Effects of Temperature and Relative Humidity on Sporangium Production of *Pseudoperonospora humuli* on Hop

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ABSTRACT

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The effects of night temperature and relative humidity (RH) on sporangium production of *Pseudoperonospora humuli* on systemically infected hop shoots were studied. Sporangium production was inhibited or was very light in hop yards when either the nightly minimum temperature was ≤5 C, or the mean RH at night was ≤64%. Production of sporangia increased with increases of nightly minimum temperature, nightly mean RH and temperature, and number of hours in which RH was ≥80%. Multiple regression analysis with minimum nightly temperature × hours with RH ≥80%, and nightly mean RH as independent variables accounted for 84% of the variation in sporangium production.

Additional key words: disease forecasting, epidemiology, *Humulus lupulus*.

Downy mildew of hop (*Humulus lupulus* L.) (a disease caused by *Pseudoperonospora humuli* (Miy. et Tak.) Wils.) occurs sporadically in the Yakima Valley of Washington (5). Epidemics of downy mildew are usually short because favorable weather in May is generally followed by hot, dry conditions in June. The greatest losses in the Yakima Valley occur from crown infections that result in death of crowns and stand reductions. Cultivars susceptible to downy mildew predominate in the area and timely applications of fungicides are needed for control.

P. humuli overwinters as mycelium in infected hop crowns in Washington (12). Shoots growing from infected crowns in the spring may become invaded by the fungus. These systemically infected shoots are typically stunted, chlorotic, have down-curled brittle leaves, and are known as "primary basal spikes." Sporangia which serve as the initial source of inoculum for a growing season are borne on the abaxial leaf surfaces of these primary basal spikes.

Sporangia are produced at night in response to a diurnal cycle when night temperature and humidity are favorable (16). In Washington, sporangium production may occur either relatively early in the season or be delayed for several weeks, depending on night temperature and humidity.

This study was initiated to relate, quantitatively, sporangium production of *P. humuli* with temperature and relative humidity (RH). Data from this study are intended for incorporation into a predictive system to be used for controlling hop downy mildew in Washington.

MATERIALS AND METHODS

Primary basal spikes were found in three hop yards of the cluster cultivar, L-1, in the springs of 1982 and 1984. The abaxial leaf surfaces of each spike were carefully examined with a 10-power hand lens to determine if sporangia were present. Nonsporulating primary basal spikes were labeled and then collected the next morning.

Sporangia that formed overnight were washed and brushed from individual leaves in either 35 or 50 ml of water. After swirling the sporangium suspension, six to eight subsamples of suspension per leaf were taken, and sporangia were counted in a hemacytometer viewed with a microscope. The area of each leaf was measured with a LI-COR model 3100 area meter and the number of sporangia per square centimeter of leaf was determined. A value for each spike was calculated from a mean of the two leaves at a node and a mean from one to three nodes. Only leaves that bore sporangia were included in determining the number of sporangia per square centimeter of leaf for a spike, unless no leaves bore sporangia. A mean number of sporangia per square centimeter was determined for each collection period from two to ten spikes (mean of five spikes). Fourteen collections were made in 1982 and 33 in 1984. Sporangia were not observed on stems in these field tests.

Temperature and RH were recorded with hygrothermographs in weather shelters 15 cm above the ground at the edge of the three hop yards. Hourly temperature and RH values were used to calculate means of temperature and RH for various time intervals from 1400 hours the day before spikes were collected up to 0600 hours the day of collection. These time intervals were chosen to correspond with the development of sporangia of *P. humuli* as observed by Yarwood (16). He reported that sporangiophores first began to appear through stomata at 2400 hours and that sporangia reached full size at 0600 hours. Linear and multiple regressions were used to analyze the data.

RESULTS

Sporangium production did not occur or was very light when the nightly minimum temperature was ≤5 C or when the mean RH between 2000 and 0600 hours was ≤64% (Table 1). Sporangia were usually produced at mean RHs ≥71% when the nightly minimum temperature was >5 C (Table 1).

Data for 1982 and 1984 were combined for analysis because linear regression equations relating number of sporangia per square centimeter of leaf to temperature, RH, or hours of RH were not significantly different for the 2 yr.

Numbers of sporangia were significantly related to mean and maximum RH, hours (from 1500 to 0600 hours) of RH ≥80%, minimum temperature, and mean temperature (Table 2). Hours of RH ≥80% from 1500 to 0600 hours accounted for more variation of sporangium production than hours of RH calculated for shorter time periods. Maximum RH only accounted for 11% ($R^2 = 0.11$) of the variation for production of sporangia (Table 2).

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Sporangium production was more closely related to the number of hours when RH was ⩾80% than mean and maximum RH or number of hours of RH ⩾70% or ⩾90%.

Mean RH accounted for more variation for sporangium production and mean temperature accounted for less when respective means included measurements made before 2400 hours than when they were not included (Table 2).

Coefficients of determination for minimum temperature and number of sporangia increased when minimum temperatures that corresponded with one or more hours of RH ⩾80% only were included in the analysis (Table 2, regression equations 12–15). Minimum temperatures corresponding with two or more hours of RH ⩾80% accounted for more variation of sporangium production than minimum temperatures corresponding with other combinations of hours of RH ⩾80%. These equations had greater regression coefficients (slopes) than equations involving other independent variables (Table 2), indicating a greater increase in sporangium production with an increase of the independent variable.

Sporangium production per square centimeter of leaf was more closely related to the product of minimum temperature and hours of RH ⩾80% than any other of the independent variables that were tested singly and listed in Table 2.

A multiple regression equation that included the product of minimum temperature and hours of RH ⩾80% (X_1), and mean RH from 2000 to 0600 hours (X_2) as independent variables accounted for 84% ($R^2 = 0.84$) of the variation for production of sporangia per square centimeter of leaf surface. Both independent variables accounted for significant structure ($P < 0.01$) in the analysis. The regression equation was: $\hat{Y} = 72{,}053 + 1{,}405\ X_1 - 1{,}253\ X_2$. Data from 1981 and 1982 were pooled because the regression planes were not significantly different ($P < 0.05$) (1,2).

Multiple regression analysis giving the above equation accounted for more variation for sporangium production and had higher F values than did analyses using other combinations of independent variables listed in Table 2.

TABLE 1. Mean number of sporangia per square centimeter of leaf produced on hop shoots systemically infected with *Pseudoperonospora humuli* in hop yards in 1982 and 1984 at various nightly mean and maximum relative humidities, hours of relative humidity ⩾80%, and minimum temperatures

Relative humidity			Minimum	Sporangia[c]
Mean[a]	Maximum	Hours >80%[b]	temp (C)	(no./cm²)
48	57	0	2.5	0
56	70	0	7.8	249
58	73	0	8.9	0
59	73	0	2.2	0
59	76	0	5.0	0
59	86	1	6.7	0
60	81	0	6.1	0
61	75	0	2.2	0
61	80	0.5	3.3	0
64	73	0	2.2[d]	0
64	84	2	4.4[d]	0
64	83	2	5.6	2,702
65	98	2	2.8[d]	475
66	93	3	3.3[d]	0
66	90	3	13.3	70,030
66	100	4	7.2	22,016
67	85	2	4.4[d]	0
68	99	1	11.1	0
68	100	2.5	3.9[d]	0
68	90	1	8.3	2,606
68	99	3	5.6	0
69	77	0	6.7	0
71	96	4	7.8	50,577
72	100	4	2.2[d]	0
73	100	4	2.5[d]	0
73	100	4	4.2[d]	0
75	95	6	6.4	5,915
75	100	5	2.5[d]	0
75	94	3	13.3	41,210
75	100	5.5	10.0	42,346
76	100	5	4.4[d]	0
76	100	3	5.6	0
79	100	5	11.1	31,317
79	100	6	2.2[d]	0
79	96	6	11.1	70,596
84	100	5	7.2	4,794
84	100	6	12.2	63,460
85	100	9	8.9	73,984
85	100	7	6.7	60,605
86	100	6	3.3[d]	0
93	100	8	5.0[d]	0
93	100	11	7.2	37,573
95	100	15	9.4	176,406
96	100	10	7.2	33,825
96	100	9	7.2	83,897
97	100	9	5.6	28,523
99	100	10	6.9	21,568

[a] Mean relative humidity from 2000 to 0600 hours.
[b] Number of hours of relative humidity from 1500 to 0600 hours.
[c] Mean number of sporangia from two to six leaves from two to 10 spikes.
[d] Nightly minimum temperature ⩽5 C, when mean relative humidity at night was ⩾64%, at which sporulation did not occur or was very slight.

DISCUSSION

Relative humidity has been stated to be the most important external factor affecting sporulation of *P. humuli* (10,16). In the arid hop-growing region of Washington, both RH and temperature influenced production of sporangia.

Royle reported that significant sporulation does not likely occur below an RH of 90% (7,10); even though slight sporulation has occurred at an RH as low as 50% (10,13). In our field study, sporulation occurred infrequently and was very light at nightly mean RHs ⩽64%. The lowest RH at which sporulation was observed was 56%. Sporulation was seen when mean RHs were ⩾71% as long as the temperature was favorable. The RH of the microenvironment of the spikes was not measured in this study, but would have been higher than that recorded at the edge of the yard (17).

Yarwood (16) observed that the temperature range for sporulation of *P. humuli* is quite large. We found a rapid increase

TABLE 2. Regression equations and coefficients of determination of regression analyses relating the number of sporangia per square centimeter of leaf produced at night on hop shoots systemically infected with *Pseudoperonospora humuli* to various independent variables

Independent variable	Regression equation[a]	R^2
1. Maximum relative humidity	$\hat{Y} = -74{,}845 + 1{,}028X$*	0.11
Mean relative humidity from:		
2. 0200 to 0600 hours	$\hat{Y} = -54{,}415 + 877X$*	0.10
3. 2400 to 0600 hours	$\hat{Y} = -75{,}391 + 1{,}165X$**	0.18
4. 2200 to 0600 hours	$\hat{Y} = -86{,}242 + 1{,}359X$***	0.24
5. 2000 to 0600 hours	$\hat{Y} = -87{,}048 + 1{,}452X$***	0.28
6. 1800 to 0600 hours	$\hat{Y} = -88{,}325 + 1{,}577X$***	0.32
7. 1600 to 0600 hours	$\hat{Y} = -88{,}875 + 1{,}696X$***	0.35
8. 1400 to 0600 hours	$\hat{Y} = -83{,}966 + 1{,}701X$***	0.33
9. Hours of relative humidity[b] ⩾70%	$\hat{Y} = -17{,}668 + 6{,}789X$***	0.40
10. Hours of relative humidity[b] ⩾80%	$\hat{Y} = -\ 7{,}528 + 6{,}642X$***	0.47
11. Hours of relative humidity[b] ⩾90%	$\hat{Y} = +\ 3{,}816 + 5{,}583X$***	0.28
12. Minimum temperature	$\hat{Y} = -20{,}290 + 6{,}397X$***	0.33
Minimum temperature on nights that have RH ⩾80% for:		
13. One or more hours	$\hat{Y} = -21{,}125 + 6{,}915X$***	0.34
14. Two or more hours	$\hat{Y} = -24{,}068 + 7{,}888X$***	0.43
15. Three or more hours	$\hat{Y} = -20{,}625 + 7{,}603X$***	0.40
Mean temperature from:		
16. 0200 to 0600	$\hat{Y} = -34{,}862 + 7{,}036X$***	0.31
17. 2400 to 0600	$\hat{Y} = -26{,}010 + 5{,}451X$**	0.20
18. 2200 to 0600	$\hat{Y} = -23{,}136 + 4{,}712X$**	0.15
19. Minimum night temperature × hours of RH ⩾80%	$\hat{Y} = -\ 8{,}396 + 992X$***	0.76

[a] * Statistically significant ($b \neq 0$) at the 5% level; ** statistically significant ($b \neq 0$) at the 1% level; *** statistically significant ($b \neq 0$) at the 0.1% level.
[b] Number of hours of relative humidity from 1500 to 0600 hours.

in sporulation with an increase of minimum and mean temperature at night, as indicated by the large regression coefficients in equations using minimum or mean temperature as an independent variable (Table 2).

In Washington, after the first emergence of systemically infected shoots, low temperatures may delay sporulation for several days or weeks, especially early in the spring. Such a delay would postpone a need for applications of fungicides. After a favorable period for sporulation, temperatures for several nights may become too cool for sporulation and then become favorable again. In California (15,16) and Europe (10), low temperatures seldom prevent sporulation for extended periods.

The quantity of sporangia produced in a hop yard is a critical factor in the epidemiology of downy mildew (11). After sporangia have formed, free water on a susceptible host for 3 hr at 19–23 C or 6 hr at 8–10 C are needed for shoot infection (8). Rainy weather in the spring often provides the warm wet weather needed for infection and also for the liberation of sporangia from spikes (3,6).

The multiple regression equation developed here will be incorporated into a forecasting system for hop downy mildew in Washington (4,14) to determine inoculum potential for a given area. Fungicide applications will then be based on inoculum potential and the likelihood of favorable weather for infection (4,14).

Forecasting models for hop downy mildew have been developed for several hop-growing areas and have been discussed elsewhere (9,10). Most of these models predict symptom development and schedule sprays to prevent a second cycle of infection. The Washington system is intended to schedule fungicide applications to prevent the first cycle of infections from sporulating primary basal spikes. This system gives short-range, specific forecasts for infection and is used in conjunction with an empirically developed model that gives a long range, general outlook for downy mildew early in the season (5).

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