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EPIDEMIOLOGY OF STRAWBERRY LEATHER ROT

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EPIDEMIOLOGY OF STRAWBERRY LEATHER ROT

Dissertation

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

by

Gary Glenn Grove, B.S., M.S.

*****

The Ohio State University

1984

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TO ODESSA GROVE AND MY ENTIRE FAMILY
ACKNOWLEDGEMENTS

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Major Field: Plant Pathology

Studies in Epidemiology. Drs. M.A. Ellis and L.V. Madden

Studies in Disease Control. Drs. M.A. Ellis and L.V. Madden
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INTRODUCTION

Leather rot of strawberry, caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., is a potentially serious disease of the cultivated strawberry (*Fragaria x ananassa* Duch.) It has been reported from many regions throughout the United States. Rose (28) first reported the disease from a number of southern states. In his initial paper, he reported the results of an extensive disease survey and performed Koch's postulates with the causal organism. Disease severity was correlated with rainfall in a subsequent paper (29). Wright, Beraha, and Smith (47,48) later reported the disease in California and Louisiana.

In Ohio, the disease was neither reported nor considered to be of economic importance until the 1981 growing season, when fruit rots resulted in fruit losses of 40-50% in some areas. Excessive rainfall during May, June, and early July of that year resulted in 20-40% crop losses due to leather rot in Knox, Lorain, Sandusky, Warren, and Wayne counties. In 1982, a much dryer year, leather rot was observed throughout the state and losses ranged from 5-10%. However, one grower in an area of localized heavy rainfall reported 30% losses of his early crop (cv. Earliglow).

All cultivars observed during disease surveys appeared susceptible to leather rot. These included cvs. Earliglow, Guardian, Klondike,
Marlate, Midway, Pochahontas, Red Chief, Robinson, Scott, and Vesper.

**Symptomatology.** *P. cactorum* can infect strawberries at all stages of development. Infection of green fruit and blossoms is common. On green fruit, diseased areas are dark brown or natural green outlined by a brown margin. As the decay spreads, the entire fruits become brown, maintain a rough texture, and are leathery. A pungent, phenolic odor is characteristic of infected green fruit. Numerous papillate, caducous sporangia characteristic of *P. cactorum* are observed upon microscopic examination of infected fruit collected during or immediately after extended rainy periods. At times, white mycelia can be observed on infected fruit. Internally, transverse sections of infected fruit show a marked darkening of each vascular bundle extending from the main bundles into each achene. A brown discoloration is characteristic of pith tissue. Abundant oospores are produced within diseased tissue and became more numerous as the decay progresses. Eventually, infected fruit form rough, shriveled mummies.

On fully mature berries, infection often results in little change to discoloration ranging from brown to dark purple. Diseased, mature fruit also have the phenolic odor, and are very bitter tasting. White mycelia can also be observed on ripe fruit. Mature fruit also become rough and leathery, and eventually form mummies during the latter stages of decay.

Due to reports of the disease following periods of excessive rainfall and the occurrence of epidemics in Ohio during wet growing seasons, an extensive study of the epidemiology of the disease was
initiated by the author. Other than Rose's paper (29), the author is unaware of any other information on the epidemiology of the disease.

Objectives of this study were to: determine the wetness durations and temperatures necessary for fruit infection and pathogen sporulation on the surface of infected fruit, to determine the mode of pathogen dispersal, and to determine the mode of survival (overwintering) of P. cactorum.
CHAPTER I

Influence of Temperature and Wetness Duration on Infection of Strawberry Fruit by Phytophthora cactorum

INTRODUCTION

Leather rot, caused by Phytophthora cactorum (Leb. & Cohn) Schroet., is a serious fruit rot disease of the cultivated strawberry (Fragaria x anassa) Duch. (8,28). First reported by Rose in 1924 in the southern United States (28), the disease has since been only occasionally mentioned (46,47,48).

Severe epidemics of leather rot have occurred in the Midwest, particularly in Ohio, during 1980 and 1981, with several growers reporting up to 40% fruit losses due to leather rot. Above average rainfall was generally associated with disease outbreaks (8,29,48).

Environmental parameters (e.g., wetness duration and temperature) conducive to infection by pathogenic fungi have been determined for several diseases (2,4,7,23,24,39). In addition to describing the symptoms and causal organism of leather rot, Rose (29) correlated disease severity with rainfall. He found leather rot to be much more prevalent in areas receiving large amounts of precipitation or those with poor drainage. Other than these observations, no other information is available on the epidemiology of this disease. The initial observations of Rose and the epidemics experienced in Ohio in
recent years, suggested that moisture and/or rainfall were important epidemiological factors favoring development of strawberry leather rot. The purposes of this study were to determine the duration of fruit wetness at specific temperatures necessary for infection of strawberry fruit by *P. cactorum*, and to develop a model to predict the level of fruit infection under both controlled and field conditions.

**MATERIALS AND METHODS**

All inoculations were performed with cultures of *P. cactorum* freshly isolated from infected strawberry fruit (cv. Midway) on pentachloronitrobenzene-benomyl-neomycin sulfate-chloramphenicol (PBNC) medium (35). For sporangial production, mycelial transfers from the edges of 3-day-old cultures were transferred to lima bean agar (35). Cultures were incubated for 7 days at 22 C in continuous light at 9,000 lux. Sporangia were washed from the surface of plates by adding 20 ml sterile distilled water (SDW) and gently swirling for 1 min. All inoculations were made with sporangial suspensions adjusted to 400/ml in SDW using a hemacytometer. Suspensions were kept at 5 C for no longer than 30 min to delay germination. All inoculations were made by applying 1 ml of suspension to each fruit with an atomizer.

**Controlled environment studies.** Strawberry plants (cv. Midway) were grown to reproductive maturity in a 1:1:1 (v:v:v) peat:sand:steam-disinfested loam mixture. Immature (green) fruit on each plant were tagged and 5-18 fruit/plant were inoculated with *P. cactorum* as previously described. To induce infection periods, inoculated plants were kept continuously wet at constant temperature in
a controlled environment chamber (Environmental Growth Chambers [EGC], Chagrin Falls, Ohio 44022) containing a Herrmidifier mister (Herrmidifier Co., Lancaster, PA 17604) inside a 1 m³ clear plastic chamber. All tests were conducted in darkness. Two plants were removed at 1, 2, 3, and 4-hr intervals and placed in a second chamber (EGC), at the same temperature as the first, for drying. Inoculations for wetness durations of less than 1 hr were performed by inoculating plants in the drying chamber. Temperature and fruit wetness in both chambers were continuously monitored with thermistors (Fenwall Electronics, Ashland, MA 01721) and printed-circuit leaf wetness sensors (Wong Labs, Cincinnati, OH 45200) connected to a microprocessor-controlled Datalogger (CR-21, Campbell Scientific, Logan, Utah, 84321). Leaf wetness sensors in the drying chamber were gently misted with an atomizer upon transfer of inoculated plants from the wetness chamber. After a 24 hr drying period, inoculated plants were removed and incubated in a third growth chamber (EGC) at 22 C with a 14 hr photoperiod at 25,000 lux. Numbers of infected and non-infected fruit were visually determined 72 hr later. Isolations were made on PENC media to verify the presence of P. cactorum. Tests were performed for nine constant temperatures between 6 and 30 C. The experiment was then repeated. The order of temperatures tested was random.

Field studies. Field studies were performed during the 1983 growing season in order to validate a regression model developed from the EGC data. Midway strawberries were also used in field studies. Fifty-1m long plots in a completely randomized design were established
in a 3-yr-old planting near Wooster, Ohio. Row spacing was 120 cm; plots to be inoculated were chosen at random. Attached, immature fruit were used in all inoculations. Fifty immature, attached fruit were tagged in each plot. Twenty-five were inoculated with a sporangial suspension of _P. cactorum_ as previously described and the remaining 25 served as uninoculated controls. Inoculations were made during natural wetness (dew or rainfall) periods or during wetness periods induced by gently misting fruit, with an atomizer, to runoff. At times, plants were covered with 1.5 x 1.5 x 1m wood-framed clear plastic chambers to prolong wetness periods. Chambers were used only at night to prevent significant alteration of radiation and temperature. Wetness, temperature, and relative humidity (Phys-Chem sensor, Phys-Chemical Research Corporation, New York, NY 10011) were continuously monitored throughout the experiment using the CR-21 datalogger. Sensors were placed immediately adjacent (within 5 cm) to inoculated fruit in the plant canopy. At least 1 hr after the conclusion of wetness periods (as determined by the wetness sensors), both inoculated and uninoculated fruit were harvested, placed on metal screens and incubated for 24 hr at 22 C with a 14 hr photoperiod at 25,000 lux. Fruit and screens were then transferred to 5 L plastic containers containing 100 ml SDW; containers were then covered with plastic wrap, sealed with tape, and incubated for an additional 48 hr at 22 C in a 14 hr photoperiod at 25,000 lux. Numbers of infected and uninfected fruit from both the inoculated and noninoculated treatments were visually determined 72 hr after fruit harvest. Isolations from all fruits were made on PBNC media to verify the presence of _P. cactorum_.

As determined by the wetness sensing grids, the combined mean drying time for both experiments was 33 min. Actual drying time for each temperature/wetness inoculation ranged from 15 to 50 min. The specific drying time for each inoculation was added to the preassigned wetness periods to give a total time of wetness duration.

Data analyses. Regression analysis was used to determine the effect of temperature ($T$) and wetness duration ($W$) on the proportion of strawberry fruit infected ($Y$) by *P. cactorum*. Analysis was based on the controlled environment studies. Properties of the regression had to include: (i) an optimum relationship between $Y$ and $T$, in which $Y$ increases to a maximum and then declines; and (ii) a positive (monotonically increasing) relationship between $Y$ and $W$, with the provisions that predicted infection cannot be less than 0.0 or greater than 1.0, regardless of the value of $W$. A logistic model was chosen of the form:

$$Y = \frac{1}{1 + \exp(-f(T, W))} \quad (1)$$

in which $\exp$ is $e$ (2.718) raised to a specified power, and $f(T, W)$ is an arbitrary function of $T$ and $W$. To handle cases when $Y$ would normally equal 0 or 1, a slightly biased estimator of $\frac{Y}{N}$ was used: $(I + 0.5)/(N+1)$, in which $I$ is the number of infected fruit and $N$ is the number inoculated. Equation 1 can be transformed to:

$$\ln(Y/(1-Y)) = f(T, W) \quad (2)$$

in which $\ln(Y/(1-Y))$ is the logit of $Y$. Linear terms were tested for making up $f(T, W)$. Those terms evaluated were: $W$, $T$, $W*T$, $T^2$, $W*T^2$, $T^3$, and $W*T^3$. All possible combinations of these terms were evaluated.
for: significance of the estimated parameters, coefficient of
determination, and pattern of residuals (22,25). The regression
analysis was performed on the data for each growth chamber experiment
separately, and then on the combined data. An F-test was conducted to
determine if the results from the two experiments were significantly
different (25).

In addition to equation 1, two other models were evaluated. The
first was an extension of Schrodter's (36) sine-model of the form

$$Y = \sin^2(f(T,W))$$  \hspace{1cm} (3)

which can be transformed to:

$$\arcsin(\sqrt{Y}) = f(T,W)$$  \hspace{1cm} (4)

In these two equations, \(\sin^2\) is the square of the trigonometric sine
function and \(\arcsin\) is the inverse sine function. All possible
combinations of the above described temperature and wetness terms were
evaluated as \(f(T,W)\).

The second model was a generalization of Analytis' "Beta" model
(1) of the form:

$$Y = pt^m(1-t)^nW^q$$  \hspace{1cm} (5)

in which \(p, m, n,\) and \(q\) are parameters, and \(t=(T-Tmin)/(Tmax-Tmin)\).
The maximum (Tmax) and minimum (Tmin) temperature were not known
precisely, but were assigned 35 and 34 C, respectively. Other values
did not significantly alter the fit of the model. Equation 5 can be
transformed to:
\[
\ln(Y) = \ln(p) + m \ln(t) + n \ln(1-t) + q \ln(W) \quad (6)
\]

RESULTS

In general, there was an increase in infection with increase in \( W \) at all \( T \) (Figs. 1 and 2). At 6°C, the maximum fruit wetness duration (4 hr in mist chamber + drying time) resulted in very low infection (average of 8.3% for the two tests). Infection levels were even less at shorter wetness durations. At 9°C, 84.6% infection was obtained at the longest wetness duration. At 12°C, 78.4% infection resulted from wetness durations between 2 and 3 hr, and almost 100% infection between 4 and 5 hr. At 15°C, between 2 and 3 hr of wetness resulted in 100% infection. An average infection level greater than 80% was obtained at wetness durations exceeding 1 hr at 17-25°C, and greater than 20% was obtained at 20-25°C for wetness durations less than 1 hr. Infection at 30°C, the maximum temperature studied, required between 2 and 3 hr of wetness to reach levels of 80% or more. Infection was negligible at durations less than 1 hr at this temperature.

Data analyses. The best logistic model representing the controlled environment data for both tests was of the form:

\[
\ln \left( \frac{Y}{1-Y} \right) = b_0 + b_1 T + b_2 W \times T + b_3 T^2 + b_4 W \times T^3 \quad (7)
\]

in which the \( b \)'s are the unknown parameters estimated from the data. Estimated parameters for both tests, and for the combined data, are presented in Table 1. An F-test indicated there was no significant difference in results between the two tests \( (P>0.50) \). All estimated parameters in the model were significant \( (P<0.05) \). The residuals had a random pattern and were normally distributed \( (25) \). The coefficient of
Fig. 1. Infection (%) of strawberry fruit by Phytophthora cactorum at different temperatures (A) and wetness durations (B) for test one. Curves represent the levels of infection at temperatures between 6 and 28 C with different wetness durations (A) and at wetness durations between 1-5 hr at different temperatures (B). Wetness duration labels in A are rounded to the next highest integer.
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<th>$Ra^2$</th>
<th>$R^*^2$</th>
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<td>-8.32</td>
<td>0.525</td>
<td>0.132</td>
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<td>-0.10 $\times 10^{-3}$</td>
<td>0.75</td>
<td>0.72</td>
<td>0.84</td>
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<td>(0.10)</td>
<td>(0.093)</td>
<td>(0.024)</td>
<td>(0.11 $\times 10^{-3}$)</td>
<td>(0.35 $\times 10^{-4}$)</td>
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<td>-7.60</td>
<td>0.452</td>
<td>0.122</td>
<td>-0.30 $\times 10^{-3}$</td>
<td>-0.10 $\times 10^{-3}$</td>
<td>0.76</td>
<td>0.73</td>
<td>0.86</td>
<td>1.25</td>
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<td></td>
<td>(0.925)</td>
<td>(0.089)</td>
<td>(0.023)</td>
<td>(0.11 $\times 10^{-3}$)</td>
<td>(0.37 $\times 10^{-4}$)</td>
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<td>-8.00</td>
<td>0.493</td>
<td>0.127</td>
<td>-0.36 $\times 10^{-3}$</td>
<td>-0.10 $\times 10^{-3}$</td>
<td>0.75</td>
<td>0.74</td>
<td>0.85</td>
<td>1.28</td>
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<tr>
<td>Experiments</td>
<td>(0.667)</td>
<td>(0.62)</td>
<td>(0.016)</td>
<td>(0.72 $\times 10^{-4}$)</td>
<td>(0.24 $\times 10^{-4}$)</td>
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*Estimated parameters for equation 7 corresponding to the intercept ($B_0$), $T(B_1)$, $WxT(B_2)$, $T^3(B_3)$ and $WxT^3(B_4)$. Numbers in parantheses under the parameters correspond to their standard deviations.

*B0 is the value of $\ln (Y/(1-Y))$ when $T=0$ and $W=0$; value of $Y$ at these conditions is equal to $1/(1+\exp(-B_0))$.
determination was fairly high ($R^2 = 0.75$ for the combined data). The coefficient of determination adjusted for degrees of freedom ($R_a^2$), which can be considerably lower than $R^2$ if unnecessary or redundant terms are in the model, was almost as large as $R^2$. Both of the coefficients are based on goodness of fit between the observed and predicted logits. We untransformed the predicted logits and determined the goodness of fit between the observed and predicted $Y$'s ($R^2$) and found that the coefficient was fairly high ($R^2 = 0.85$ for combined data).

Inspection of the model (equation 7) revealed that there was a linear and cubic relationship between logits and $T$. There was also an interaction of $W$ and $T$ as well as $W$ and $T^3$. This indicates that the response to $T$ is not consistent for all wetness periods. Predicted values of $\ln(Y/1-Y)$, and therefore $Y$, were calculated for temperatures between 1 and 5 hr, based on the parameters for the combined data. Bell-shaped curves were produced when $Y$ was plotted versus $T$ (Fig. 3). The curve spread out as $W$ increased and the $T$ at the optimum infection level shifted slightly to the left. There was a monotonic increase in $Y$ with increase in $W$ at all $T$ (Fig. 4); the shape of the curves varied with the value of $T$. Equation 7 was rearranged to determine the hours of wetness required for a specific level of infection (e.g., 50%) at temperatures between 6 and 30 C (Fig. 5). For $Y=0.50$, greater than 6 hr were required at 6 C, ~2 hr were required at 12 C and <1 hr was required at 21 C (Fig. 5). The same type of curve is produced for other levels of $Y$. It was impossible to get values of $Y<0.25$ around the optimum temperature (~21 C).
Fig. 3. Effect of temperature on predicted infection of strawberry fruit by *P. cactorum* at wetness durations of 1-5 hr. Curves were generated using equation 7 with parameters in Table 1 for the combined tests.
Fig. 4. Effect of wetness duration on predicted infection at temperatures of 6, 12, 18, 24, and 30 °C. Curves were generated using equation 7 with parameters listed in Table 1 for the combined tests.
Fig. 5. Combinations of temperature and wetness durations necessary to predict 10, 25, 50, 75, 90% infection. Curves were generated using equation 7 with parameters listed in Table 1 for the combined tests.
Neither the sine or Beta models fit the data as well as the logistic. The same temperature and wetness terms were significant with the sine and logistic models. For the combined data, the sine model (equation 3) had an $R^2$ equal to 0.75. The Beta model (equation 5) had an even poorer fit to the data with $R^2=0.70$.

For field validation, the logistic equation, with the coefficients for the growth chamber study, was used to predict infection level. Hours of wetness and the average temperature during the wetness period in the field were used in the prediction equation. The observed $Y$'s from the field inoculations ($I$) were regressed on the predicted $Y$'s from the growth chamber prediction model ($P$) (Fig. 6). An unbiased prediction model should have a slope of one and an intercept of zero for the regression. The estimated equation was:

$$I = -0.09 + 0.996(P)$$  (8)

The intercept of -0.09 was not significantly different from 0, and the slope of 0.996 was not significantly different from 1.0 ($P>0.25$). The coefficient of determination equaled 0.83 for the equation. The correlation between observed and infected values was 0.91.

DISCUSSION

Results clearly indicate that wetness period ($W$) and temperature ($T$) are significant factors influencing the infection ($Y$) of immature strawberry fruit by *P. cactorum* and that equation 7 is a valid model predicting the level of infection at different values of $W$ and $T$. Coefficients of determination ($R^2$) of 0.75, 0.76, and 0.75 for tests
Fig. 6. Observed proportion of strawberry fruit infected by *P. cactorum* versus the predicted proportion of infected fruit based on equation 7 with the parameters listed in Table 1 for the combined tests (\(R^2=0.83\)). Temperatures and wetness durations input to the equations were measured in the field.
one, two, and the combined tests, respectively, indicate that a relatively high proportion of the variability of infection levels experienced in the test are accounted for by the components of the model. Coefficients of determination adjusted for degrees of freedom ($R_a^2$) were also relatively high, indicating the importance of various terms in the model. Low values of $R_a^2$ relative to $R^2$ indicate redundancy, i.e., that some components of the model may not be necessary (17, 22, 25). This clearly was not the case with our results. Kranz (17) recommends obtaining $R^2$ values for both the transformed and untransformed values, as $R^2$ values obtained for transformed values measure the explained variability of the transformed variable. $R^2$ values obtained for untransformed $Y$ values ($R^2_a$) in this study were also high (0.85) for the combined tests. An interaction between $W$ and $T$ was also evident upon inspection of the model.

The effect of temperature was most pronounced at short wetness durations, e.g., 1 hr wetness at 6°C resulted in a much lower infection level than 1 hr at 21°C. At the short wetness durations, optimum infection occurred over a relatively short $T$ range, whereas at long wetness periods, high infection occurred over a wide range. The logistic model with the environmental terms in equation 7 provided the best fit of the controlled environment infection data. The generalizations of the sine and Beta models were clearly inferior to the logistic. The Beta model might be improved with different estimates of $T_{min}$ and $T_{max}$. However, we have no reason to believe that $T_{min}$ and $T_{max}$ are constant for different levels of $W$. 
Field validation resulted in linear equation 8 with intercept and slope not significantly different from 0 and 1, respectively, indicating that the growth chamber derived model produced unbiased predictors of field infection. The coefficient of determination ($R^2=0.83$) was acceptable (25). The model obtained in this study accurately predicted the levels of strawberry fruit infection at different wetness durations and temperatures in the field. Several wetness/temperature combinations recorded in the field experiments resulted in high predicted infection levels; however, observed infection for these periods was substantially lower (see data points at right-hand side of Fig. 6). Perhaps sensor dryness failed to correspond to actual fruit dryness during these periods or the wetness duration cannot be extrapolated this far beyond the maximum of 5 hr used in the growth chamber.

The wetness period required for the infection of unripe strawberry fruit by *P. cactorum* was exceedingly short over a broad range of temperatures. Substantial levels of infection (>80%) could result from a 1-2 hr wetness period between 17 and 25 C; the optimum temperature for infection was ~21 C. Progressively longer wetness periods were required to produce correspondingly high levels of infection as the temperature was increased or decreased away from the optimum. No temperature maxima and minima were found, although the longest wetness periods at the lowest temperature tested failed to result in infections >10%.
The importance of wetness and temperature on the epidemiology of several diseases caused by airborne \((P. \text{ infestans}; P. \text{ phaseoli})\) and/or water splashed \((P. \text{ palmivora}, P. \text{ citrophthora}, P. \text{ lateralis}, P. \text{ capsici}, \text{ and } P. \text{ syringae})\) Phytophthora spp. is well documented in the literature \((6,14,30,40,44)\). Infection of potato leaves \((Solanum \text{ tuberosum } L.)\) by \(P. \text{ infestans}\) can result from 6 - 8 hr wetness periods at 15 C; infection can result from 12 - 24 hr periods at 12 and 24 C \((6,30)\). Schlub \((34)\) has reported infection of bell pepper \((Capsicum \text{ sativum } L.)\) by \(P. \text{ capsici}\) under various temperatures and wetness periods. Periods of at least 24 hr are necessary for > 50% infection at 15 or 31 C, while 4 hr at 27 C results in 60% infection. Clearly, an interaction between temperature and wetness periods, similar to that revealed in \(P. \text{ cactorum}\), exists in the two forementioned examples. Gerlach et. al.\((9)\) reported that infection of \(Pieris \text{ japonica } (\text{Thunb.})\) D. Don by \(P. \text{ citrophthora}\) zoospores requires inoculum exposures of 15, 2, and 4 hr at 12, 24, and 32 C, respectively. Infection of papaya \((Carica \text{ papaya } L.)\) fruit by \(P. \text{ palmivora}\) can occur after a 15 min exposure to a zoospore suspension \((7,14)\). Hunter and Kunimoto \((14)\), and Dao \((7)\) reported that surface sterilization of fruits after several hours' exposure to a zoospore suspension failed to prevent infection. As expected, the interaction between temperature and wetness, as well as temperature optima, are different for each of these examples, and the relative effect of each is unique to each host: pathogen relationship. However, no Phytophthora spp. appears to require shorter wetness periods for infection than \(P. \text{ cactorum}\) and \(P. \text{ palmivora}\).
Microcomputer-based disease forecasting systems have been developed utilizing environmental parameters (i.e., wetness period and temperature) similar to those described here (16, 18). Unfortunately, wetness periods and temperatures falling within the optimal range found for strawberry infection occur frequently during the Ohio strawberry season (Grove, Madden, and Ellis, unpublished). Dew periods of much greater than 1 hr are quite common and the mean temperature falls well within the optimal range for infection. Other epidemiological factors, in addition to the conditions described here, contribute to the development of leather rot epidemics. For instance, investigations indicate that caducous sporangia that form on the surface of infected strawberry fruit are readily dispersed by water drops (Grove, Madden, and Ellis, unpublished). Research in progress concerning conditions favoring sporulation and pathogen dispersal will contribute to our knowledge of this disease.
CHAPTER II

Influence of Temperature and Wetness Duration on Sporulation of Phytophthora cactorum on Infected Strawberry Fruit

INTRODUCTION

Epidemics of leather rot of strawberry (Fragaria x anassa), caused by Phytophthora cactorum (Leb. & Cohn) Schroet., have resulted in significant quality and yield losses in Ohio's fruit crop in recent years. Excessive rainfall and/or flooded conditions favor disease development (8,29).

Numerous sporangia characteristic of P. cactorum have been observed microscopically on the surfaces of infected fruit collected during or immediately after wetness periods in the field (8,25). Dispersal of sporangia by water splash mechanisms has been demonstrated (Grove et.al, in preparation). These observations have indicated that under favorable environmental conditions, a potential source of secondary inoculum readily forms on infected fruit surfaces. Rainfall following such conditions could result in epidemics of the severity experienced in Ohio during recent years.

Investigations have indicated that a film of water is necessary for sporangial production by P. cactorum on infected strawberry fruit (Grove, Madden, and Ellis, unpublished). Environmental parameters
(i.e. wetness duration and temperature) conducive to infection, and a regression model accurately predicting infection at different temperatures and wetness durations, have been described (13). The purposes of this study were to: determine the temperatures and wetness durations necessary for sporangial production on the surface of infected fruit, and to develop a model predicting sporangial production at different temperatures and wetness durations.

MATERIALS AND METHODS

Inoculum production and inoculation. Day-neutral strawberry plants (cv. Tristar) were used in all experiments. Plants were grown to reproductive maturity in a 1:1:1 (v:v:v) peat: sand: steam-disinfested silt-loam mixture in the greenhouse. For each temperature:wetness duration tested 48, detached, immature (green) fruit were washed with five 500 ml aliquots of sterile distilled water (SDW), surface sterilized in 5% chlorox for 30 sec, rinsed five times with 500 ml SDW, and inoculated with a zoospore suspension of P. cactorum. All inoculations were performed with cultures freshly isolated from infected strawberry fruit (cv. Tristar) on pentachloronitrobenzene-benomyl-neomycin sulfate-chloramphenicol (PBNC) medium (35). Mycelial transfers from the edges of three-day-old cultures were made to lima bean broth (35). Broth cultures were incubated 96 hr at 22 C in continuous light at 9,000 lux. Cultures were then refrigerated for 30 min at 5 C, followed by a return to 22 C. Zoospores were released approximately 30 min following removal refrigeration. All inoculations were made with zoospore suspensions
(10,000/ml) in SDW brought to final concentration with a hemacytometer. Fruit were placed in 30 ml of inoculum contained in 15 cm diam plastic petri plates and incubated at 22 C in continuous light at 9,000 lux for 4 hr. Inoculated fruit were then rinsed with five 500 ml aliquots of SDW to remove any mycelia, zoospores, or sporangia adhering to the fruit surface and then air dried for 1 hr at 22 C at 30-40% RH in continuous light at 9,000 lux.

Incubation. After drying, fruit were placed on elevated screens contained in 1 L clear-glass jars and incubated 72 hr at 20 C in 70% ± 5% in a 14 hr photoperiod at 25,000 lux. Relative humidity in the incubation jars was controlled with glycerol:water mixtures in a closed system (15,27,33). Each incubation jar was fitted with a #10 rubber stopper into which two pieces of 8 mm diam glass tubing were inserted. One 15 cm long section served as a humidified air input and extended to within 5 cm of the jar bottom. A second 8 cm long section served as a humidified air output and extended 5 cm into the jar through the rubber stopper. Both pieces of tubing entered the incubation jars through holes drilled in the rubber stopper. Humidified air was obtained by bubbling air from a Cole-Palmer vacuum:pressure pump (Cole-Palmer Instrument Company, Chicago, Ill 60648) into a 500 ml glycerol:water solution contained in a 1 L Ehrlemmyer flask. A #9 rubber stopper, fitted with two pieces of 8 mm diam glass tubing, was used to seal the flask. One 20 cm piece of glass extended into the solution to within 5 cm of the flask bottom. Air entered the solution through an aquarium stone connected to the tubing. Humidified air above the solution exited via the second piece of glass tubing. The second piece of glass
tubing, 10 cm in length, extended through the rubber stopper to 5 cm above the glycerol:water solution and served as the humidified air output. Before entering the solution flask, air was filtered through glass wool contained in a 2.5 cm diameter piece of glass tubing. Both ends of the filter were sealed with #6 cork stoppers. Five cm pieces of 8 mm diam glass tubing extended into the filter through holes drilled in the stoppers. Solution flask, incubation jars, air filter, and pump were connected in series with 8 mm diameter latex tubing (Arthur Thomas Company, Philadelphia, PA 19105). Humidified air leaving the glycerol:water solution entered the incubation bottles, flowed through the bottles, and entered the vacuum port of the pump. All humidity-control apparatus except the pump, were autoclaved at 200 C at 20 psi for 30 min prior to each temperature:wetness test. The entire apparatus was placed in a controlled-environment chamber (Environmental Growth Chambers [EGC], Chagrin Falls, Ohio 44022) and the incubation temperature (20 C) controlled to ± 0.5 C. Incubation jars were positioned horizontally to ensure uniform lighting. Temperature and relative humidity within the closed system were continuously monitored with sensors (Phys-Chem Sensor, Phys-Chemical Research Corporation, New York, NY 10011) connected to a microprocessor-controlled Datalogger (CR-21, Campbell Scientific, Logan, Utah 84321). Temperature in the EGC was continuously monitored with thermistors (Penwall Electronics, Ashland, MA 01721) also connected to the Datalogger.
Sporangia production. Following 72 hr incubation, inoculated fruit were exposed to various wetness durations by placement in a second EGC containing a 1 m$^3$ clear-plastic chamber enclosing a Herrmidifier mister (Herrmidifier Company, Lancaster, PA 17604). Each fruit was placed on a 2.5 cm$^2$ screen contained in an open 9 cm diameter petri plate. Fruit were gently misted with SDW using an atomizer prior to placement in the wetness chamber. Eight fruit were sampled for sporangial formation at 0, 3, 6, 12, 16, and 24 hr wetness durations at 10, 12.5, 15, 20, 25, 27.5, 30 C. The order of temperatures tested was random. Temperature and wetness were continuously monitored within the mist chamber with thermistors and printed-circuit wetness sensors (Wong Labs, Cincinnati, OH 45200) connected to a second Datalogger.

The 14 hr photoperiod was maintained throughout the experiment. Fruit were removed from incubation bottles at the conclusion of 14 hr light period and placed in wetness for 10 hr dark. Lights in the wetness chamber were turned on after 10 hr incubation. Light intensity within the wetness chamber was 20,000 lux.

Sporangial counts were made by removing eight fruit and respective petri plates at random from the wetness chamber at the above listed times. Water from the mister settling into each plate was added to a 50 ml graduated cylinder and brought to a final volume of 30 ml with SDW. Each fruit and respective 30 ml suspension were transferred to 50 ml plastic centrifuge tubes. Tubes were sealed with #6 rubber stoppers and vortexed at high speed for 2 min. Wash suspensions were transferred to 9 cm diam plastic petri plate bottoms and allowed to settle for 5 min. Sporangia at the bottom of plates were counted at 40
X. Twenty fields were counted in each plate. Fruit length (mm),
diameter at calyx end (mm), and weight (g) were recorded for each
fruit. The experiment was conducted twice.

Data Analyses. Because strawberry fruit did not have
identical sizes, sporangial production was divided by fruit surface
area to calculate sporangia/mm². As fruit shape was generally conical,
the formula for surface area (S) of a cone:

\[ S = \pi rs \]  

in which \( r \) = fruit radius (mm) and \( s \) = fruit length (mm), was used to
determine fruit surface area (mm²).

Regression analysis was used to determine the effect of
temperature (T) and wetness duration (W) on the number of sporangia (Y)
on the surface of strawberry fruit infected with P. cactorum.
Properties of the regression model had to include: (i) an optimum
relationship between \( Y \) and \( T \), in which \( Y \) increases to a maximum and
then declines; and (ii) a positive (monotonically increasing)
relationship between \( Y \) and \( W \). A model was chosen of the form:

\[ \ln(Y) = f(T,W) \]  

in which \( \ln(Y) \) is the natural logarithm of \( Y \), and \( f(T,W) \) represents an
arbitrary function of \( T \) and \( W \). The following linear terms were
evaluated for making up \( f(T,W) \): \( W, T, W^2, T^2, W^3, T^3 \). All
possible combinations of these terms were evaluated for: significance
of the estimated parameters, coefficient of determination, and pattern
of residuals (17,22,25). The regression analysis was performed on the
data for each experiment separately, and then on the combined data. An
F-test was conducted to determine if the results from the two experiments were significantly different (25).

RESULTS

Sporangial production occurred between 12.5 and 27.5°C, with an optimum temperature of ~20°C (Fig. 7). In general, sporangial production increased with increased wetness duration between between 12.5 and 27.5°C (Fig 8). Sporangia were absent at 10 and 30°C, although mycelial growth was profuse at 30°C. At 12.5°C, sporangia were first evident after 12 hr wetness; 24 hr resulted in 49 sporangia/mm² (Fig 8). Three hours wetness resulted in 10.7, 14.6, 8.5, and 4.2 sporangia/mm² at 15, 20, 25, and 27.5°C, respectively. Sporangial production increased to 523.1, 589.2, 149.6, and 49 sporangia/mm² at 15, 20, 25, and 27.5°C after 24 hr wetness, respectively. One hundred or more sporangia were produced between 15 and 25°C with W>16 hr of wetness. Only at 24 hr were more than 200 sporangia/mm² observed.

Data Analyses. The best model representing the sporulation data was of the form:

\[ \ln(Y) = b_0 + b_1 T + b_2 W T + b_3 T^3 + b_4 W T^3 \]  

(11)

in which the \(b\)'s are unknown parameters estimated from the data. Estimated parameters for the combined data, and for both tests, are presented in Table 2; all estimated parameters were significant (\(P<0.01\)). An F-test indicated there was no significant difference between the two tests (\(P>0.60\)). The residuals had a random pattern and were normally distributed (25). The coefficient of determination was
Fig. 7. Sporangial production per mm$^2$ by P. cactorum on infected fruit surfaces at different temperatures (10-30 C) and four wetness durations for the combined tests.
Fig. 8. Sporangial production per mm\(^2\) by P. cactorum on infected fruit surfaces at different wetness durations (0-24 hr) for five temperatures for the combined tests.
Table 2. Estimated parameters of equation 11 for temperature (T) and wetness duration (W) together with the coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R_a^2), and the standard error about the regression curves (S).

<table>
<thead>
<tr>
<th></th>
<th>b_0</th>
<th>b_1</th>
<th>b_2</th>
<th>b_3</th>
<th>b_4</th>
<th>R^2</th>
<th>R_a^2</th>
<th>S</th>
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<td>Test 1</td>
<td>-6.11</td>
<td>0.621</td>
<td>1.3x10^-2</td>
<td>-4.6x10^-4</td>
<td>-1.29x10^-5</td>
<td>0.870</td>
<td>0.839</td>
<td>0.54</td>
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<td></td>
<td>(1.54)</td>
<td>(0.120)</td>
<td>(2.2x10^-3)</td>
<td>(9.6x10^-5)</td>
<td>(3.8x10^-6)</td>
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<tr>
<td>Test 2</td>
<td>-5.17</td>
<td>0.554</td>
<td>1.3x10^-2</td>
<td>-4.0x10^-4</td>
<td>-1.3x10^-5</td>
<td>0.837</td>
<td>0.796</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>(1.67)</td>
<td>(0.130)</td>
<td>(2.5x10^-3)</td>
<td>(1.1x10^-4)</td>
<td>(4.3x10^-6)</td>
<td></td>
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<tr>
<td>Combined</td>
<td>-5.68</td>
<td>0.589</td>
<td>1.3x10^-2</td>
<td>-4.3x10^-4</td>
<td>-1.3x10^-5</td>
<td>0.854</td>
<td>0.838</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>(1.061)</td>
<td>(0.082)</td>
<td>(1.6x10^-3)</td>
<td>(6.8x10^-5)</td>
<td>(2.6x10^-6)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

^a Estimated parameters for equation 11 corresponding to intercept (b_0), T (b_1), W*T (b_2), T^3 (b_3), W*T^3 (b_4). Numbers in parentheses under the parameters correspond to their standard deviations.
fairly high \( R^2 = 0.854 \) for the combined data. The coefficient of
determination adjusted for degrees of freedom \( R^2 \), which can be
considerably lower than \( R^2 \) if a model was to contain redundant or
unnecessary terms, was almost as large \((17,22,25)\). Both of the
coefficients are reflective of the goodness of fit between observed and
predicted log values. The coefficient of determination for actual
sporangia values \( R^2 \) was determined to be 0.79 for the combined data.

Use of the logarithms stabilized variances and linearized the
relationship between sporangia numbers and wetness duration. The \( T \) and
\( T^3 \) terms in the model accounted for the observed optimum-type
relationship between \( \ln(Y) \) and temperature. There was also an
interaction of \( W \) and \( T \), as well as \( W \) and \( T^3 \). This indicated a
differential response of \( \ln(Y) \) to \( T \), depending upon wetness duration.
Predicted values of \( \ln(Y) \) were calculated for wetness durations between
0 and 24 hr and for temperatures between 12.5 and 27.5°C. Based on the
terms in equation 11, bell-shaped curves were produced when \( \ln(Y) \) was
plotted vs. \( T \), and straight lines were produced when \( \ln(Y) \) was plotted
vs. \( W \) (Figs. 9, 10). The interaction of \( W \) and \( T \) is clearly shown in Fig.
10 by the crossing prediction lines. The predicted \( T \) optimum can be
calculated by taking the first derivative of equation 11. Although
dependent on \( W \), the optimum \( T \) varied by less than 1°C between 6 and 24
hr wetness. The optimum equaled 20.6 at 12 hr and 20.2°C at 24 hr.

DISCUSSION

Results clearly indicated that wetness duration \( (W) \) and
temperature \( (T) \) are significant environmental factors influencing
sporulation of \textit{P. cactorum} on infected strawberry fruit. Equation 11
Fig. 9. Effect of temperature on predicted sporangial number (per mm²) of *P. cactorum* at wetness durations of 6, 12, 16, and 24 hr. Curves were generated using equation 11 with parameters listed in Table 2 for the combined tests.
Fig. 10. Effect of wetness duration on predicted sporangial number (per mm²) of *P. cactorum* at temperatures of 12.5, 15, 20, 25, and 27.5 C. Curves were generated using equation 11 with parameters listed in Table 2 for the combined tests.
was accepted as the best model predicting the natural log of sporangia at values of $\bar{W}$ between 3 and 24 hr and $\bar{T}$ between between 12.5 and 27.5 C. Coefficients of determination of 0.87, 0.837, and 0.854 for tests one, two, and the combined data, respectively, indicate that a relatively high proportion of the variability in sporangial numbers in the tests was accounted for by the components of the model. The similarity of $R^2$ and $R^2$ values is further evidence of the importance of the various components in the model (17, 22, 25). An interaction between $\bar{W}$ and $\bar{T}$ was also evident upon inspection of the model. As wetness duration increased, the range of temperatures over which sporulation occurs broadened.

Results obtained in this study differ somewhat from findings of Rose (28) who reported a temperature optimum for sporulation in vivo falling between 10 and 20 C. Since we observed no sporulation at 10 C, our temperature findings appear somewhat higher. However, the optimum sporulation temperature, and the temperature range over which sporulation occurs, could be influenced by pathogen isolate, host cultivar, or other environmental conditions (31).

Although direct comparison between in vitro and in vivo sporulation parameters are at best artificial (31), free water has been reported to enhance sporulation of F. cactorum grown on solid medium; exposure of 24 hr old cultures to 12 hr free water resulted in significant increases in sporangial number (45). Our results indicated that a film of free water was required for sporangial production in vivo, and that the sporulation response was first detected following a wetness duration of 3 hr at between 15 and 25 C.
Temperature and moisture requirements for sporangial production in vivo are quite variable among other Phytophthora spp. Trujillo (41) reported that sporangial production of \( \textit{P. colcasiæ} \) after 2-3 hr at 100% RH. \( \textit{P. infestans} \) has been reported to sporulate between 13-26 C (6). Eight hour periods of 100% RH within the optimal temperature range of 18-22 C were required for sporulation on infected potato \((\textit{Solanum tuberosum})\) tissue. Sporulation at temperatures outside this range required progressively longer exposure to 100% RH. Rocha and Maihado (26) reported sporangial formation in \( \textit{P. palmivora} \) occurring at 70-90% RH, but not at 100%. Gerlach et. al. (9) reported sporulation on \( \textit{Pieris japonica} \) infected with \( \textit{P. citrophthora} \) after 4 hr immersion in salt solutions at 16-28 C. However, a minimum of 7 hr immersion was needed at 8 and 32 C. Temperature and moisture requirements and interactions, are evidently somewhat unique to each host: pathogen system.

The influence of temperature and wetness duration on infection of strawberry fruit by \( \textit{P. cactorum} \) has been reported previously (13). A regression model with identical terms (eq. 7) as for sporangia production (eq. 11) was developed for describing infection as a function of \( T \) and \( W \). Wetness periods required for infection were exceedingly short, e.g., >80% of the fruit were infected between 17-25 C with a wetness duration of 2 hr. Due to the shortness of the wetness periods required for infection, and the relatively frequent occurrence of these conditions during the Ohio strawberry season (Grove, Madden, and Ellis, unpublished), we felt that other disease cycle components were the determining factors for leather rot epidemics. Although
C, sporangia are much more abundant at longer wetness durations. The temperature range is also broader as wetness duration increases. We feel that sporangia production and subsequent splash dispersal (Grove, Madden, and Ellis, in preparation) are key components for the development of epidemics. We plan to incorporate our results into a predictive system for scheduling fungicide applications.
CHAPTER III
Splash Dispersal of *Phytophthora cactorum*
from Infected Strawberry Fruit

INTRODUCTION

Excessive rainfall in Ohio during the 1980 and 1981 growing seasons was associated with heavy losses in the state's strawberry (*Fragaria x anassa*) crop due to fruit rots (8). Leather rot, caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., accounted for up to 40% of the losses (8). Fruit and blossoms infected with *P. cactorum* were observed 0-20 cm above the soil surface, with relative numbers of each decreasing with increased height. Numerous papillate sporangia, characteristic of *P. cactorum*, were microscopically observed on the surface of infected fruit collected during or immediately following extended wetness periods. Previously, leather rot epidemics occurred during or immediately following periods of excessive rainfall (28,29).

During the 1982 and 1983 growing seasons, in which precipitation was equal to or below the long-term average, respectively, occurrence of leather rot was sporadic with losses generally ranging 0-5% (8). Diseased fruit observed at those times were generally in contact with soil. However, one grower in an area of localized heavy rainfall experienced losses due to aerial blossom and fruit infection by *P. cactorum* (Ellis and Grove, unpublished).
Environmental parameters (wetness duration and temperature) conducive to fruit infection by _P. cactorum_ have been reported previously (13). Temperature and wetness conditions favoring high infection rates occur nightly in Ohio during the strawberry fruiting season (Grove, Ellis, and Madden, unpublished), indicating that other moisture related factors contribute significantly to the development of leather rot epidemics. The occurrence of aerial blossom blights and fruit rots after precipitation, in addition to speculation by Rose (28), has indicated that splash dispersal of infective _P. cactorum_ propagules may be an important component in the development of leather rot epidemics.

Dispersal of pathogenic fungi via rain splash mechanisms has been well documented (9,12,14,20,40,43,44). The purposes of this study were: to demonstrate the role of water splash in the dispersal of infective _P. cactorum_ propagules and to quantify the effects of water drop size and velocity at impact on distance traveled by propagules.

**MATERIALS AND METHODS**

**Splash studies.** All studies were performed using detached strawberry fruit (cv. Tristar) obtained from plants grown to reproductive maturity in a 1:1:1 (v:v:v) peat:sand:steam-disinfested loam mix. All inoculations were performed with cultures of _P. cactorum_ freshly isolated from infected strawberry fruit (cv. Tristar) on pentachloronitrobenzene-benomyl-neomycin sulfate-chloramphenicol medium (PBNC) (35). For sporangial production, mycelial transfers from the edges of of 3-day-old cultures were transferred to lima bean agar (35).
Cultures were incubated for 7 days at 22 C in continuous light at 9,000 lux. Sporangial germination was induced by flooding each culture with 20 ml sterile distilled water (SDW) followed by refrigeration at 5 C for 30 min. Zoospore concentrations were adjusted to 10,000/ml with SDW and a hemacytometer 30 min following removal from refrigeration. Fruit were washed with detergent and SDW for 5 min, rinsed with 5-100 ml aliquots of SDW, surface sterilized for 30 sec with 9:1 (v:v) SDW:chlorox solutions, and then rinsed with 5-100 ml aliquots of SDW. Ten ml of inoculum and fruit were then transferred to plastic petri plates and incubated for 4 hr at 22 C in continuous light at 9,000 lux. Inoculated fruit were then placed on metal screens contained in a controlled-environment chamber (Environmental Growth Chambers [EGC] Chagrin Falls, Ohio 44022) and incubated 48 hr at 20 C in a 14 hr photoperiod at 25,000 lux. Fruit were then transferred to a second EGC containing a 1 m³ clear-plastic chamber enclosing a Herrmidifier mister (Herrmidifier Co., Lancaster, PA 17604) and kept continuously wet for 24 hr at 20 C continuous light at 25,000 lux. Sporangial production was then verified by microscopic examination at 40X. One sporulating fruit was used for each splash test.

Splash tests were performed by impacting water droplets with an average diameter of 0.41 or 0.026 cm on infected fruit surfaces. All tests were performed in a closed stairwell at 18-22 C. The drop sources were a 50 ml glass buret and a 100 ml plastic syringe for the 0.41 mm and 0.026 mm drops, respectively.
Drop diameters were determined by releasing a fixed number of drops, calculating average drop volume and then using the equation for the volume of a sphere to estimate average drop diameter. Terminal velocities for the 0.41 and 0.026 cm diam drops are 850 and 217 cm/s, respectively (32). In tests incorporating the 0.41 cm drops, the glass buret was positioned 8.5, 3.0, or 1.0 m above the infected fruit. Ninety-six 9 cm diameter plastic petri plates containing 15 ml PBNC media (35) medium were used in each test. Plates were arranged level with, 4 cm above, and 4 cm below the infected fruit on 120 x 8.5 x 4 cm (lwh) boards. Plates were positioned in straight-line horizontal distances of 29-120 cm, 13-120 cm, and 10-100 cm above, below, and level with the source, respectively. The three plate levels were "staggered", i.e., each line of plates from the source was only at one level to avoid interference with drop flights. Plate tops were removed immediately prior to the beginning of each experiment. Water droplets, at a rate of 48 drops/min were allowed to impact on the fruit surface for 14.5 min. Plates were covered immediately after the experiment and incubated for 72 hr at 22 C in continuous light at 9,000 lux. Colony numbers in each plate were determined by visual inspection and by microscopic examination at 40X.

In tests incorporating 0.026 cm drops, the 100 ml plastic syringe was placed 2.0 and 0.5 m above sporulating fruit. Ninety-six 5.5 cm diameter plastic petri plates containing 7.5 ml PBNC media (35) were positioned on 120 x 8.5 x 4 cm (lwh) pine boards level with, 4 cm above, and 4 cm below the infected fruit at horizontal distances of 6-54, 20-68, and 13-76 cm, respectively. Prior to each test, 0.5 ml
SDW was added to each plate to enhance sporangial germination. Plates were uncovered at the start of the experiment. Drops, at a rate of 48 drops/min, were allowed to impact on the fruit for 14.5 min. Plates were then covered and incubated 72 hr at 22 C in continuous light at 9,000 lux. Colony numbers were determined visually and by microscopic examination at 40X. Experiments with the large and small drops were each conducted twice.

**Plant to plant spread.** Attached, immature strawberry fruit were inoculated by applying 1 ml of a zoospore suspension of *P. cactorum* to each fruit with an atomizer. Inoculum was prepared and brought to final concentration as described previously. Plants were inoculated in a controlled-environment chamber (EGC) containing a 1m³ clear-plastic enclosure containing a Herrmidifier mister (Herrmidifier Co. Lancaster, PA). Inoculations were performed in the EGC; plants were then incubated 6 hr in the wetness chamber in continuous darkness at 22 C. Plants were removed and incubated an additional 72 hr at 22 C in a 14:10 photoperiod at 25,000 lux. To induce sporulation, plants were returned to the wetness chamber and incubated in continuous wetness for 24 hr and 22 C in a 14 hr photoperiod at 12,000 lux.

For splash experiments, 6-10 attached, immature fruit (cv. Tristar) were tagged on each plant. Plants were placed 15 and 30 cm from the sporulating fruit in a closed stairwell and "staggered" to prevent the plants of 15 cm from shielding the plants at 30 cm. Six plants were positioned at each respective distance; two were covered with clear plastic bags and four were left uncovered. Water drops, 0.41 cm dia, were dropped from a height of 8.5 m onto the surface of
exposed sporulating fruit for 14.5 min at a rate of 48 drops/min. Following the splash experiment, all plants were transferred to the EGC wetness chamber and incubated for 24 hr at 22 C in a 14 hr photoperiod at 25,000 lux. Plants were then transferred to a second EGC at 20 C in a 14 hr photoperiod at 25,000 lux. Proportions of diseased fruit were determined visually 72 hr after the splash test. Tissue sections from each fruit were placed on PBN medium (35) to verify the presence of P. cactorum.

**Data analyses.** Drop velocities for each drop source and release height were determined using the formula:

\[ V = \left(\frac{a}{b}\right)^{0.5} \tanh \left(\cosh^{-1} \left(\exp(bZ)\right)\right) \]  

in which \( a = g(w-p) \) and \( b = \frac{3pc}{4Dw} \), with \( V \)=velocity of the drop (cm/s), \( D \)=drop diam (cm), \( p \)=density of air \( (0.00129 \text{ g/cm}^3) \), \( w \)=density of water \( (1 \text{ g/cm}^3) \), \( c \)=drag coefficient \( (0.559) \), \( g \)=acceleration due to gravity \( (980 \text{ cm/s}^2) \), and \( Z \)=vertical distance coefficient (cm) from the release point to the sporulating fruit \( (32) \). Regression analysis was used to determine the effects of drop velocity (cm/s) at impact \( (V) \), level of plate (cm) relative to inoculum source \( (L) \), and horizontal distance from inoculum source \( (D) \) on the number of colonies \( (Y) \) of P. cactorum obtained in petri plates arranged about the inoculum source. A simple model was chosen of the form:

\[ \ln(Y) = b_0 + b_1(D) \]  

in which \( \ln(Y) \) is the natural logarithm of colony number, and \( b_0 \) and \( b_1 \) are parameters estimated from the data. Kiyosawa & Shiyomi \( (19) \) used this model for studying dispersal gradients in the field. The dispersal coefficient \( (b_1) \) represents the steepness of the colony
gradient away from the source; \( b_0 \) is an indication of "source strength", i.e. the effective number of propagules at the source. Preliminary data analyses was performed on each combination of release height, test, replication, and plate level. Results from preliminary splash tests indicated that \( \ln(Y) \) was linearly related to distance. Equation 13 was expanded to evaluate the effect of drop velocity, plate level, and interactions on \( \ln(Y) \). Stepwise regression was used to evaluate the significance of: \( V \), \( D \), \( L \), \( L^2 \), \( V^*D \), \( V^*L \), and \( D^*V^*L \). All regression models with combinations of these terms were evaluated for: significance of estimated parameters, coefficient of determination \( (R^2) \), \( R^2 \) adjusted for degrees of freedom \( (R^2_a) \), and pattern of residuals \( (22,25) \). The regression analysis was performed on tests 1 and 2, and then on the combined data. An F-test was conducted to determine if the results from the two tests were significantly different \( (25) \). Data analyses using 0.41 and 0.026 cm drops were at all times kept separate due to the different volumes of water used and the different plate sizes.

RESULTS

Sporangia, zoospores, and mycelial fragments were observed microscopically at 40X in petri plates upon completion of each experiment. Colonies of \( P. caactorum \) developed at distances of up to 120 and 50 cm from the inoculum source as a result of water-splash the 0.41 and 0.026 cm drops, respectively. Greater drop velocities resulted in more total colonies and in greater horizontal dispersal in all cases. Colony numbers were generally highest in petri plates
positioned 4 cm below the inoculum source. No colonies were observed 4 cm above the source when the fruit was impacted by the 0.026 drops at 202.8 cm/s.

The best overall model representing the dispersal of *P. cactorum* propagules by splash mechanisms for both drop sizes was of the form:

\[ \ln(Y) = b_0 + b_1D + b_2V + b_3V^*L \quad (14) \]

in which the \( b \) are unknown parameters estimated from the data. Estimated parameters for all tests, and for the combined data, are presented in Tables 3 and 4. An F-test indicated a significant difference between tests one and two involving the 0.41 cm drop \((P<0.05)\), but no significant difference between the two tests involving the 0.026 cm drop \((P>0.20)\). The major difference between the two tests with the large drops was represented by the values of \( b_0 \), the predicted \( \ln(Y) \) when \( D, V \), and \( L \) equal zero. Although \( b_0 \) does not have a direct meaning because \( V \) in our study never came close to zero, the parameter is proportional to the number of sporangia on the fruit surface. The other parameters for the large drop tests were of the same magnitude and sign. For both drop sizes, the residuals had a random pattern and were normally distributed. Coefficients of determination \((R^2)\) were fairly high, e.g. \( R^2 = 0.856 \) and \( 0.794 \) for 0.41 and 0.026 cm drops, respectively, for the combined data. Coefficients of determination adjusted for degrees of freedom \((R_a^2)\) were almost as large as \( R^2 \) in both cases, indicating significant parameter estimates \((17,22,25)\).

Inspection of the model revealed a negative relationship between \( \ln(Y) \) and \( D \), substantiating the observed decline in colony numbers as distance from the infected fruit increased. There was a positive
Table 3. Estimated parameters of equation 14 for velocity (V), distance (D), and level (L), together with the coefficient of determination ($R^2$), $R^2$ adjusted for degrees of freedom ($R^2_a$), and the standard error about the regression curves (S) for 0.41 cm drop.

<table>
<thead>
<tr>
<th></th>
<th>Estimated parameters</th>
<th>$R^2$</th>
<th>$R^2_a$</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$b_0$</td>
<td>$b_1$</td>
<td>$b_2$</td>
<td>$b_3$</td>
</tr>
<tr>
<td>Test 1</td>
<td>-2.081</td>
<td>-0.054</td>
<td>0.010</td>
<td>-0.907x10^{-4}</td>
</tr>
<tr>
<td></td>
<td>(.295)</td>
<td>(.21x10^{-2})</td>
<td>(.45x10^{-3})</td>
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</tr>
<tr>
<td>Test 2</td>
<td>1.714</td>
<td>-0.064</td>
<td>0.006</td>
<td>-0.932x10^{-4}</td>
</tr>
<tr>
<td></td>
<td>(2.62)</td>
<td>(.002)</td>
<td>(.40x10^{-3})</td>
<td>(.27x10^{-4})</td>
</tr>
<tr>
<td>Combined</td>
<td>0.361</td>
<td>-0.058</td>
<td>0.007</td>
<td>-0.859x10^{-4}</td>
</tr>
<tr>
<td></td>
<td>(.271)</td>
<td>(.002)</td>
<td>(.41x10^{-3})</td>
<td>(.26x10^{-4})</td>
</tr>
</tbody>
</table>

*aEstimated parameters for equation 14 corresponding to intercept ($b_0$), D ($b_1$), V ($b_2$), and L*V ($b_3$). Numbers in parentheses under the parameters correspond to their standard deviations.
Table 4. Estimated parameters or equation 14 for velocity (V), distance (D), and level (L) together with the coefficient of determination (R²), R² adjusted for degrees of freedom (Rₐ²), and the standard error about the regression curves (S) for 0.026 cm drop.

<table>
<thead>
<tr>
<th></th>
<th>Estimated parameters</th>
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<th>Rₐ²</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b₀</td>
<td>b₁</td>
<td>b₂</td>
<td>b₃</td>
</tr>
<tr>
<td>Test 1</td>
<td>-22.14</td>
<td>-0.093</td>
<td>0.128</td>
<td>-0.105x10⁻²</td>
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<tr>
<td></td>
<td>(4.55)</td>
<td>(.011)</td>
<td>(.022)</td>
<td>(.218x10⁻³)</td>
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<tr>
<td></td>
<td>Test 2</td>
<td>-25.35</td>
<td>-0.122</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>(4.64)</td>
<td>(.11)</td>
<td>(.22)</td>
<td>(.226x10⁻³)</td>
</tr>
<tr>
<td>Combined</td>
<td>-24.53</td>
<td>-0.110</td>
<td>0.140</td>
<td>-0.120x10⁻²</td>
</tr>
<tr>
<td></td>
<td>(3.34)</td>
<td>(.008)</td>
<td>(.016)</td>
<td>(.162x10⁻³)</td>
</tr>
</tbody>
</table>

a Estimated parameters for equation 14 corresponding to intercept (b₀), D (b₁), V (b₂), and L·V (b₃). Numbers in parentheses under the parameters correspond to their standard deviations.
relationship between \( \ln(Y) \) and \( V \) which indicated that more colonies were formed (more sporangia dislodged) as impaction velocity increased. There was also an interaction between \( V \) and \( L \), indicating that the number of colonies at each plate level was not consistent at all drop velocities.

Predicted values of \( \ln(Y) \) were calculated using equation 14 with estimated parameters for the combined tests for drop velocities of 314, 673, and 445 cm/s for the 0.41 cm drop, and 202.75 and 216.64 for the 0.026 cm drop (Figs. 11-12). (Values of \( D \) used in equation 14 were 15-125 cm and 10-58 cm for the large and small drops, respectively (Figs. 11-12). Predicted values of \( \ln(Y) \), and therefore \( Y \), increased with increasing drop velocities and decreased with increasing distance from the inoculum source (Figs. 11-12). The same relationship was observed at each level. Although numbers of colonies decreased from 4 cm below to 4 cm above the inoculum source at all velocities, the differences in colony number between levels increased as velocity increased.

Plant to Plant Spread. Of the uncovered plants used in splash tests, infection of fruit was 100% at both distances from the source. Leather rot symptoms failed to develop on any plants covered with plastic bags during the splash test.

DISCUSSION

Propagules of \( P. \) cactorum were readily dispersed from infected strawberry fruit via splash mechanisms, and the number colonies were significantly related to drop velocity \( (V) \), and the distance of the propagule trap from the source \( (D) \). Negative values of the dispersal
Fig. 11. Effect of impact by 0.41 cm diam water drops at three velocities on the predicted colony number of *F. cactorum* 4 cm above, level, and 4 cm below the inoculum source at various distances away from the source. Curves were generated using equation 14 with parameters listed in Table 3 for the combined tests. Range of distances used in the prediction equation correspond to the location of petri plates at the three levels. Velocity of water drops was determined using equation 12.
Fig. 12. Effect of impact by 0.026 cm diam water drops at three velocities on the predicted colony number of P. cactorum 4 cm above, level, and 4 cm below the inoculum source at various distances away from the source. Curves were generated using equation 14 with parameters listed in Table 4 for the combined tests. Range of distances used in the prediction equation correspond to the location of petri plates at the three levels. Velocity of water drops was determined using equation 12.
coefficent ($b_1$) were obtained in all cases. Negative gradients (and dispersal coefficients) have been shown to indicate dispersal from an inoculum source via wind (12) and splash mechanisms (9,19,20). Positive values of $b_2$ indicated that increasing drop velocities ($V$) resulted in greater total colony numbers at all distances. The effect of velocity at impact was expected since momentum at impact ($velocity\times drop\ diameter$) powers the splashing of water drops with spores (32). Although there was a decline in colony number as plate level increased from 4 cm below the inoculum source to 4 cm above the source, the steepness of this vertical gradient was not consistent for all drop velocities. The gradient steepness increased as $V$ increased as indicated by the significant $V*L$ interaction.

Coefficients of determination (Tables 3 and 4) for tests utilizing 0.41 and 0.026 cm drops were high, indicating that a large amount of variability experienced in the tests were explained by the model. Coefficients of determination adjusted for degrees of freedom ($R^2_a$) were also high, indicating the importance of various terms in the model. Unnecessary or redundant terms in the model would have resulted in much lower $R^2_a$ values (17,22,25). Splash dispersal of P. eactorum propagules from infected to healthy fruit was evident by the high infection levels experienced in test plants, and by the failure of plants covered during the test to develop leather rot symptoms. However, a negative dispersal gradient was not observed, indicating that either too few fruits were used in the test, or that plants were not placed at distances sufficient enough to detect the negative gradient.
Splash dispersal of infective propagules within the genus *Phytophthora* has been documented or suggested (9,14,20,40,43,44). Hunter and Kunimoto (14) reported that epidemics of *P. palmivora* blight of papaya (*Carica papaya* L.) were favored in large part by dispersal of sporangia by wind-blown rain. They also speculated on the presence of a similar mechanism contributing to outbreaks of *P. colocasiae* blight of taro. Gerlach et al. (9) showed that zoospores of *P. citrophthora* were readily dispersed to potted *Pieris japonica* (Thunb.) D. Don by splashing water; lesion incidence on infected plants in the field was shown to follow a negative vertical gradient. Outbreaks of *P. syringae* fruit rot of apple (*Malus* sp.) were in large part related to the incidence of heavy rains in the United Kingdom; it was believed that inoculum was splashed into tree canopies from infested leaf debris on the orchard floor (43,44). Aerial infections of cedar (*Chamaecyparis lawsonia* (Murr.) Parl) have been shown to be dependent upon dispersal of *P. lateralis* sporangia from stem cankers and infected lower branches (40). The incidence of *Rhododendron* leaf lesions, caused by *P. parasitica*, followed a negative vertical infection gradient with height from container bases; occurrence of lesions was found to be highest on plants in close proximity to areas prone to flooding during irrigation (20). Demonstration of splash dispersal of *P. cactorum*, in addition to the forementioned reports of similar phenomena, emphasizes the importance of the mechanism in development of aerial epidemics caused by various *Phytophthora* spp.
Raindrops reaching the ground usually vary between 0.02 and 0.5 cm diameter, with small drops being more numerous than large ones (5). However, significant numbers of drops larger than 0.2 cm diameter can originate from convective clouds, such as cumulus (3). Drop sizes studied here fall well within those ranges. The larger drop could easily approximate water dripping from within the strawberry canopies. We feel that drop sizes and velocities studied here could easily result in dispersal of _P. cactorum_ propagules from the ground into plant canopies, and disperse sporangia, mycelia, and/or zoospores both within and between rows of strawberry plants. Splash dispersal of _P. cactorum_, in addition to meteorological factors favoring infection and sporulation, probably contributed to the severe aerial epidemics of leather rot experienced in recent years.
CHAPTER IV

Overwinter Survival of Phytophthora cactorum in Infected Strawberry Fruit

INTRODUCTION

Epidemics of strawberry leather rot, caused by Phytophthora cactorum (Leb. & Cohn) Schroet., have resulted in significant yield and quality losses in Ohio (8). In 1981, it was estimated that 20 to 40% of Ohio's strawberry crop was lost to leather rot (8). Very little information has been published on this disease and the overwintering nature of P. cactorum in strawberry fields has not been described.

Survival of several Phytophthora spp. in infected host tissue has been reported (6,37,42). Phytophthora palmivora has been reported to survive in infected rubber tissue (42). Phytophthora cinnamomi has been recovered from Banksia grandis roots up to 2 yr following plant death (37). Gerlach et. al. (10) reported survival of P. citrophthora in diseased Pieris japonica tissue. Kuks and Benson (20) reported the survival of P. parasitica in infected Rhododendron tissue.

In our observations in Ohio, strawberry fruits infected with P. cactorum eventually dry up to from mummies in the later stages of decay (8). We have observed oospores in fruit mummies obtained from laboratory inoculations and naturally infected fruit in the field (8).
The purpose of this study was to determine if mummified fruit could serve as a source of overwintering inoculum for leather rot.

MATERIALS AND METHODS

Laboratory inoculations were performed with cultures of *P. cactorum* freshly isolated from infected strawberry fruit (cv. Midway) on pentachloronitrobenzene-benomyl-neomycin sulfate-chloramphenicol medium (PBNC) (35). Mycelial transfers from the edges of 3-day-old cultures were transferred to lima bean agar (35). Cultures were incubated for 7 days at 22°C in continuous light at 9,000 lux. Sporangia production was first observed after 4 days' incubation. Sporangial germination was then induced by flooding each culture with 20 ml sterile distilled water (SDW) followed by refrigeration for 30 min at 5°C. Plates were removed from refrigeration and brought to 22°C and zoospores were produced in 30 min. Zoospore concentration was adjusted to 10,000/ml in SDW using a hemacytometer.

Fruits were obtained from plants (cv. Midway) grown to reproductive maturity in a 1:1:1 (v:v:v) peat:sand:steam-disinfested silt-loam mixture in the greenhouse. Immature (green) fruit were harvested and washed with detergent in SDW. Each fruit was then rinsed 5 times with 50 ml SDW and surface sterilized in 100 ml of a 10% chlorox solution for 30 sec, then rinsed again in five-50 ml aliquots of SDW.

Twenty ml of inoculum was transferred to 9 cm diam plastic petri plates; 5 fruits were then placed in inoculum for 4 hr. Inoculated fruit were then placed in 50 x 50 cm plastic containers, sealed with
clear plastic wrap, and incubated 72 hr at 22 C in continuous light at 9,000 lux. Containers were then uncovered and inoculated fruit were allowed to incubate an additional 11 d at 22 C in continuous light. Relative humidity in the room was 30-40%. Tissue sections were taken daily from infected fruit and observed microscopically at 40 X for oospore production. Oospores were first observed 4 d following inoculation.

Mummified fruit (5) were then placed between 2-6 cm pieces of 130 μm nitex cloth; the pieces were sewn together with monofilament fishing line. A total of 16 fruit pouches, each containing 5 mummified fruit, were prepared in this manner and buried 1 cm below the soil surface in a strawberry field (cv. Midway) at the conclusion of the fruiting season. Locations of buried fruit pouches were marked with wooden stakes. Pouches were buried on July 1, 1982, and retrieved April 15, 1983. The experiment was conducted again in 1983-1984.

Upon retrieval, 3 mummies from each pouch were placed in 50 ml aliquots of SDW and ground for 1 min at high speed in a Sorvall Omni-Mixer. Each suspension was further diluted by the addition of 250 ml SDW. Ten ml of suspension was then transferred to 9 cm diam plastic petri plates and incubated 14 d hr at 22 C in continuous light at 9,000 lux. Daily microscopic examinations at 40 X were made over a 14 d period.

The remaining two fruit from each pouch were placed on moistened filter paper in 9 cm diam plastic petri plates and covered. Following a 72 hr incubation period at 22 C in continuous light at 9,000 lux, strawberry seedlings were observed emerging from the mummified fruit.
tissue. Each seedling was transferred to 10 ml SDW in 9 cm diam plastic petri plates, covered, and incubated 24 hr at 22 C in continuous light at 9,000 lux. Seedlings were then observed microscopically at 40 X, and then transferred to petri plates containing PBNC medium (35). Plates were incubated 72 hr at 22 C in continuous light at 9,000 lux.

Twenty-five mummified strawberry (cv. Midway) fruit that formed in the previous season were collected on the soil surface of a strawberry field at Wooster, OH, in April 1983 and 1984. The field had a history of leather rot the previous several years. Twenty mummies were chosen at random and each mummie was placed in 50 ml SDW and ground for 1 min at high speed in a Sorvall Omni-Mixer. Each mummie suspension was then further diluted by the addition of 250 ml sdw. Ten ml of suspension was then transferred to 9 cm diam plastic petri plates and incubated 14 d at 22 C in continuous light at 9,000 lux. Daily microscopic examinations at 40 X were made over a 14 d period. The five remaining mummies were placed on moistened filter paper in 9 cm diam plastic petri plates and covered. Following a 72 hr incubation period at 22 C in continuous light at 9,000 lux, strawberry seedlings were observed emerging from the mummified fruit tissue. Each seedling was transferred to 10 ml SDW in 9 cm diam plastic petri plates, covered, and incubated 24 hr at 22 C in continuous light at 9,000 lux. Seedlings were then observed microscopically at 40 X, and then transferred to petri plates containing PBNC medium (35). Plates were incubated 72 hr at 22 C in continuous light at 9,000 lux.
RESULTS

Germinating oospores were observed in all (buried and nonburied) mummified fruit suspensions 4-14 d after placement in SDW. Numerous papillate sporangia, characteristic of *P. cactorum*, were observed microscopically at the distal portions of germ tubes emerging from oospores contained in the suspensions (Fig. 13). Indirect germination of several sporangia connected via a germ tube to oospores was observed.

Sporangia of *P. cactorum* in or on the root tip and hypocotyl regions of strawberry seedlings obtained from overwintered mummies (Fig. 14). Strawberry seedlings emerging from naturally occurring mummies in the field were also colonized by *P. cactorum*. *P. cactorum* was isolated from all strawberry seedlings that developed on mummified fruit.

DISCUSSION

Our results suggest that *P. cactorum* can overwinter in mummified tissue from infected strawberry fruit. The pathogen was readily observed on and recovered from both buried and nonburied fruit during two years of testing.

Sporangia, mycelia, chlamydospores, and oospores of *P. cactorum* have all been reported to have survival value in soil (21,38). Survival in soil for up to 14 and 35 days have been reported for mycelia and sporangia, respectively (11,21,38). Sporangial and mycelial viability in soil has been shown to depend upon soil conditions. Sneh and McIntosh (38) reported that mycelia were unable to survive freezing and that the ability of sporangia to survive
Fig. 13. Germinating oospore (X 2,500) from overwintered fruit mummy.
Fig. 14. Sporangia (X 2,500) of *P. cactorum* on strawberry seedling originating from overwintered fruit mummy.
temperatures <10°C depended upon soil moisture; sporangial survival occurred only if soil was saturated prior to freezing and then only if frozen <4 hr.

Oospores, sporangia, and mycelia are quite common in or on strawberry fruit infected with _P. cactorum_. However, overwinter survival of sporangia and/or mycelia in mummified fruit tissue is not likely (38). The retrieval of _P. cactorum_ 9 mo following burial exposure at the soil surface, and with frozen soil conditions of 1–3 mo, indicate that oospores are the most probable survival propagules.

The implications of mummified fruit as a primary source of inoculum for leather rot are obvious. In areas where leather rot is a serious problem, sanitation (removal of infected fruit) should be considered as an important disease control measure.
SUMMARY

Wetness duration and temperature have a direct influence on the infection of strawberry fruit by \textit{P. cactorum} and on subsequent sporulation of the pathogen on infected strawberry fruit.

Fruit infection increased with increased wetness duration (0-5 hr) at all temperatures tested (6-30 C). For each wetness duration, infection increased up to the optimum temperature (21 C), and then declined. At temperatures between 17 and 25 C, 1-5 hr wetness resulted in $>$80% infection. A multiple regression model was developed to describe infection as a function of wetness and temperature.

Sporulation of \textit{P. cactorum} on the surface of infected fruit required a film of free water, and occurred between 12.4 and 27.5 C. Sporangial production increased with increased wetness duration (0-24 hr) at temperatures between 10 and 27.5 C. For each wetness duration, sporulation increased up to the optimum temperature (~20 C) and then declined. Sporangia were present after 3 hr wetness between 15 and 25 C. A multiple regression model was developed to describe sporangial production as a function of temperature and wetness duration.

It was found that propagules (zoospores, mycelia, and sporangia) were readily dispersed from infected fruit surfaces by water splash mechanisms. Colony number in petri plates containing selective medium positioned up to 1 m from the inoculum source followed a negative
gradient with increased distance from the source. A multiple regression model was developed describing colony number as function of distance and water drop velocity at impact. Plant to plant dispersal of inoculum from infected to healthy fruit was demonstrated using potted plants.

The presence of germinating oospores in overwintered, mummified fruit indicated that *P. cactorum* was capable of surviving the winter in infected host tissue. Infected seedlings arising from overwintered fruit mummies were also noted, and indicated a possible additional source of primary inoculum. Therefore, mummies that develop from fruit infected by *P. cactorum* probably serve as an excellent source of primary inoculum for the the disease.
CONCLUSION

A hypothetical disease cycle of leather rot of strawberry has been developed from research and observations in this study (Fig. 15). *P. cactorum* can survive as oospores in mummified fruit at or immediately beneath (1 cm) the soil surface, thus serving as a source of primary inoculum. Under favorable soil conditions in the spring, oospores germinate, producing sporangia. Dispersal of sporangia and/or zoospores can occur via water splash mechanisms (rainfall or irrigation) to surrounding fruit, resulting in primary infections.

Infection occurs in the presence of free water on the fruit surface. Wetness durations as short as 1 hr between 17 and 25 °C can result in high levels of infection. As temperatures increase or decrease away from the optimum temperature (~21 °C), progressively longer wetness durations are required for infection.

Formation of dispersal and/or infection units (sporangia) on infected fruit surfaces occurs in the presence of free water and is favored by temperatures between 15-25 °C, with the optimum temperature ~20 °C. Sporangia can form at wetness durations of 3 hr at 15-25 °C, but are produced at 12.5 and 27.5 °C under wetness durations of ≥ 12 and 6 hr, respectively. Longer wetness durations result in greater sporangial production at temperatures within the sporulation range. Infective propagules
(sporangia, mycelia, and/or zoospores) are dispersed by splash mechanisms to nearby healthy fruit, resulting in secondary infections under the proper temperature/wetness conditions.

As infected fruit mummify, oospores are formed within fruit tissue. Mummified fruit eventually fall to the soil, where the pathogen overwinters as oospores within mummified tissue, thus completing the disease cycle.

It is our intention to utilize information contained herein in the development of a microcomputer-based disease forecasting system for leather rot. Parameters to be measured and used to identify meteorological situations of "high-risk" include: wetness duration, temperature, and rainfall. Accurate determination of temperature and wetness duration are necessary for accurate prediction of infection and/or sporulation periods; measurement of rainfall could identify situations in which dispersal of P. cactorum propagules is most likely to occur. The proper monitoring of the forementioned parameters could result in more effective use of protectant fungicides and prevent leather rot epidemics.
Fig. 15. Disease cycle of leather rot of strawberry, caused by *Phytophthora cactorum*. 
LIST OF REFERENCES


