Relationships of Soil Temperature and Moisture to Clubroot (*Plasmodiophora brassicae*) Severity on Radish in Organic Soil

B. A. THUMA, Graduate Research Associate, R. C. ROWE, Associate Professor, and L. V. MADDEN, Assistant Professor, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Ohio State University, Wooster 44691

ABSTRACT

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Effects of rainfall, soil temperature, and soil moisture on clubroot severity on radish (cultivar Scarlet Knight) were investigated in naturally infested Carlisle muck soil. Cumulative rainfall in the first 2–3 wk, soil temperature throughout the growth period, and interaction of soil temperature with soil moisture were the variables most highly correlated with disease development. Regression equations were developed to predict severity of clubroot as a function of these variables.

Additional key words: *Raphanus sativus*

Clubroot, caused by *Plasmodiophora brassicae* Wor., is a major disease of cruciferous crops worldwide (1,4,8,15,16). The disease is characterized by distorted swellings on roots of susceptible plants (4,8,15). In cases of severe infection, plants are usually stunted and often wilt as a result of abnormal proliferation of infected cells interfering with water uptake (4,8). In Ohio, clubroot is primarily a concern in the production of fresh-market cruciferous vegetables on organic soils (10,11).

Liming, crop rotation, and soil drainage have been standard recommendations for clubroot control (4,7,8). Because of the high buffering capacity of organic soils, control by liming is not feasible (4,8,14). Improving soil drainage has reduced losses but has not provided reliable control, especially during periods of excessive rainfall (4,11). Current use of complex multiple-cropping systems by Ohio fresh-market vegetable growers limits the usefulness of crop rotation because of the long viability of *P. brassicae* resting spores (4,8). Resistance to clubroot in radishes has been identified (10) but will probably not be of long-term use because of the potential development of virulent races of the pathogen (1,3,13). Fungicides have also been ineffective on organic soils (5,6,11).

Most studies with *P. brassicae* have been conducted on mineral soils (2,9). This work was initiated to investigate the effects of such environmental variables as rainfall, soil temperature, air temperature, and soil moisture on clubroot development in organic soil. The purpose was to explain the wide variability in symptom development often reported by commercial radish growers and to devise a preliminary system for predicting disease severity.

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MATERIALS AND METHODS

Pathogen isolation and identification. Clubbed roots of radish (*Raphanus sativus* L. 'Scarlet Knight') naturally infected with *P. brassicae* were collected from a naturally infested Carlisle muck soil near Hartville in northeastern Ohio. Inoculum was prepared using a modifica-

tion of the methods of Buczacki et al (3). Firm clubbed roots washed in tap water were comminuted at high speed for 1 min in a Waring Blendor in about 500 ml of sterile distilled water (SDW). This suspension was filtered through cheesecloth and centrifuged at 500 *g* for 3 min. The pellet was resuspended in SDW and recentrifuged six or seven times. The resulting pellet containing *P. brassicae* resting spores was resuspended and stored in SDW. If the inoculum was not to be used immediately, it was refrigerated at 4 C as long as 1 wk. Concentrations of spores in inoculum were determined with a hemacytometer. To keep fresh inoculum available for use in temperature studies during winter months, *P. brassicae* was maintained in the greenhouse in roots of Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr. 'Michihli') initially inoculated with resting spores collected from naturally infected radishes at the Hartville location.

Physiologic specialization in *P. brassicae* is complex, but a system of race determination has been defined by Buczacki et al (3) that uses a set of 15 host plants known as the European clubroot differential (ECD) set. Seeds of the ECD set were planted in an autoclaved mixture of Wooster silt loam and commercial peat (5:1, v/v) contained in autoclaved wooden flats measuring 36 × 54 × 8 cm. Seeds were spaced about 6 cm apart within each row, with 6 cm between rows, and inoculated at planting by placing 1 ml of a suspension of 10^8 *P. brassicae* resting spores per milliliter of the Hartville isolate over each seed. The experiment was replicated three times using 30 plants per differential host. Plants were maintained in the greenhouse with a 16-hr photoperiod with supplemental lighting of 10.5–10.8 klux. Air temperatures in the greenhouse throughout these studies ranged from 12 to 33 C, with a mean daily temperature of 20.5 C. Host plants were harvested after 6 wk and visually rated for disease severity on a scale of 0–3 according to Buczacki et al (3). ECD determination indicated a value of 16/02/30. This is similar to race 6 (1,10,13), which has been reported frequently in the midwestern United States on *Brassica* spp. (13), including on radish (10).

Field studies. Field plots were established in Carlisle muck soil on a commercial vegetable farm near Hartville in 1980 and 1981. The plot was arranged so that single rows of radish (cultivar Scarlet Knight) were planted weekly using a hand-pushed planter. The soil was disked and rolled smooth before planting and rows were spaced 0.5 m apart, with about 50 plants per meter to approximate commercial spacing. The entire 90-m row length was subdivided by stakes into six replicate sections of 15 m each.

Diazinon 4EC was drenched over each row with a watering can 1 wk after planting at a rate of 307 L/ha to control cabbage root maggots (*Hylemya brassicae* Bouché). Carbaryl 50W and dimethoate 4EC were applied occasionally to the entire plot for control of flea beetles (*Phyllotreta* sp.) and aphids (Homoptera: Aphididea), respectively.

In 1980, three tensiometers (Jet Fill Tensiometer, Model 2725, Soil Moisture Equipment Corp., Santa Barbara, CA 93100) were spaced 30 m apart along the edge of the plot to monitor soil moisture. Daily readings were recorded in centibars of soil suction, with the daily average of the three instruments used for analysis. A double-roofed white wooden instrument shelter housed a recording two-point thermograph (Model T601, Weather Measure Corporation, Sacramento, CA 95800) with one probe recording air temperature (inside the shelter) and the other recording soil temperature (buried 15 cm deep). Rainfall was recorded daily by a rain gauge located near the shelter.

Weekly plantings began on 2 May and ended on 4 September, similar to a commercial radish planting schedule, resulting in 18 plantings. The entire 90-m row of radishes was harvested after 6 wk. Each plant was rated visually for clubroot severity on a 0–5 scale: 0 = no visible infection; 1 = very small swelling on a lateral root, with no visible infection of bulb; 2 = small club on a lateral root with a diameter of <1 cm; 3 = medium-sized club, 1–2 cm diameter or more than one small club; 4 = large club with a diameter of >2 cm or more than one medium-sized club; and 5 = bulb severely clubbed or more than one large club.

In 1981, the plot was established in the same field and location. Single rows were planted on a weekly basis as before except each 15-m replicate was further subdivided into three 5-m sections. One 5-m section from each of the six 15-m replicates was selected randomly and harvested from each row at the end of 4-, 5-, and 6-wk growth periods. Environmental monitoring devices were as in 1980 except both probes of the thermograph recorded soil temperature, one at a depth of 10 cm and the other at 15 cm. Planting began on 5 May and ended on 3 September. Harvested plants were evaluated as before.

A standard curve to convert tensiometer centibar readings to soil moisture content was determined. Carlisle muck soil was spread thinly on kraft paper on a laboratory bench and some samples were allowed to air-dry for varying time periods, whereas others were atomized with varying amounts of water. Soil samples were mixed and aliquots of about 1 L were placed in plastic bags inside a wire basket and allowed to equilibrate for 30 min. Four readings with four different tensiometers were taken on each sample. Tensiometers were placed in the soil in plastic bags and the soil was packed around each probe to maintain good contact with the porous ceramic bulb of each instrument. Tensiometers were allowed to equilibrate 20 min before a reading was taken. Soil samples (about 10 cc) were removed from the area immediately surrounding the porous bulb section of each tensiometer and moisture content was determined by oven-drying for 24 hr at 105 C.

Field data analysis. A disease rating for each replicate was calculated by averaging individual plant ratings. Correlation analysis was used to compare disease ratings of field-grown radishes with the following environmental variables: average soil moisture in grams of water per 100 g oven-dried soil at each week during the season (M1–M6), cumulative rainfall in centimeters for each week (R1–R6), and cumulative daily degree days of soil temperature above 12.2 C for each week at 15 cm deep in 1980 and at 10 and 15 cm deep in 1981 (DD1–DD6). A degree day is defined as the daily mean temperature minus a base temperature, T_{base}. Regressing disease ratings in 1980 on mean soil temperature revealed that 12.2 C was an adequate estimate of T_{base}.

A multiple regression equation was developed to describe the relationship between disease ratings (Y) and environmental variables and their interactions. A stepwise procedure was then used to remove nonsignificant variables. Variables used in the stepwise regression analysis were the weekly values of soil moisture, degree days of soil temperature, and rainfall measurements most highly correlated with Y as well as the two- and three-way interactions of these variables.

The Minitab statistical package was used for all regression and correlation analysis (12). For each regression equation, the following were determined: coefficient of determination (R^2), coefficient of determination adjusted for degrees of freedom (R^2_a), standard deviation around the regression line (S), and F-statistic (F).

Controlled-temperature studies. Carlisle muck soil was autoclaved 6 hr, then mixed with commercial vermiculite (3:2, v/v) to alleviate soil compaction. The soil mix was placed in autoclaved wooden flats measuring 20 × 38 × 8 cm and seeded with cabbage (*Brassica oleracea* var. *capitata* L. 'Danish Ballhead'), Chinese cabbage (cultivar Michihli), mustard (*Brassica juncea* (L.) Coss. var. *crispifolia* Bailey 'Giant Southern Curled'), and radish (cultivar Scarlet Knight). Flats were planted with 10 seeds per box, with three boxes per host per soil temperature. Seeds were spaced about 5.5 cm apart in two rows 8 cm apart and inoculated as before with 0.5 ml of inoculum placed on each seed before covering. Flats were arranged randomly in a 1.6-m controlled environmental chamber (Model 13, Environmental Growth Chambers, Chagrin Falls, OH 44022) with a 12-hr photoperiod at 25.5 klux. Soil moisture

Table 1. Correlations of clubroot disease ratings with field-measured environmental variables

	Environmental variable[a]																	
Year	DD1	DD2	DD3	DD4	DD5	DD6	M1	M2	M3	M4	M5	M6	R1	R2	R3	R4	R5	R6
1980	0.581	0.568	0.600	0.663	0.632	0.667*[b]	0.253	0.374	0.436	0.432	0.422	0.457*	0.498	0.587*	0.560	0.422	0.378	0.314
1981																		
4 wk	0.251	0.253	0.367	0.443*	···	···	−0.009	−0.027	−0.032	−0.072*	···	···	0.227	0.326*	0.226	0.188	···	···
5 wk	0.071	0.117	0.252	0.342	0.420*	···	−0.089	−0.73	0.033	0.036	−0.197*	···	0.336	0.359	0.421*	0.312	0.198	···
6 wk	−0.044	−0.003	0.150	0.264	0.379	0.473*	−0.031	−0.120	−0.068	−0.018	−0.157	−0.250*	0.360	0.368	0.447*	0.409	0.307	0.305

[a] DD1–6 = Cumulative degree day units of soil temperature for each week of the growing period measured at 15-cm depth in 1980 and 10 cm in 1981. Degree day is defined as the daily mean temperature minus a base temperature equal to 12.2 C. M1–6 = Mean soil moisture content (MOIS) at the end of each week calculated from daily tensiometer values of centibars of soil suction (TENS) using the equation: $MOIS = 160e^{0.009(TENS)}$, where TENS <0. R1–6 = Cumulative rainfall for each week in centimeters.

[b] Asterisks denote highest correlation coefficient in each group.

Table 2. Comparison of radish clubroot severity observed in the field in 1980 with predicted disease severity using regression equation: $Y = 0.597 + 0.00205\ (DD6) + 0.000306\ (DD6 \times R2)$[a]

	Clubroot disease index		
Harvest date	Observed	Predicted	Deviation
19 June	0.26	0.17	+0.09
26 June	0.39	0.22	+0.17
3 July	0.11	0.58	−0.47
11 July	0.74	0.92	−0.18
17 July	0.21	0.46	−0.25
24 July	0.21	0.45	−0.24
30 July	0.22	0.41	−0.19
7 August	0.73	0.75	−0.02
15 August	0.47	0.69	−0.22
21 August	1.33	1.15	+0.18
28 August	1.14	1.69	−0.55
4 September	1.91	1.54	+0.37
12 September	1.48	1.44	+0.04
18 September	1.80	1.05	+0.75
25 September	0.86	0.67	+0.19
4 October	0.46	0.30	+0.16
11 October	0.16	0.08	+0.08
18 October	0.06	−0.04	+0.10

[a] DD6 = Degree days of soil temperature for entire 6-wk growth period; R2 = total rainfall (cm) for the first 2 wk.

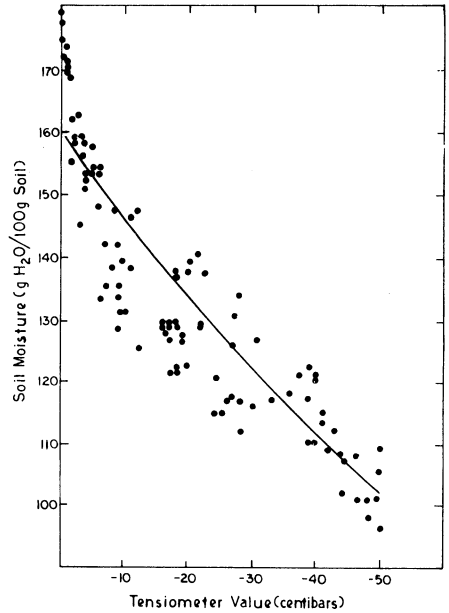

Fig. 1. Soil moisture content (MOIS) of Carlisle muck soil in relation to tensiometer centibar values (TENS). Standard curve generated according to:
$MOIS = 160e^{0.009\ (TENS)}$, TENS <0.

was kept at or near field capacity by daily watering. Plants were grown 6 wk at mean soil temperatures of 13.9, 14.4, 18.9, 22.8, 25.0, 26.7, and 27.8 C. Soil temperatures were monitored with recording thermographs and thermometers and did not fluctuate more than ±2 C. After 6 wk, plants were harvested and rated for clubroot severity as described previously. A mean rating was determined for each flat.

RESULTS

The standard curve for conversion of tensiometer centibar values to soil moisture content is presented in Figure 1. The relationship between soil moisture (MOIS) in grams per 100 g oven-dried soil and tensiometer values (TENS) in negative centibars was described by:

$$\ln(MOIS) = 5.07 + 0.009(TENS),\ TENS < 0$$

$$R^2 = 0.845\ R^2_a = 0.842\ S = 0.080\ F = 407.44,$$

where ln is the natural log transformation. This equation was rearranged to:

$$MOIS = 160e^{0.009\ (TENS)}\ TENS < 0.$$

At centibar values approaching zero, soil moisture rises sharply. Zero centibar values were recorded on soil samples with moisture levels ranging from 172 to 209 g water per 100 g oven-dried soil. It is obvious that centibar values >−2 are unreliable because it was not possible to get an acceptable fit of the data as values approached zero.

In 1980, rainfall and soil temperature were most correlated with disease severity (Table 1). Although degree days based on air temperature were significantly correlated with disease severity, those based on soil temperature were more highly correlated and thus were used in the regression analysis.

Stepwise regression analysis produced the predictive equation:

$$Y = -0.597 + 0.002051(DD6) + 0.000306(DD6 \times R2)$$

$$R^2 = 0.732\ R^2_a = 0.696\ S = 0.329\ F = 20.48,$$

where DD6 is degree day units of soil temperature for the entire 6-wk growth period and R2 is total rainfall in centimeters during the first 2 wk. This equation was significant at $P < 0.001$. Table 2 presents mean observed disease index values at harvest compared with values predicted from the regression equation.

Data collected in 1981 were analyzed in the same way at the end of 4, 5, and 6 wk of growth (Table 1). Although degree days for soil temperature at both 10- and 15-cm depths were significantly correlated with disease severity, those measured at 10 cm produced a higher correlation and thus were used in regression analysis.

Regression analysis on disease index data for 4-wk-old radishes did not produce a significant equation. Data from radishes harvested at 5 wk produced a significant equation to predict disease severity:

$$Y = -0.530 + 0.0631(DD5) + 0.1278(R3) - 0.00043(DD5 \times M5)$$

$$R^2 = 0.589\ R^2_a = 0.486\ S = 0.495\ F = 5.74,$$

where DD5 is degree days of soil temperature for the entire 5-wk growth period, R3 is total rainfall in centimeters for the first 3 wk, and M5 is the mean soil moisture content for the entire 5-wk growth period. This equation was significant at $P < 0.01$. Results of observed mean disease index values at harvest compared with values predicted

from this regression equation are presented in Table 3.

Regression analysis on harvest data at the end of 6-wk growth produced the regression equation:

$$Y = 0.963 + 0.0734(DD6) + 0.1324(R3) - 0.00052(DD6 \times M6)$$

$$R^2 = 0.605 \quad R^2_a = 0.498 \quad S = 0.535 \quad F = 5.62,$$

where DD6 is degree days of soil temperature for the entire 6-wk growth period, R3 is total rainfall in centimeters for the first 3 wk, and M6 is the mean soil moisture content for the entire 6-wk growth period. This equation was significant at $P<0.01$. Table 3 compares mean disease index values at harvest with the predicted values based on this regression equation.

Results of controlled-temperature studies of disease severity on cabbage, Chinese cabbage, mustard, and radish at seven soil temperatures are in Figure 2. At about 14 C, variable levels of disease severity were observed. With increasing temperatures, bell-shaped curves were observed for all crops with maximum severities at 21–22 C. At 27.8 C, the highest soil temperature studied, all four crops were under temperature stress and did not thrive. Disease also declined markedly.

DISCUSSION

There has been some question of the accuracy of tensiometers for soil moisture determination in organic soils. Accurate determinations are dependent on maintaining good contact between soil and the

Table 3. Comparison of radish clubroot severity observed in the field in 1981 with predicted severity using regression equation $Y = 0.530 + 0.0631$ (DD5) $+ 0.1278$ (R3) $- 0.00043$ (DD5 × M5) when harvested at the end of 5 wk[a] and $Y = 0.963 + 0.0734$ (DD6) $+ 0.1324$ (R3) $- 0.00052$ (DD6 × M6) when harvested at 6 wk[b]

Harvest date	Clubroot disease index					
	5 wk			6 wk		
	Observed	Predicted	Deviation	Observed	Predicted	Deviation
18 June	0.40	0.17	−0.11	0.77	1.01	−0.24
25 June	0.17	0.23	−0.06	0.83	0.54	+0.29
2 July	1.02	1.12	−0.10	1.28	1.38	−0.10
9 July	1.36	1.39	−0.03	1.74	1.44	+0.30
15 July	0.82	1.31	−0.49	1.32	1.95	−0.63
22 July	1.59	0.52	+1.07	1.53	1.33	+0.20
30 July	2.10	1.37	+0.73	2.47	1.41	+1.06
6 August	0.34	0.94	−0.60	0.76	1.13	−0.37
13 August	0.81	0.96	−0.05	1.97	1.53	+0.44
20 August	1.97	1.82	+0.15	2.33	2.07	+0.26
27 August	1.39	1.30	+0.09	1.38	1.92	−0.54
3 September	0.09	0.66	−0.57	0.07	0.81	−0.74
10 September	0.04	0.45	−0.41	0.03	0.24	−0.21
17 September	0.05	0.11	−0.06	0.35	0.36	−0.01
24 September	0.57	0.34	+0.23	0.80	0.52	+0.28
1 October	0.27	0.08	+0.19	0.31	...[c]	...
7 October	0.05	...	...	0.11	...	...
15 October	0.09	...	...	0.31	...	...

[a] DD5 = Degree days of soil temperature for the 5-wk growth period, R3 = total rainfall (cm) for the first 3 wk, and M5 = mean soil moisture content for the 5-wk growth period.
[b] DD6 = Degree days of soil temperature for the 6-wk growth period, R3 = total rainfall (cm) for the first 3 wk, and M6 = average soil moisture content for the 6-wk growth period.
[c] No values because of missing soil moisture data (tensiometers removed before freezing).

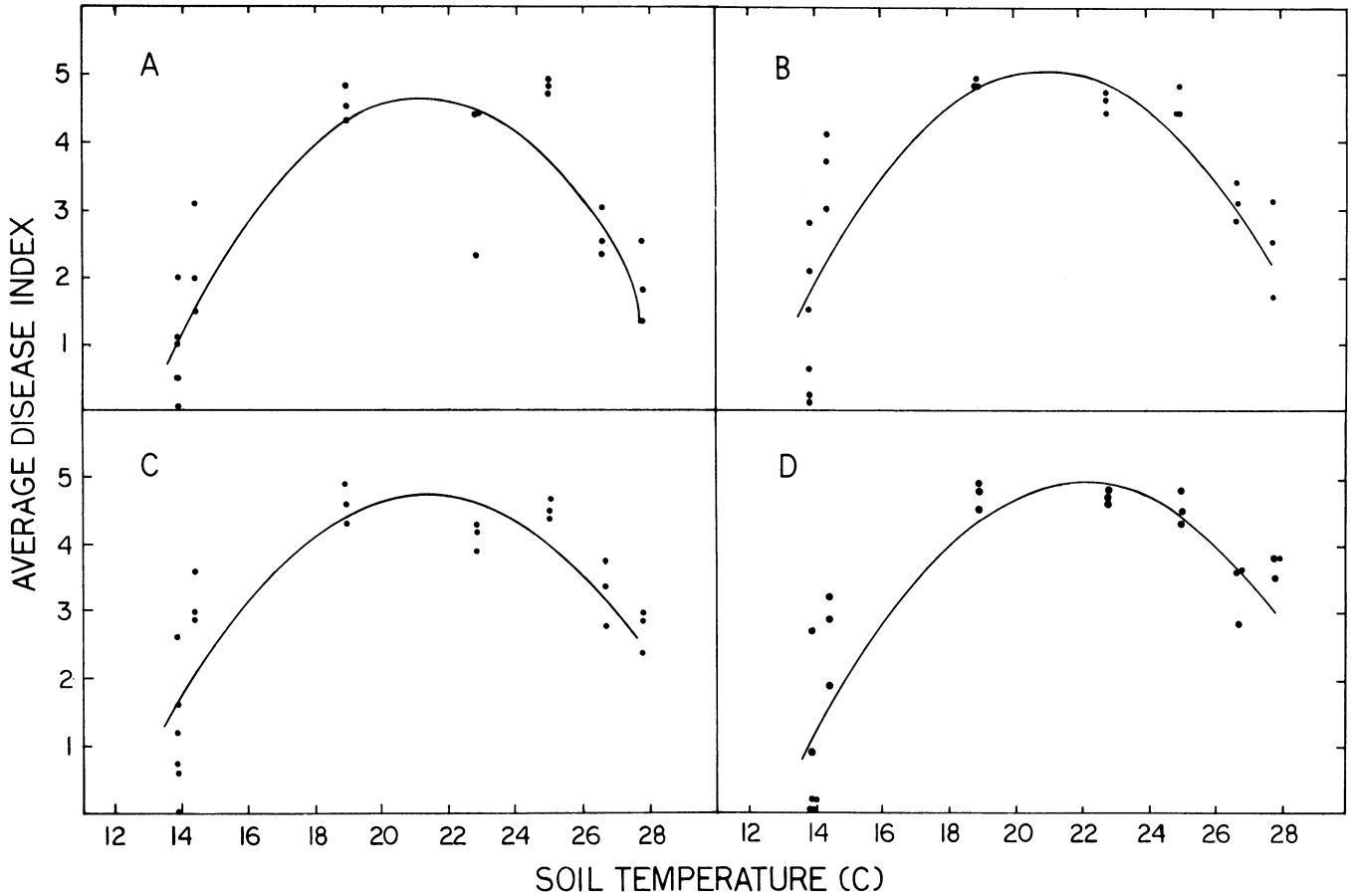

Fig. 2. The effect of constant soil temperature on symptom development of clubroot (*Plasmodiophora brassicae*) on **(A)** cabbage, **(B)** Chinese cabbage, **(C)** mustard, and **(D)** radish. Predicted curves (solid lines) were based on a regression equation of the form $Y = B_0 + B_1 T + B_2 T^2$, where Y is disease index, T is temperature (C), and the Bs are parameters estimated from the data on each crop.

porous ceramic bulb of the instrument. Widely fluctuating readings may occur if good contact is not maintained. Carlisle muck is a well-decomposed fine-textured organic soil. Maintenance of contact is, therefore, not difficult if the soil is well packed around the instrument when it is initially installed. Tensiometers are not suitable for soil moisture determinations on organic soils high in undecomposed plant debris or in artificial mixes prepared with vermiculite and bark chips, etc., because adequate contact with the bulb cannot be maintained. In our studies, we did not encounter difficulty in maintaining contact between soil and tensiometer bulbs nor did we observe widely divergent readings between replicate instruments. Because the nonlinear regression equation explained 85% of the moisture variability as a function of tensiometer values below zero centibars, we believe that tensiometers provided an acceptable and practical measure of soil moisture under field conditions.

A combination of several environmental factors was necessary to significantly predict clubroot disease severity during both seasons. Soil temperature based on degree days was important throughout the growth period. It was a significant variable in all final regression equations, except for the 4-wk harvest, when no variables were significant. Although a predictive equation significant at $P=0.05$ could not be developed after 4 wk of growth, total rainfall in the first 2 wk of growth and soil temperature throughout the growing period were the likely variables of importance because this equation was significant at $P<0.10$. In the other predictive equations, the variables of importance were soil temperature throughout the growth period, rainfall in the seedling stage (the first 2 wk in 1980 and the first 3 wk in 1981), as well as the interaction of soil temperature with soil moisture throughout the growth period in the 1981 equations. The multiple regression equations explained 59–73% of the variability in disease ratings. The equations for the 5- to 6-wk harvests were very similar. Disease ratings increased as a linear function of both soil temperature and rainfall and increased as a negative function of the interaction of soil temperature and moisture. Although moisture, as represented by rainfall, during the seedling stage was positively related to disease development, for unexplained reasons, moisture in combination with soil temperature was negatively related. This negative interaction was not observed in 1980.

The influence of high soil moisture in the seedling stage is most probably related to root hair infection. This has been noted previously in cabbage (2), where it was proposed that primary infection of root hairs by zoospores arising from germinating *P. brassicae* resting spores occurs within 2 wk after planting in the infested soil.

Our conclusion that soil temperature throughout the growth period of the host has a significant effect on disease severity also concurs with work on cabbage (2). In our study, controlled soil temperature experiments yielded bell-shaped disease severity curves with maxima at 21–22 C, closely following the host's temperature-growth responses. Similar relationships have been found with other crucifers (9). It seems that once infection has occurred, further disease development is related primarily to growth of the host plant rather than to direct environmental effects on the pathogen itself.

The predictive equations for 1981 and 1982 were developed only from the data collected during those years and represented the best fit of the data obtained through ordinary least squares regression analysis. Further data and independent model validation studies are needed to develop predictive equations with general applicability. Prediction of clubroot severity in the field from monitored environmental variables offers some possibilities for disease management, especially through irrigation scheduling. If periods of extensive rainfall occur soon after seeding of radish in heavily infested soils, growers should be aware of the potential consequences and harvest the crop as early as possible before advanced symptoms develop. Further studies may help refine predictive models and identify other environmental factors of significance in clubroot development.

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