampled immediately after inoculation (time zero assessment). Two tagged leaves from each of two plants per treatment were sampled at the end of the 24-h cycle on days 1, 2, 3, 5, and 7 after inoculation. Two leaf disks excised from the middle leaflet of each leaf (a total of eight disks per treatment per sampling time) were prepared for microscopic observation, and conidial and appressorial numbers were quantified as described previously. The experiment was repeated once.

Survival experiments. Survival of *C. acutatum* on symptomless leaves was investigated using leaves remaining on inoculated plants that had been exposed to the wet/dry treatment regimes in the intermittent wetness experiment. At the end of the 7-day

TABLE 1. Quadratic regression analysis results for the effects of temperature on numbers of germinated conidia, conidia producing secondary conidia, total conidia, and appressoria on the surface of strawberry leaves inoculated with *Colletotrichum acutatum* and incubated under continuous wetness^z

Count	Incubation period (h)	y intercept	Linear slope	Quadratic slope	R ²	P value for lack of fit	Temperature optimum (°C)	SE for temperature optimum
Germinated conidia	6	-54.9	6.79	-0.13	0.91	0.88	26.5	0.9
	12	-57.6	8.37	-0.18	0.81	0.80	23.8	0.7
	18	—	—	—	—	—	—	—
	24	—	—	—	—	—	—	—
	36	—	—	—	—	—	—	—
	60	—	—	—	—	—	—	—
	84	—	—	—	—	—	—	—
	132	—	—	—	—	—	—	—
Conidia producing secondary conidia	6	—	—	—	—	—	—	—
	12	-16.0	1.77	-0.04	0.50	0.019*	24.5	1.8
	18	-17.9	1.92	-0.03	0.61	0.29	28.3	4.4
	24	-15.3	1.80	-0.04	0.66	0.005*	24.0	1.1
	36	-7.14	0.87	-0.02	0.23	0.69	24.4	3.1
	60	-1.62	0.23	-0.005	0.13	0.18	23.1	3.3
	84	—	—	—	—	—	—	—
	132	—	—	—	—	—	—	—
Total conidia	6	-3.27	3.81	-0.08	0.81	0.055	25.4	0.4
	12	-0.86	4.31	-0.09	0.71	0.61	25.2	0.4
	18	-29.5	7.21	-0.13	0.48	0.041*	27.5	1.1
	24	-52.6	10.7	-0.22	0.61	0.13	24.6	1.5
	36	-73.9	13.7	-0.28	0.60	0.10	24.1	1.8
	60	-77.0	13.9	-0.30	0.64	0.10	23.4	1.8
	84	-86.2	15.7	-0.35	0.55	0.097	22.4	1.3
	132	-43.0	10.8	-0.25	0.57	0.15	21.4	2.0
Appressoria	6	-61.4	7.56	-0.16	0.94	0.66	24.4	1.3
	12	-78.9	10.6	-0.24	0.91	0.71	22.6	1.6
	18	-41.9	7.36	-0.16	0.61	0.70	22.6	3.9
	24	-42.1	8.13	-0.18	0.49	0.37	22.6	1.4
	36	-34.2	8.83	-0.21	0.80	0.67	20.9	1.5
	60	-48.3	10.6	-0.26	0.84	0.49	20.5	1.4
	84	-14.8	8.03	-0.21	0.81	0.62	19.1	1.3
	132	-33.0	10.8	-0.28	0.88	0.47	19.2	1.6

^z * indicates significant lack of fit at $P = 0.05$. — indicates linear regression results are not reported if standard error (SE) for temperature optimum > 5 .

incubation period, plants were dried with fans and all inoculated leaves were tagged to distinguish them from newly emerging, noninoculated leaves. The plants were then transferred to a greenhouse bench where they were maintained at $30 \pm 10^\circ\text{C}$ and drip-irrigated to prevent free moisture on leaves. In both runs of the experiment, one tagged leaf was sampled from each of four plants per treatment at 1, 2, 4, 6, and 8 weeks after transfer to the greenhouse bench. In the second run, leaves were also sampled on the day of transfer from the chambers to the greenhouse bench (week 0). Sampled leaves were bagged and frozen overnight in order to kill leaf tissue and incubated for 7 days at 25°C and 100% relative humidity in moist chambers, as described previously, to induce acervular development by *C. acutatum* (28). The total number of acervuli on the middle leaflet of each leaf, counted with a dissecting microscope ($\times 10$), was divided by the leaflet area, measured with a leaf area meter (Model 3100; Li-Cor, Lincoln, NE), to estimate the number of acervuli per square centimeter.

Data analysis. Quadratic regression analysis was performed on data from the temperature experiment using the general linear models (GLM) procedure of SAS (SAS Institute, Cary, NC). Temperature optima and confidence intervals for each type of measurement were estimated based on Fieller's theorem. Linear regression analysis was performed using the GLM procedure on data for the intermittent wetness experiment, excluding data for the 0 h wetness treatment. Survival on leaves was analyzed separately for each run of the experiment according to a randomized

complete block design with two replications. Area under the curve (AUC) values were calculated and compared using Duncan's multiple range test. Nonlinear regression analysis was performed using SigmaPlot 2000 (SPSS, Chicago, IL) to determine the relationship between the number of acervuli formed on leaves after freezing and the number of appressoria on leaves at week 0 of the survival experiment (i.e., day 7 of intermittent wetness experiment). Statistical significance of all analyses was expressed at the $P \leq 0.05$ threshold.

RESULTS

Temperature experiments. *C. acutatum* conidia germinated, formed appressoria, and produced secondary conidia at all temperatures studied (Fig. 1). The timing and magnitude of these processes were, however, significantly affected by temperature. An optimum temperature range of 23.0 to 27.7°C was observed for conidial germination within 12 h after inoculation (Table 1; Fig. 1A), but by 18 h the number of germinated conidia was similar among temperatures (Fig. 1B). After 60 h, the number of germinated conidia tended to decrease with increasing temperature, probably due to cell lysis. Conidia appeared unusually swollen and highly vacuolated at 35°C starting 12 h after inoculation.

Temperature significantly influenced production of secondary conidia on the surface of symptomless leaves, determined by the

Fig. 2. Conidial and appressorial populations of *Colletotrichum acutatum* on strawberry leaves incubated at 25°C and different wet/dry treatment regimes: A, Germinated conidia; B, conidia producing secondary conidia; C, total conidia, and D, total appressoria. Data shown are averaged over both runs of the experiment.

number of conidia observed with a secondary conidium attached to a conidial or hyphal phialide (19). The optimum temperature for secondary conidiation ranged from approximately 21.3 to 32.7°C between 12 and 36 h after inoculation (Table 1). Secondary conidiation was rare at 10°C and at sampling times of >36 h after inoculation (Fig. 1D to F). Production of secondary conidia increased the total number of conidia on the surface of symptomless leaves, particularly at 25 and 30°C, at which total conidia more than doubled during the experiment (Fig. 1G to I). Although the number of germinated conidia began to decrease by 60 h, this decrease was counterbalanced by high numbers of ungerminated conidia produced by secondary conidiation at these temperatures.

Production of appressoria was favored by cooler temperatures than conidial germination and secondary conidiation (Fig. 1J to L). The optimum for appressorial production ranged from 17.6 to 26.5°C and decreased with time after inoculation (Table 1). For example, although appressorial production was faster at 23 to 26°C 6 h after inoculation, the largest number of appressoria was observed at 17.6 to 20.8°C 132 h after inoculation. Overall, the least favorable temperature for appressorial production was 35°C (Fig. 1J to L).

Intermittent wetness. Conidial germination, secondary conidiation, and appressorial development were significantly affected by wet/dry treatment regime at 25°C (Fig. 2). Conidial germination increased with increasing daily period of wetness duration, indicated by the positive slopes of the regression (Table 2), and never occurred in the continuously dry treatment (Fig. 2A). The number of germinated conidia was largest 1 day after inoculation in all treatments with ≥8 h wetness per day, and then decreased over time in all treatments.

Production of secondary conidia occurred mainly within 1 day after inoculation and was rare at later sampling times (Fig. 2B). Secondary conidiation was observed only in treatments with >4 h of wetness per day and increased with increasing wetness period within 1 day after inoculation (Table 2). Consequently, total conidial numbers were greater under continuous wetness than in any other treatment 1 day after inoculation. Total conidia then tended to decrease over time in all treatments.

Appressorial production increased with daily wetness duration at each sampling time (Fig. 2D; Table 2) and, in contrast to conidia, did not tend to decrease over the period of the experiment. Wetness duration also affected appressorial melanization (L.

F. S. Leandro, unpublished data). Melanization never occurred on appressoria formed with 4 h wetness and was most pronounced in treatments with ≥12 h wetness, in which ≥90% of appressoria became melanized.

Survival. The number of acervuli formed on leaves after freezing and incubation treatment decreased with time after transfer of plants, which had been inoculated and incubated under different intermittent wetness regimes, to the greenhouse bench (Fig. 3). There were significantly more acervuli on leaves incubated under continuous wetness than on leaves from any other treatment in both runs of the experiment (Table 3). In the first run, acervular numbers were also significantly higher in the treatment with 16 h wetness than in those with shorter wetness durations (Table 3). At each sampling time, the number of acervuli formed on leaves was

Fig. 3. Number of acervuli formed by *Colletotrichum acutatum* on strawberry leaves after the following treatment regime: plants were spray-inoculated with *C. acutatum* conidia, incubated at 25°C under six different wet/dry treatment regimes for 7 days, and maintained in the greenhouse under dry conditions for up to 8 weeks. At each sampling time during the latter period, leaf samples were excised, frozen overnight, and incubated at 25°C and 100% relative humidity for 1 week to induce acervular development. Data shown are for the second run of the experiment.

TABLE 2. Linear regression analysis of effects of intermittent wetness on numbers of germinated conidia, conidia producing secondary conidia, total conidia, and appressoria on the surface of strawberry leaves inoculated with *Colletotrichum acutatum* and incubated at 25°C^z (data for the treatment with 0 h wetness was not included in the analysis)

Count	Incubation period (days)	y intercept	Linear slope	R ²	P value for lack of fit
Germinated conidia	1	0.90	1.95	0.75	0.0017*
	2	1.69	1.43	0.67	0.0004*
	3	1.21	1.00	0.43	0.062
	5	1.85	0.40	0.35	0.10
	7	-0.93	0.55	0.43	0.0014*
Conidia producing secondary conidia	1	-0.21	0.06	0.41	0.50
	2	-0.007	0.002	0.09	0.97
	3	0.000	0.000	—	—
	5	-0.016	0.002	0.08	0.47
	7	-0.026	0.004	0.09	0.56
Total conidia	1	34.91	1.05	0.32	0.0004*
	2	33.05	0.24	0.11	0.20
	3	26.68	-0.12	0.09	0.0077*
	5	23.82	-0.73	0.31	0.012*
	7	13.12	-0.14	0.02	<0.0001*
Appressoria	1	-1.67	2.01	0.79	0.024*
	2	-5.12	2.50	0.78	0.60
	3	-6.04	2.46	0.74	0.078
	5	-9.59	3.02	0.82	0.0005*
	7	-8.25	2.53	0.73	0.17

^z * indicates significant lack of fit with *P* = 0.05.

closely related ($r^2 \geq 0.95$) to the number of appressoria on the leaves before freezing (Fig. 4).

DISCUSSION

This study indicates that temperature and the duration of intermittent wetness periods affect germination, secondary conidiation, and appressorial development of *C. acutatum* on symptomless strawberry leaves. The optimum temperature range of 23 to 27°C observed for conidial germination is similar to that previously reported for germination and mycelial growth rates of *C. acutatum* in culture (7,21,37,39) and for infection and sporulation on strawberry fruit (18,39). Others have reported reduced or no growth of *C. acutatum* on agar at temperatures above 33°C (7,18,39) and attributed low incidence of strawberry fruit infection at 35°C to high pathogen mortality (39). In our study, higher incidence of conidial lysis at 35°C may explain the decrease in conidial populations observed on leaves under continuous wetness at this temperature. Reports on the effects of wetness duration on *C. acutatum* have been limited to studies on strawberry fruit (39) or in vitro (11). Fruit rot incidence increased with wetness duration (39), and germination on glass slides was greatly reduced when relative humidity was below 91% (11). In our study, conidial germination was also favored by increasing wetness duration, and was very low when daily wetness periods were ≤ 4 h.

We previously reported the production of secondary conidia by *C. acutatum* on symptomless strawberry leaves at 26°C during continuous wetness (19). Induction of secondary conidiation by other fungal species has been associated with high inoculum density (35,38), germination autoinhibitors (23,38), nutrient limitation (5,35,38), and high temperature stress (3,38). Temperature and wetness effects on secondary conidiation of *C. acutatum* had not been investigated previously. In our study, secondary conidiation was observed as soon as 12 h after conidial germination between 20 and 30°C, suggesting a potential for rapid inoculum increase on symptomless plants under favorable environmental conditions. The fact that most secondary conidiation was detected within 24 h after inoculation in both temperature and wetness experiments suggests that *C. acutatum* may lose its capacity to multiply in this manner after prolonged periods, possibly due to depletion of conidial energy reserves. The nutrient source for such multiplication is unclear, but previous studies with *C. acutatum* showed that the presence of the host was not required (19).

Appressoria serve as infection (9,34) and survival (29,32,34) structures in *Colletotrichum* spp. Appressorial development by this group of fungi is affected by several factors including nutrient stress, host surface characteristics, phylloplane microorganisms, temperature, and light intensity (9,32). In our study, appressorial production was favored by cool temperatures and was severely impaired at 35°C. Similar results have been obtained on agar,

where *C. acutatum* showed an optimum range for appressorial production at 20 to 25°C but lost the capacity to produce these structures at 30°C (21). As with germination and secondary conidiation, appressorial production and melanization increased with wetness duration, but in contrast to conidia, appressorial populations tended to remain stable over the course of the experiment. Appressorial melanization has been associated with protection against desiccation, UV light, unfavorable temperatures, and microbial activity (9,29). Vulnerability of unmelanized appressoria to some of these factors may explain the decrease in appressorial numbers over time on leaves incubated with 8 h wetness per day, because a higher proportion of appressoria remained unmelanized in this treatment compared to those with longer wetness durations.

The broad temperature and wetness tolerance of appressorial production by *C. acutatum* suggests it is well adapted to survival on strawberry leaves under conditions that are suboptimal for conidial germination and multiplication. More rapid appressorial production at 23 to 26°C compared with other temperatures may, however, indicate that the pathogen has the ability to form survival structures even when optimum conditions for germination and secondary conidiation are present for relatively short periods.

In our study, *C. acutatum* survived for up to 8 weeks on strawberry leaves under dry greenhouse conditions. In a similar study (12), this fungus survived for over 3 months on symptomless foliage of strawberry and nonhost plants. *C. acutatum* was also shown to survive for up to 6 months on mummified strawberry fruit (40), 9 months in soil (7), and 2 years on pine debris (30). Survival of the fungus on strawberry and other hosts may occur as melanized appressoria (2,30,34), conidia (12,31), or quiescent infections (12). The strong relationship between appressoria and acervuli on leaves in our experiments suggests that *C. acutatum* survived dry conditions primarily as appressoria. Although the number of acervuli decreased rapidly over time, even the lowest acervular densities observed in our study might give rise to substantial inoculum levels because each acervulus contains many conidia.

Multiplication of *C. acutatum* on symptomless foliage prior to appearance of fruit rot or other symptoms may set the stage for epidemics that occur in strawberry production fields. Early suppression of fungal multiplication could therefore delay the onset

TABLE 3. Survival of *Colletotrichum acutatum* on strawberry leaves incubated at 25°C under different wet/dry treatment regimes for 7 days and maintained under dry conditions on a greenhouse bench for up to 8 weeks; survival was then assessed by freezing the leaves overnight and incubating at 25°C and 100% relative humidity for 7 days to stimulate formation of acervuli^z

Treatment	Number of acervuli (cm ⁻² leaf area)	
	Run 1	Run 2
0 h wet/24 h dry	0.0 c	0.0 b
4 h wet/20 h dry	0.0 c	0.0 b
8 h wet/16 h dry	0.8 c	0.9 b
12 h wet/12 h dry	4.4 c	23.7 b
16 h wet/8 h dry	14.8 b	62.2 b
24 h wet/0 h dry	44.8 a	336.6 a

^z Values followed by the same letter within columns are not significantly ($P \geq 0.05$) different according to Duncan's multiple range test. Values are area under the curve for acervuli formed on leaves over the survival period.

Fig. 4. Exponential relationship between the number of appressoria of *Colletotrichum acutatum* on strawberry leaves at the start of an 8-week survival period and the number of acervuli induced to form on leaves by freezing and incubating at 25°C and 100% relative humidity for each sampling time. During the survival period, the plants were maintained in the greenhouse under dry conditions. Appressoria were counted on leaves at the end of a 7-day incubation period at 25°C under six different wet/dry treatment regimes. Data shown are for the second run of the experiment.

of epidemics and reduce disease levels. For example, reduction of *C. acutatum* inoculum on transplant foliage may contribute to the effectiveness of fungicide dips used in Israel to control strawberry anthracnose (14). Although most evidence for the role of symptomless foliage as a source of inoculum for strawberry anthracnose originates from controlled environment studies (12,19), symptomless leaves provided sufficient inoculum to initiate fruit rot development in preliminary field trials (41). These preliminary results corroborate the relevance of our findings to the behavior of *C. acutatum* under field conditions.

This study provides strong evidence that *C. acutatum* can become established, persist, and produce inoculum on symptomless strawberry leaves under a range of temperatures and intermittent wetness periods. In addition to our previous work at 26°C under continuous wetness (19), we have now shown that secondary conidia and appressoria may be produced under environmental conditions typical of many temperate strawberry-producing areas in the world. Survival of *C. acutatum* on strawberry leaves has implications for long-distance dissemination on transplants, as well as proliferation and dissemination of the fungus within production fields. Furthermore, asymptomatic foliage of other plant species, including weeds, has been recently suggested as an additional inoculum source for *C. acutatum* (12). The environmental biology of *C. acutatum* on symptomless foliage should therefore be considered when developing management practices for strawberry anthracnose.

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