A Sensor for Monitoring Moisture of Wheat Residues: Application in Ascospore Maturation of *Pyrenophora tritici-repentis*

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ABSTRACT

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An electrical impedance sensor was constructed for monitoring water content and water potential (ψ) of wheat straw residues. The sensor consisted of two stainless-steel sewing needles mounted parallel on a pair of insulated terminals and inserted into the straw. Excitation voltage (V_x = 500 mV) was applied to the sensor from a data logger, and residual voltage from the sensor (V_s) was measured. Electrical impedance of the sensor was recorded as V_s/V_x. Five of the sensors performed similarly when evaluated under alternating dry and humid conditions in a moisture apparatus and under field conditions. Readings for sensors made with straw collected in the field at 0, 2, and 8 mo after wheat was harvested decreased logarithmically with declining water content of the straw elements and increased exponentially as ψ of the elements increased. Readings for sensors made with 8-mo-old straw were lower at all water contents and at most ψ values (except near −1.2 to −2.5 MPa and −40 to −50 MPa) than were those for sensors made with 0- or 2-mo-old straw, which did not differ significantly. Sensors were used to monitor residue moisture in relation to ascospore maturation of *Pyrenophora tritici-repentis* in wheat stem residues kept in a controlled moisture apparatus or in the field. Significant linear relationships were found between numbers of mature asci in pseudothecia and accumulated hours when ψ of the residues ≥ -2.5 MPa.

Host residues on the soil or projecting above the soil are important inoculum sources of many pathogens that attack aboveground parts of plants. The pathogens utilize residues as substrates for growth, survival, and reproduction (1). Moisture in the residues is a key variable influencing these processes (3,4), although its effects on pathogens are understood chiefly from studies done under controlled or semicontrolled conditions. Field studies have been limited by lack of a practical method for monitoring residue moisture. Measurements of wetness on green leaves and of atmospheric humidity have not been good indicators of residue moisture status (12,13). An ability to monitor residue moisture would facilitate studies of the dynamics and management of important residue-borne pathogens, such as *Pyrenophora tritici-repentis* (Died.) Drechs., the cause of tan spot of wheat (*Triticum aestivum* L.).

Water exists in plant residues in a state of dynamic equilibrium with atmospheric moisture and with water in any contiguous soil or organic matter (2). Water flows into and out of residues, usually as vapor, from higher to lower water potential (6). Cuticles of dead tissues usually are partially disintegrated and do not form continuous barriers to water movement. Within the residues, adsorptive and cohesive (capillary) forces attract water to the tissue matrix, thus forming the matric component of the residue water potential ($\psi_{residue}$). An osmotic component of $\psi_{residue}$, resulting from ions and molecules in the water, is also likely to be important at certain stages of residue decomposition. Because pathogens respond to total water potential (4), methods for continuous measurement of $\psi_{residue}$ are needed for field studies of pathogens in residues (13).

James et al (6) described an electrical impedance probe for monitoring moisture in dead onion leaves. The sensor gave only a rough indication of water content of the dead leaves and often failed in the field when contact between electrodes and residue was lost. The present paper describes an impedance sensor, modified from that of James et al, suitable for continuous estimation of ψ of wheat stem residues. The sensor was used to study the dynamics of residue moisture in relation to the maturation of ascospores of *P. tritici-repentis* under fluctuating conditions in humidity chambers and in the field. Earlier workers reported relationships of $\psi_{residue}$ and pseudothecial production by the pathogen under controlled conditions (7,10, 11).

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MATERIALS AND METHODS

Sensor design and evaluation. The impedance sensor consisted of two stainless-steel sewing needles, each 3.8 cm long, mounted parallel and 1 cm apart on a pair of insulated electrical terminals (Radio Shack stock no. 274-679) (Fig. 1A). The needles, which functioned as electrodes, were inserted crosswise through pieces of wheat straw internodes. The straw was collected from the field 2 mo after the wheat was harvested. Voltage (500 mV) was applied to the sensor from a data logger (model 21X, Campbell Scientific Inc., Logan, UT) through a circuit in which two resistors (56 and 47 kiloohms) were incorporated to format the output signal (Fig. 2). Alternating current was used to avoid polarizing water in the straw elements. In field use, the sensor was in contact with the earth, directly or via crop residues; accordingly, two blocking capacitors (10 μF) also were included in the circuit to prevent further polarization of water. Impedance of electrical current by the sensor was expressed as the ratio of the measured voltage (V_s) recorded on the data logger and the excitation voltage (V_x), which was the voltage product of $R_2/(R_1 + R_2 + R_s)$ (Fig. 2). Eight circuits were assembled on one circuit board to allow concurrent use of up to eight sensors.

Impedance characteristics of five sensors were compared by subjecting them to alternating dry and humid periods when the relative humidity was 60–70% and near 100%, respectively. The tests were done in a Plexiglas moisture chamber positioned inside a growth cabinet. Moisture was provided to the chamber by an ultrasonic humidifier. Duration of humid and dry periods was controlled by an electrical timer that turned the humidifier on and off every 12 hr for 10 consecutive days. The chamber was kept dark and at 15 C during wet periods and illuminated and at 25 C during dry periods. Sensor readings (V_s/V_x) were recorded at 60-sec intervals and averaged hourly by the data logger. Mean readings for each wet and dry period, computed from the average hourly values for each of the five sensors, were compared using analysis of variance (ANOVA) with a standard *F* test.

A similar study was done in which each electrode was inserted into the second internode of upright wheat stubble in the field (Fig. 1B). The five sensors were positioned about 15 cm above the soil and about 2 m apart in a plot of continuous winter wheat at the Arkell Research Station, near Guelph. Sensor readings were measured at 60-sec intervals and averaged hourly by the data logger during a 30-day period. Mean daily values computed from hourly averages for each of the five sensors were compared using ANOVA as before.

Water content of straw in relation to impedance. Sensors inserted into straw collected from the field immediately after harvest and at 2 and 8 mo after harvest were used to examine water content of the straw elements in relation to impedance. There were five replicate sensors per treatment. Each sensor was tested by disconnecting it from circuit wiring and immersing the straw elements, mounted on the electrodes, in deionized water for 10 min to saturate the straw. The elements were then removed from the water, and droplets adhering to the straw were detached by manually shaking the sensor or by lightly touching the droplets with paper tissue. The sensor was immediately weighed, then reconnected to the circuit wiring, and a reading was obtained on the data logger. Readings and sensor weight subsequently were recorded at intervals of about 10 min while the straw elements air-dried and until the readings stabilized at or near zero. The final straw weight was regarded as the air-dry weight. The amount of water in the straw elements at each weighing time was calculated by subtracting the weight of the air-dried sensor from the observed weight and was expressed as a proportion of the weight of water in the elements when fully saturated. Relationships of this proportion and sensor reading were described using regression techniques (8).

Water potential of straw in relation to impedance. About 20 pieces of straw internodes, each about 10 cm long, were collected from the field at 0, 2, and 8 mo after harvest. The straw pieces were saturated with deionized water and adhering water droplets were removed as described above. The pieces were then placed on paper towels on the laboratory bench. Successive groups of four straw pieces were taken, at random, immediately after wetting and subsequently at intervals of 8–10 min. Each group was mounted on the electrodes of a sensor and the readings were recorded. The straw pieces were then removed from the electrodes, cut into segments about 1 cm long, and transferred into a specimen chamber (14 mm deep and 14 mm in diameter) of a thermocouple-psychrometer apparatus (Decagon Devices Inc., Pullman, WA). The straw was transferred rapidly to minimize loss or gain of moisture. The chamber was immediately sealed within the instrument, and moisture of the straw and air in the chamber was allowed to equilibrate during 20 min (7). Temperature and wet bulb depression of the chamber atmosphere were then measured by thermocouples in the instrument, which were connected to a digital recorder (model SC-10A, Decagon Devices). A psychrometric formula for the instrument was used to calculate ψ of the chamber atmosphere and thus of the straw. The psychrometer was calibrated against salt solutions of known ψ prior to the study. The relationship between water potential and electrical impedance of the straw was evaluated by regression techniques (8).

Atmospheric moisture apparatus. An apparatus was constructed to control atmospheric moisture at five different levels simultaneously. The apparatus consisted of a Plexiglas chamber (125 cm long, 55 cm wide, and 25 cm high) divided crosswise into five compartments of equal size. Moisture was supplied to the compartments from an ultrasonic humidifier positioned adjacent to one end of the apparatus. Moisture from the humidifier passed through a 5-cm-diameter PVC tube mounted in the upper portion of the chamber and into each compartment via 0.5-cm holes in the side of the tube. The distal end of the tube was sealed. Numbers of holes leading into successive compartments from the humidifier were five, four, three, two, and one. The number of holes and distance from the humidifier allowed a stepwise reduction in the amount of moisture entering successive compartments. A 3-mm gap between the lid and the walls of the apparatus permitted outflow of air and drying of the chamber air when the humidifier was turned off. Operation of the humidifier was controlled by an electrical timer to provide alternating humid and dry periods. The entire apparatus was kept in a growth chamber to provide control of temperature and light regimes and low ambient humidity.

Controlled residue moisture and ascospore maturation. The atmospheric moisture apparatus and impedance sensors were used to study periods of residue wetness and dryness in relation to ascospore maturation by *P. tritici-repentis*. Wheat stem residues naturally infested with the pathogen were collected in a plot of continuous, zero-tillage winter wheat at the Arkell Research Station on 8 March 1990, when mature ascospores were first observed. The residues were cut into 10-cm segments and placed as a single layer on vermiculite about 2 cm deep in the bottom of each compartment of the apparatus. The photoperiod was 12 hr, and light- and dark-period temperatures were 25 and 15 C, respectively. Moisture status of residues in each compartment was monitored by inserting the electrodes of an impedance sensor through four residue segments. Readings for each sensor were recorded at 60-sec intervals and averaged hourly by the data logger. The values were transformed to those of water potential (MPa) using the appropriate

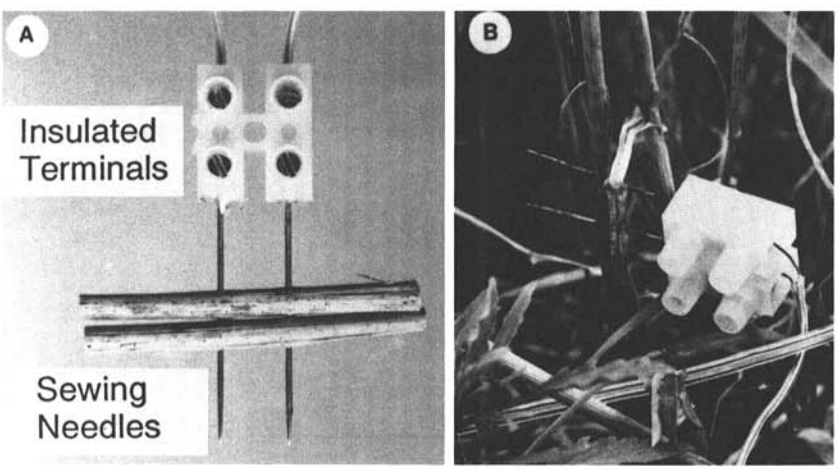

Fig. 1. Impedance sensor consisting of two stainless-steel sewing needles mounted parallel on a pair of insulated terminals and inserted into wheat stem residues in **(A)** the laboratory and **(B)** the field.

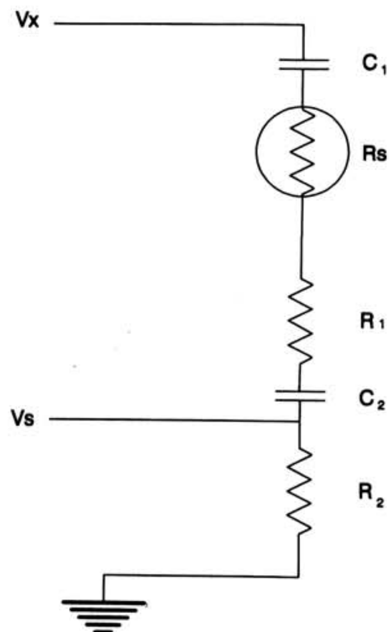

Fig. 2. Generalized diagram of circuit used for impedance sensor: V_x is excitation voltage (500 mV), V_s is measured voltage, R_s is sensor resistance (kiloohms), C_1 and C_2 are 10-μF capacitors, and R_1 and R_2 are 56- and 47-kiloohm resistors, respectively.

calibration curve for the type of residue. Duration of daily wetness periods ranged from 6 to 8 hr and was controlled by an electrical timer connected to the humidifier. During wet periods, a control signal from the data logger turned the humidifier on and off, by means of a switch relay, each time the reading of the sensor in the wettest compartment was equivalent to $\psi_{residue}$ of −2.5 and −0.5 MPa, respectively. Contents of four replicate samples of 10 pseudothecia from residue segments taken at random in each compartment were examined on a light microscope, and asci containing mature ascospores were counted. This procedure was repeated at 2-day intervals until all ascospores had matured. The study was repeated using light- and dark-period temperatures of 15 and 5 C, respectively. Relationships between cumulative hours of $\psi_{residues}$ greater or equal to −2.5 MPa and mean numbers of mature asci contained in 10 pseudothecia were evaluated by linear regressions (8). The threshold value of −2.5 MPa was based on earlier observations of water potential in relation to growth and sporulation of *P. tritici-repentis* (7,10).

Field residue moisture and ascospore maturation. Impedance sensors were used to study relationships between moisture and ascospore maturation of *P. tritici-repentis* in stubble in five 1 × 1 m field plots in an area of continuous zero-tillage wheat at the Arkell Research Station during 21 March to 4 June and 24 May to 27 June 1990. Stubble for the second study period was lifted from the plot on 21 March, stored at −20 C, and returned to the plot on 24 May. One sensor, with electrodes inserted through four pieces of standing stubble at about 10 cm above the soil, was used for each period in each plot. Readings for each sensor were measured at 60-sec intervals and transformed to values of water potential (MPa) using calibration curves for the particular residues. Air temperature at 15 cm above the soil was monitored with a thermistor (model 107, Campbell Scientific) connected to the 21X data logger. Four replicate samples of 10 pseudothecia were taken at 5-day intervals from the leaf sheath of the second internode of random pieces of standing stubble in each plot. The pseudothecia were examined for asci containing mature ascospores as before. The relationship between cumulative hours of $\psi_{residue}$ greater or equal to −2.5 MPa and the mean number of mature asci within 10 pseudothecia was evaluated by linear regressions (8).

RESULTS

Impedance of sensors. Readings for the five sensors did not differ significantly ($P = 0.812$) when compared during cycles of alternating dry and humid conditions in the moisture chamber. Readings were about 0.4 in 60–70% relative humidity and 19.0 in 100% relative humidity. The readings increased from the low to high levels during 10–15 min after the humidifiers were turned on at the beginning of the humid periods, and decreased from the high to low levels during 60–90 min at the end of the humid periods.

Sensor readings also were not significantly different ($P = 0.786$) in the field test; readings were low (about 0.4–0.6) during dry periods and high (about 19) during periods of dew or rain.

Impedance in relation to water content. Readings for sensors in straw collected at 0, 2, and 8 mo after harvest decreased logarithmically with declining water content of the straw elements (Fig. 3); thus, electrical impedance of the straw elements increased logarithmically with decreasing water content. The relationships for sensors made with straw collected at 0 and 2 mo after the wheat was harvested did not differ significantly. However, readings for the sensors made with straw left in the field for 8 mo after harvest were significantly ($P \leq 0.05$) lower at all water contents than observed for sensors made with 0- or 2-mo-old straw. Loss and absorption of water were faster in 8-mo-old straw, which was partially decomposed, than in 0- or 2-mo-old straw, which showed little sign of decomposition.

Impedance in relation to water potential. Sensor readings increased exponentially as ψ of the straw elements increased (Fig. 4); thus, electrical imped-

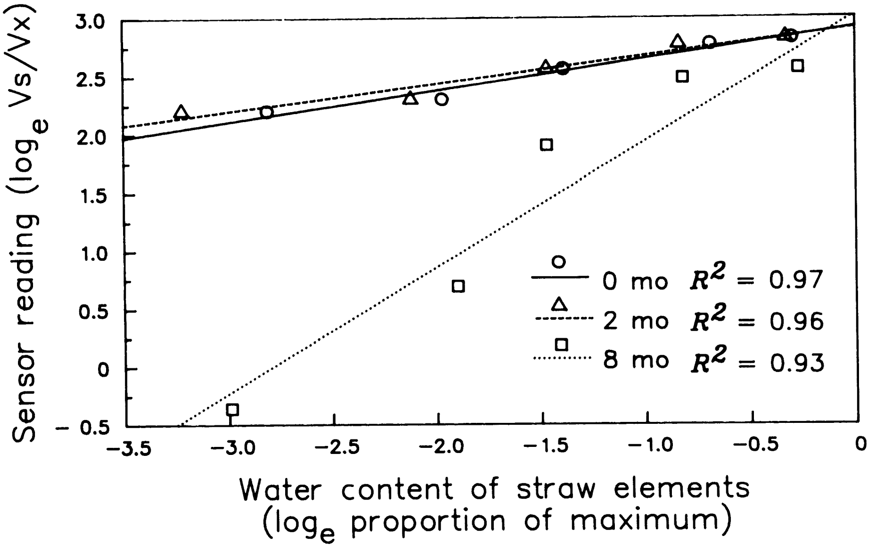

Fig. 3. Sensor readings (Vs/Vx) on the data logger (Y) in relation to water content of the straw elements of impedance sensors (X). Logarithmic regressions (log$_e$ XY) were computed for elements utilizing straw collected from a wheat plot at the following times after harvest: 0 mo ($Y = 2.91 + 0.27X$), 2 mo ($Y = 2.91 + 0.24X$), and 8 mo ($Y = 3.02 + 1.08X$).

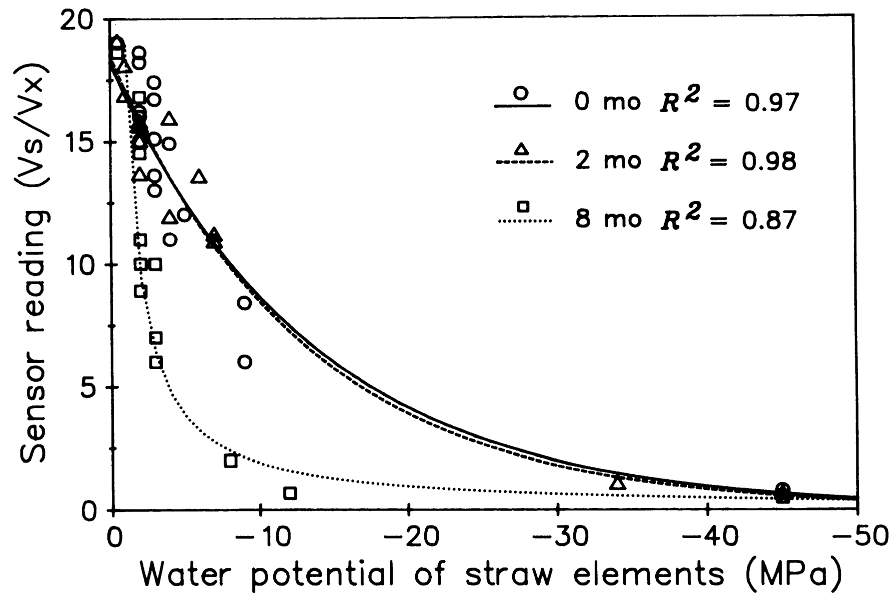

Fig. 4. Sensor readings (Vs/Vx) on the data logger (Y) in relation to water potential of the straw elements of impedance sensors (X). Exponential regressions were computed for elements utilizing straw collected from a wheat plot at the following times after harvest: 0 mo ($Y = 18.1\ e^{-0.074X}$), 2 mo ($Y = 18.4\ e^{-0.078X}$), and 8 mo ($Y = 19.2\ e^{-1.008X}$).

ance of the straw elements increased exponentially with decreasing ψ. The state of decomposition of wheat residues affected the relation of electrical impedance and ψ. The partially decomposed 8-mo-old residues absorbed and lost water faster than the 0- or 2-mo-old residues. At a given sensor reading, water potential values were significantly lower ($P \leq 0.05$) in straw collected 8 mo after harvest than in straw collected 0 or 2 mo after harvest. Differences in $\psi_{residue}$ due to straw weathering, however, were very small in the ψ ranges of −1.2 to −2.5 and −40 to −50 MPa.

Residue moisture in relation to ascospore maturation. Significant linear relationships were found in regression analyses of number of mature asci present in pseudothecia from wheat residues and the accumulated number of hours of $\psi_{residue} \geq -2.5$ MPa, both in the atmospheric moisture apparatus and in the field (Table 1). For each regression line obtained, the probability that the slope value was zero was very low ($P = 0.0001$). When tested for equality, the slopes for the regressions were found to be significantly different ($P \leq 0.05$) in all possible combinations. The average air temperatures in the field during the test periods of 21 March to 4 June and 24 May to 27 June were 7.1 and 17.4 C, respectively.

DISCUSSION

The impedance sensor was simple yet effective for monitoring water content and ψ of wheat straw residues. Readings for the sensors were highly correlated with water content of the straw elements, as determined gravimetrically, and with ψ measured with the thermocouple-psychrometer apparatus. The sensor and required circuitry were easily constructed from low-cost materials.

Effectiveness of the sensor for monitoring moisture in straw contrasted with the relative ineffectiveness of the impedance probe developed earlier for monitoring moisture in dead onion leaves (6). Weak correlations between probe output and water content of onion residue were attributed to varying electrolyte concentration in dead tissue, structural differences among tissues near the electrodes of the probe, and faulty contact between the probe and the dead leaf tissues. In the present study, electrolyte concentration of wheat stem residues after harvest was probably low and consequently may have had little effect on sensor readings. Structural ridigity of the electrodes and of the wheat straw residues was conducive to good electrical contact between the impedance sensor and the residues. Use of fixed resistors in the sensor avoided adjustment errors that may arise during calibration of variable resistors, such as were used for the onion residue probe (6). Use of resistors with kiloohm values different from those in the present study or use of high-resistance wire, however, would result in sensor readings that do not match those we report.

Chemical and structural changes in wheat stem residues during weathering and decomposition (5) alter the moisture characteristics of the residues (9,10) and likely accounted for the observed differences in response to residue water of sensors made with 0- or 2-mo-old residues compared with 8-mo-old residues. Because relationships between residue water content or ψ and sensor readings ultimately change over time, precautions are needed in using the sensors. Wheat residues of similar age and with a similar history of weathering and decomposition should be utilized for replicated sensors. Calibration curves of sensor readings in relation to water content and ψ should be recalculated periodically when sensors are used for long-term monitoring. When monitoring $\psi \geq -2.5$ MPa, recalibration may not be necessary if small imprecisions can be tolerated. The changes in the moisture characteristics of the decomposing wheat straw showed trends similar to those reported for decaying leaves of oak (*Quercus robur* L.) and beech (*Fagus sylvatica* L.) (3) and to observations of decaying stubble of two wheat cultivars (9).

The impedance sensor may underestimate water associated with wheat stem residues when the water is in the form of discrete droplets on the surface of the residues. The droplets tend to form during dew periods on relatively fresh straw, probably in response to hydrophobic layers of cuticle and epicuticular wax remaining in the residues. Loss of hydrophobic materials by weathering or decomposition may render older residues unfavorable for droplet formation. Water in the discrete droplets is not estimated by the sensor, which responds only when water forms continuous films or deposits in or on the residues, thus producing an electrical pathway between the electrodes.

The impedance sensor allowed $\psi_{residue}$ to be monitored in relation to ascospore maturation of *P. tritici-repentis* in the field as well as under controlled conditions. The studies indicated that accumulated hours of $\psi_{residue} \geq -2.5$ MPa was strongly related to ascospore maturation. This relationship may explain field observations in Ontario (J. M. C. Fernandes and T. D. W. James, *unpublished*) that ascospores matured earlier on prostrate stubble than on upright stubble; duration of residue wetness (≥ -2.5 MPa), monitored with the impedance sensor, averaged 35% longer when the stubble was prostrate. Persistent wetness of prostrate stubble may favor breakdown of cellulose, hemicellulose, and other structural components of the straw (5) and thereby increase the matric potential at any given water content of the straw (3,9). Findings in the present study enlarged on earlier observations of water potential and residue moisture in relation to production and survival of pseudothecia of the pathogen (7,10,11,15).

The variability among the slopes for the regression lines (Table 1) indicated that at least one other factor was important in limiting maturation of ascospores. The differences in temperature of the wet periods, which varied markedly among the experiments, likely were important in contributing to the observed differences in slopes (10). Wright and Sutton (14) used day-degree accumulation to forecast incidence of pseudothecia that had matured in the spring. The prediction, however, was different for periods that included long duration of high water potential in the residues and periods that included days when the residues were dry for most of the time.

The impedance sensor was designed to monitor wetness in wheat residues but could be adapted to measure moisture in residues of many other crops. The low cost of adding this sensor to others used for monitoring weather variables in crops makes it a useful tool in studying the epidemiology of residue-borne pathogens.

Table 1. Linear regression analyses of mean numbers of mature asci of *Pyrenophora tritici-repentis* per 10 pseudothecia (Y) in relation to accumulated number of hours (X) when $\psi_{residue}$ was ≥ -2.5 MPa, in a moisture apparatus and in the field

Experiment	df	Regression equation	Slope probability[a]	R^2
Moisture apparatus				
15 C/5 C[b]	9	$Y = 5.26 + 0.21X$	0.0001	0.92
25 C/15 C	10	$Y = 5.03 + 0.40X$	0.0016	0.73
Field				
21 March–4 June[c]	9	$Y = -8.80 + 0.28X$	0.0001	0.89
24 May–27 June	29	$Y = -12.00 + 0.49X$	0.0015	0.90

[a] $P < |T|$ for slope coefficient.

[b] Mean temperature of daily 12-hr light and dark periods.

[c] Period of study.

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LITERATURE CITED

1. Bruehl, G. 1987. Soilborne Plant Pathogens. Macmillan, New York. 368 pp.
2. Campbell, G. S. 1977. An Introduction to Environmental Biophysics. Springer-Verlag, New York. 159 pp.
3. Dix, N. J. 1985. Changes in the relationship between water content and water potential after decay and its significance for fungal successions. Trans. Br. Mycol. Soc. 85:649-653.
4. Duniway, J. M. 1982. Soil-plant-water relations and disease. Pages 307-325 in: Biometereology in Integrated Pest Management. J. L. Hatfield and I. J. Thomason, eds. Academic Press, New York.
5. Harper, S. H. T., and Lynch, J. M. 1985. Colonisation and decomposition of straw by fungi. Trans. Br. Mycol. Soc. 85:655-661.
6. James, T. D. W., Sutton, J. C., and Rowell, P. M. 1984. Monitoring wetness of dead onion leaves in relation to Botrytis leaf blight. Pages 627-632 in: Proc. Br. Crop. Prot. Conf. Vol. 2.
7. Pfender, W. F., Pacey, C. A., and Zhang, W. 1988. Saprophytic growth and pseudothecia production by *Pyrenophora tritici-repentis* in plant tissue held at controlled water potentials. Phytopathology 78:1205-1210.
8. SAS User's Guide. 1988. Release 6.03 ed. SAS Institute, Cary, NC. 1,028 pp.
9. Summerell, B. A., and Burgess, L. W. 1988. Moisture characteristics of decomposing stubble of two wheat cultivars. Soil Biol. Biochem. 20:963-964.
10. Summerell, B. A., and Burgess, L. W. 1988. Factors influencing production of pseudothecia by *Pyrenophora tritici-repentis*. Trans. Br. Mycol. Soc. 90:557-562.
11. Summerell, B. A., and Burgess, L. W. 1989. Factors influencing survival of *Pyrenophora tritici-repentis*: Water potential and temperature. Mycol. Res. 93:41-45.
12. Sutton, J. C. 1988. Predictive value of weather variables in the epidemiology and management of foliar diseases. Fitopatol. Bras. 13:305-312.
13. Sutton, J. C., Gillespie, T. J., and James, T. D. W. 1988. Electronic monitoring and use of microprocessors in the field. Pages 99-113 in: Experimental Techniques in Plant Disease Epidemiology. J. Kranz and J. Rotem, eds. Springer-Verlag, Heidelberg.
14. Wright, K. H., and Sutton, J. C. 1990. Inoculum of *Pyrenophora tritici-repentis* in relation to epidemics of tan spot of winter wheat in Ontario. Can. J. Plant Pathol. 12:149-157.
15. Zhang, W., Pfender, W. F., Adee, E., and Nus, A. 1989. Effect of selected conservation-tillage practices on wetness duration of wheat straw residue and ascocarp production by *Pyrenophora tritici-repentis*. (Abstr.) Phytopathology 79:1163.