

Epidemiology of *Cercospora* Footrot of Wheat: Spore Production

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ABSTRACT

Maximum sporulation by *Cercospora herpotrichoides* occurred at 10 C and decreased to insignificant levels above 20 C and below 0 C, when incubated at constant temperature. Under alternating high and low temperatures, the fungus continued to sporulate moderately when temperatures were in the optimal range (8-12 C) as long as the duration below 0 C did not exceed 14 hrs. Sporulation was reduced or prevented if daily temperatures were above 20 C for 10 hr, even if favorable temperatures (8-12 C) occurred during the rest of the day.

A value which reflects the influence of fluctuating temperature regimes on sporulation was developed which was designated the "Daily Thermal Sporulation Coefficient"

Additional key words: eyespot, sporulation, temperature.

(DTSC). This value is based on the temperature-sporulation curve for *C. herpotrichoides* and is a function of the total hours of favorable and unfavorable temperatures that occur daily.

Active sporulation periods are defined as periods of at least 2-3 weeks during which the humidity near the soil remains near saturation, the air temperature is above freezing for more than 8 hr/day, and the average DTSC is above 50. If these conditions are maintained continuously, maximum levels of sporulation can occur for up to 50 days. Sporulation periods can be identified from field temperature data and used to assess the seasonal epidemic potential.

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Footrot of wheat, caused by *Cercospora herpotrichoides* Fron., is a serious disease of winter wheat in certain areas of eastern Washington and Oregon and western Idaho. Disease development is favored by the cool, damp fall and spring weather that occurs in areas with high rainfall and/or elevation. During the winter in these areas, temperatures below 0 C occur only at irregular intervals and precipitation is either in the form of rain or snow.

The annual incidence of footrot varies with the amount of inoculum available, the prevailing weather conditions, and the methods of wheat culture. The fungus sporulates abundantly at temperatures between 5 and 15 C on infected stubble remaining from previous crops, and subsequent infections of the basal leaf sheaths occur under similar conditions (1, 2, 3, 4, 5, 6). Low levels of sporulation have been reported at constant temperatures as low as 0 C (1) and under fluctuating field temperatures with lows of -3 C to -4 C (4, 7).

Frequent rains are necessary to assure inoculum dispersal and the high humidity required for sporulation and infection (3, 4, 9). In areas where footrot is annually a serious problem, abundant moisture usually is present from late fall through early spring. Temperature then, has the greatest influence on spore production.

The purpose of this study was to evaluate the influence of temperature on sporulation as related to the development of *Cercospora* footrot epidemics.

MATERIALS AND METHODS.—Spores of *C. herpotrichoides* were obtained by growing the fungus on potato dextrose agar (PDA) slants at 10 C for 2-4 weeks. Conidia were washed from the agar surface with distilled water containing a trace of Tween 20 to minimize clumping. The suspension was centrifuged and the spores resuspended in distilled water to avoid nutrient contamination from the culture medium.

Mycelial plugs, nearly free of spores, were obtained by

cutting out 5-mm plugs with a No. 2 cork borer 1 cm behind the leading edge of 3- to 6-week-old colonies grown on PDA plates at 20 C.

Naturally colonized stubble used in certain studies was collected from harvested fields located near La Grande or Pendleton, Oregon. The amount of sporulation from mycelial plugs or naturally colonized stubble was determined by agitating the plug or stubble piece in a 10 ml test tube containing 0.5 ml distilled water on a Vortex mixer for 15 sec. The number of spores in the wash water was estimated with a haemocytometer.

RESULTS AND DISCUSSION.—*Influence of temperature on sporulation.*—Mycelial plugs of *C. herpotrichoides* were placed on water agar plates and incubated in the dark at various temperatures. Sporulation near and below 0 C was evaluated with a temperature gradient plate (10) that produced a linear gradient of temperatures from 5 C to -5 C. Water agar was poured on the plate and three rows of 18-20 mycelial plugs were placed about 1 cm apart on the agar along the temperature gradient. The plate was covered with a plastic hood to insure high humidity. After 2 weeks of incubation, the amount of sporulation at each temperature was determined. Maximum sporulation of *C. herpotrichoides*, when incubated at constant temperatures, occurred at 10 C and decreased to insignificant levels above 20 C and below 0 C (Fig. 1-A).

Considering the daily patterns of fluctuating temperatures that occur under field conditions, studies were initiated to investigate the effects of daily periods of favorable and unfavorable temperatures on sporulation. Water agar plates containing mycelial plugs were incubated in the dark and manually shifted from a high to a low temperature to simulate daily temperature fluctuations, on a 10-hr "day" and 14-hr "night" schedule.

The amount of sporulation after 2 weeks of incubation under three constant "night" temperatures and varying

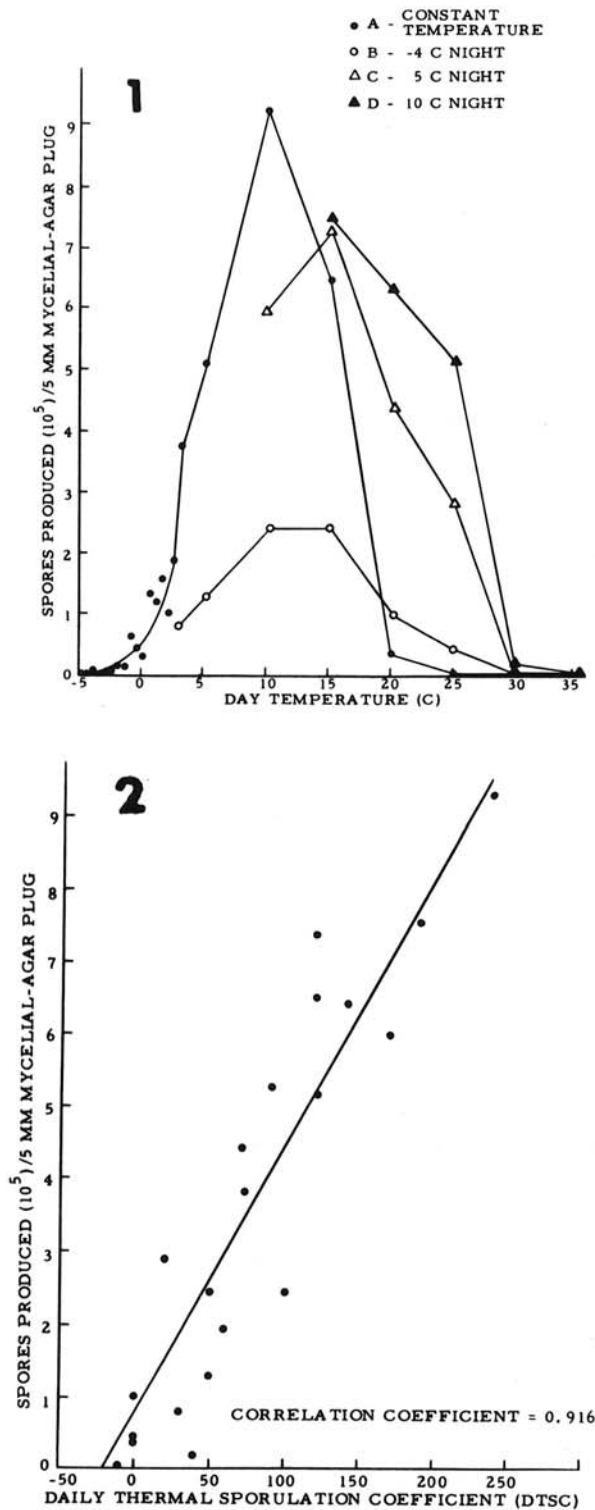


Fig. 1-2. 1) The effect of a constant temperature (A) and alternating 10-hr "day" and 14-hr "night" temperatures (B, C, D) on sporulation by *Cercospora herpotrichoides*. 2) Sporulation by *C. herpotrichoides* as a function of Daily Thermal Sporulation Coefficients (data of Figure 1).

"day" temperatures is shown in Fig. 1-B, C, D. *C. herpotrichoides* continued to sporulate moderately even though 14-hr periods of freezing temperatures occurred daily, provided 10-hr periods of favorable temperatures (5 C to 20 C) were also maintained (Fig. 1-B). If 10-hr periods of moderately high temperatures (20 C to 25 C) were maintained, sporulation was sub-optimal even if optimal temperatures occurred during the 14-hr "night" period (Fig. 1-C, D). However, if higher temperatures (30 C to 35 C) were maintained during the 10-hr period, sporulation decreased to near zero regardless of the temperatures which occurred during the 14-hr period.

Development of a method to evaluate the effect of fluctuating temperatures on sporulation.—The total amount of sporulation occurring under any fluctuating daily temperature regime should be related to (i), the effect of specific temperatures on the ability of the fungus to sporulate, and (ii) the duration of each temperature period that occurs during the daily cycle. Considering the constant temperature-sporulation curve for *C. herpotrichoides* (Fig. 1-A) an arbitrary set of values, herein designated Equivalent Unit Values (EUV's) were assigned to changes in temperature based on the sporulation response. Temperatures from 0-10 C were assigned ascending EUV's of 0-10 and those from 11-20 C, descending EUV's of 9-0. Temperatures below 0 C were all assigned EUV's of 0 since their effect is equal in temporarily inhibiting sporulation by decreasing metabolic activity. Temperatures above 20 C [the optimal temperature for vegetative growth (3, 8, 11)] were assigned decreasing negative numbers; i.e., 21 C = -1, 22 C = -2, etc., since active suppression of sporulation increases in this range as temperatures rise.

EUV's can be used to calculate a numerical value based on the ability of the fungus to sporulate under any set of daily temperatures, herein designated the Daily Thermal Sporulation Coefficient (DTSC). A DTSC is calculated for any set of conditions by multiplying the number of hours incubated at each temperature by the corresponding EUV and summing these products over a 24-hr period. The data of Fig. 1 after conversion to DTSC values show a linear relationship (Fig. 2) indicating that sporulation is a function of the total hours of favorable and unfavorable temperatures occurring daily as expressed by DTSC values.

DTSC's can be calculated from field data taken on a recording thermograph chart even though temperatures fluctuate constantly. The value is computed by calculating the area under the curve between the 0-C and 10-C lines on the chart by counting grid squares. The area under the curve above the 10-C line is then calculated and subtracted from the previous value, the remainder being the DTSC. The area under the curve below the 0-C line is ignored since all EUV's below 0 C are equal to 0. In this manner field temperature data can be easily converted to DTSC's and used to evaluate the effect of any temperature profile on sporulation. This method of temperature profile evaluation could be adapted to any organism by assigning EUV's on the basis of the temperature-response curve of the organism in question.

Influence of the duration of nightly freezing temperatures on sporulation.—To further assess sporulation when "night" temperatures are below 0 C, the temperature gradient plate was used to evaluate the

effects of the duration of daily periods of freezing temperatures. The gradient plate was modified to provide a constant plate temperature of -5°C during the "night" and a gradient running from 0°C to 10°C during the "day" (10). Since agar is unsatisfactory under conditions of continual freezing and thawing, a layer of fine, washed, white sand was placed on the gradient plate and wet with distilled water to near saturation. After 2 weeks of incubation in the dark, sporulation of mycelial plugs was evaluated as a function of the "day" temperature. This procedure was repeated several times, varying the length of the below 0°C period.

The amount of sporulation that occurred under the various temperature regimes was evaluated by using DTSC values (Fig. 3). The distribution of these points indicates that sporulation fell into three zones as a function of the DTSC. Below a DTSC of 50, sporulation was minimal. In the range of 50-120, sporulation was moderate; whereas, above 120 heavy sporulation occurred. Sporulation did not occur when the length of the freezing period exceeded 14 hr. It must be assumed that the fungus requires at least 10 hr per day of above 0°C temperatures to resume the metabolic activity necessary for spore production. Figures 3 and 4 both show a DTSC of 0 to be the lower limit of sporulation. This is to be expected as this value is equivalent to a continuous temperature of 0°C .

Potential seasonal sporulation on naturally infested stubble.—One-cm sections of infested wheat stubble were washed in running water, placed on wet cheesecloth in a covered plastic container and incubated in the dark at 10°C . At monthly intervals, the number of spores produced on each stubble piece was determined. The stubble pieces were washed after each sampling to remove any remaining spores and returned to the incubation chamber for continued sporulation. Maximum levels of sporulation diminished after 50 days of continuous sporulation, and decreased to minimal levels after 100 days (Fig. 4).

In the wheat-growing areas of eastern Oregon where foot rot is a problem, conditions favoring active sporulation occur only sporadically from November to May, the total rarely exceeding 100 days. At any one location, sporulation is limited only by weather patterns and can be expected whenever conditions are favorable. In western Oregon where ideal conditions of temperature and moisture prevail almost continuously, starting in October, the inoculum soon would be exhausted. This may partially explain the almost total absence of foot rot west of the Cascade Mountains, in spite of the very favorable climate for this disease.

The stubble pieces that had been incubated for nine months under ideal conditions for sporulation in the laboratory, were allowed to dry out and were placed on the soil surface (July) in a fallow field near La Grande, Oregon. After 2 months of exposure, *C. herpotrichoides* could not be reisolated from these stubble pieces. This period of high summer temperatures and desiccation effectively eliminated the fungus after it had been exhausted by 9 months of continuous sporulation. This information is important since most wheat stubble is left standing through the winter to control erosion. In higher rainfall areas favorable to foot rot development, the infected portions of this stubble may sporulate quite

heavily during this period. Trashy fallow of this stubble the following spring may be more advantageous than mold board plowing, as is often practiced, since this would keep more of the infected stubble on the surface exposing it to high temperatures and desiccation during the summer.

Identification of active sporulation periods under field conditions.—To monitor epidemic development during a given season, it is desirable to be able to identify active sporulation periods from field temperature data. DTSC values can be used if the lag period between the onset of favorable conditions and initiation of sporulation is known. To determine this time period, 2-cm sections of actively sporulating, naturally colonized stubble were washed in running water to remove all spores, and incubated on a wet-sand temperature gradient plate. The stubble pieces were initially incubated under conditions unfavorable to sporulation, consisting of 10 hr "nights" at a constant -5°C and 14 hr "days" at a constant 3°C (DTSC = 42). After 2 weeks, the -5°C "night" was reduced to 6 hr and the "day" temperatures were raised to provide a gradient running from 5°C to 15°C (DTSC = 90-180). No significant sporulation occurred during this period. Stubble pieces were then sampled every 2 days to determine the time required to resume sporulation as a function of the day temperature. At warmer temperatures ($13-15^{\circ}\text{C}$), sporulation began in a week to 10 days, but at

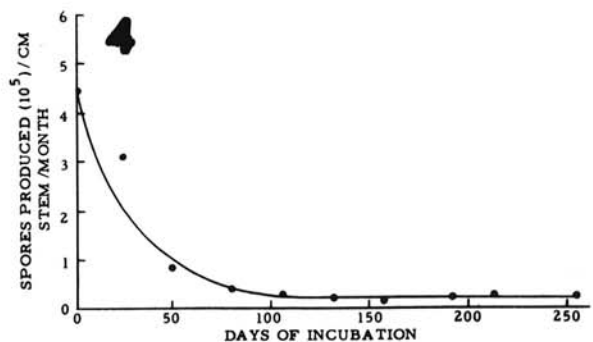
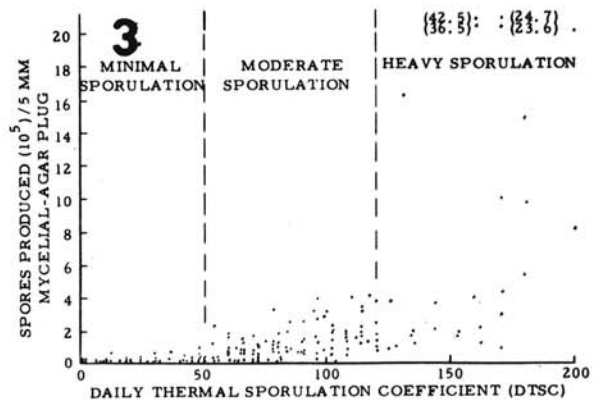


Fig. 3-4. 3) Sporulation zones based on Daily Thermal Sporulation Coefficients. 4) Rate of production by *Cercospora herpotrichoides* on naturally infested stubble under continuous incubation at 10°C .

cooler temperatures (5-12 C), sporulation resumed only after 2 weeks.

Active sporulation periods can be defined as any period of at least 2-3 weeks duration in which the humidity near the soil remains near saturation, the air temperature is above freezing for more than 8 hr/day, and the average DTSC is above 50. Low levels of sporulation may take place outside this range, but are of little significance in the epidemic development of foot rot. These periods can be identified from field temperature data and used to assess seasonal epidemic potential.

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