# Use of Epidermal Cell Responses to Evaluate Resistance of Winter Wheat Cultivars to *Pseudocercosporella herpotrichoides*

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## ABSTRACT

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Epidermal cell responses (papilla formation, penetrations stopped within epidermal cells, and hypersensitivity) on the first-leaf sheath were used to determine the percent successful penetrations on susceptible and resistant winter wheat seedlings inoculated with conidia of *Pseudocercosporella herpotrichoides*. The greatest disease development occurred when inoculated plants were incubated in high relative humidity at 15 C and decreased at 10, 20, and 5 C, respectively. Differences among resistant and susceptible cultivars were significant at both 10 and 15 C, although the greatest differentiation between Cappelle-Desprez (resistant) and VPM-1 (highly resistant) occurred at 10 C. The most reliable method of differentiating cultivars for resistance to *P. herpotrichoides* included incubation of inoculated plants at 10 C and a disease rating based on fungal penetration attempts stopped by papillae or within epidermal cells in both hypersensitive and nonhypersensitive cells. The coleoptile was associated with infection of the first-leaf sheath and the formation of elliptical lesions occurred 0.9–2.3 cm above where the leaf sheath attached to the crown. Lesions were more distinctive on resistant than susceptible cultivars, and more resistant responses occurred near the edges than at the centers of the lesions. No significant differences in pathogenicity were found among four isolates of *P. herpotrichoides* tested.

Strawbreaker foot rot (eyespot), caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton, is the most widespread, chronic disease of winter wheat (*Triticum aestivum* L.) in the Pacific Northwest region of the United States (26). Currently, this disease is controlled with a single foliar application of a benzimidazole fungicide in the spring. Resistant cultivars would be more desirable since they are more economical and reliable, and because the fungus has become resistant to benzimidazole fungicides in Europe (2,5). Currently, none of the widely grown commercial winter wheat cultivars available in the Pacific Northwest have adequate levels of resistance to *P. herpotrichoides*. Wheat breeders have been working to incorporate foot rot resistance into adapted cultivars, but the selection of resistant cultivars is a slow process because plants must be grown to maturity in the field over several years (25).

Seedling tests for resistance to *P. herpotrichoides* offer the advantages of shorter screening time and a more uniform environment than screening in the field. However, the present seedling tests (2,23,31) need to be improved to be able to differentiate highly resistant individuals from resistant individuals. This inability to clearly differentiate progeny containing different levels of resistance leads to low heritabilities when these seedling tests are used to analyze segregating generations.

In wheat seedlings, the coleoptile and senescent tissues (coleoptile and leaf sheaths) promote infection by *P. herpotrichoides* (4,17,25,27,29), while epidermal cell responses including papilla and halo formation (10,12,24,25), cell wall thickening (14,35,36), and hypersensitive responses (3,4,16,25) in the leaf sheaths serve as barriers to fungal penetration and colonization (1). These resistance responses occur in both resistant and susceptible cultivars but are more abundant and well-developed in resistant cultivars (3,4,10). These responses also appear to establish resistance or susceptibility early in pathogenesis, because subsequent growth through leaf sheaths is reported to be independent of cultivar (31).

Epidermal cell responses to attempted penetration by *P. herpotrichoides* may provide a practical means of assessing the resistance of wheat, since seedling resistance is correlated with adult plant resistance (23,25). Murray and Ye (25) found that the percentage of penetration sites with papillae, penetrated papillae, and total successful penetrations in winter wheat seedlings were positively correlated with field resistance of mature plants to *P. herpotrichoides*. However, disease development must be increased and environmental variation must be reduced before this seedling test can be used to screen individual plants. These improvements may be achieved by finding the most reliable combination of epidermal responses (papilla formation, penetrations stopped within epidermal cells, and hypersensitive response) for establishing resistance and assessing the effect of temperature on these responses.

Temperature, humidity, coleoptile placement, and isolates of the pathogen influence disease development (4,13,21,31,32). Several studies have documented the ranges and optimum temperatures for infection based on symptom development or the number of leaf sheaths penetrated. Bateman and Taylor (3) found that *P. herpotrichoides* penetrated the coleoptile more rapidly at 17 C than 12 C. Higgins and Fitt (18) reported leaf sheaths were penetrated more rapidly at 10–15 C than at 5–10 C. Defosse (11) found the optimum temperature for infection of the first-leaf sheath was at or below 15 C. Dickens (13) reported that 4 wk after inoculation there were more infected tillers at 8–13 C than at 17–18 C; he failed to find infection at 25–26 C. Oort (27) tested temperatures from 5–25 C and found the optimum temperature for growth of the pathogen in the plant was 5–9 C. Schrödter and Fehrmann (30) reported that infection occurred from 4 to 13 C with an optimum about 8–9 C. Scott (31) found that the number of leaf sheaths penetrated by *P. herpotrichoides* increased with temperature over the range of 6–18 C. Bateman and Taylor (3,4) have shown that initial infection of the first leaf sheath depended on infection of the coleoptile and the rate of spread of hyphae through the coleoptile tissue. They found that plants with coleoptiles had more infections than plants with the coleoptiles removed (4). The extent of infection may also be influenced by the isolates of *P. herpotrichoides* included in the inoculum. Previous work has indicated that significant interactions between cultivars and isolates can occur, but the interactions were not consistent between experiments (32). Thus, there was no evidence of physiological specialization (32).

The purpose of this study was to improve the seedling test (25) used to assay for resistance to *P. herpotrichoides* by determining the effect of temperature on epidermal cell responses to attempted penetration, the combination of epidermal cell responses to use in establishing a disease rating that gives the greatest differentiation among highly resistant and resistant cultivars, and the influence of coleoptile position on disease development. Secondary objectives were to evaluate the variability in pathogenicity among isolates used in these experiments and investigate the level of resistance in common local commercial cultivars and breeding lines using this seedling test. A preliminary report has been published (37).

## MATERIALS AND METHODS

**Cultural conditions.** The cultural practices used in all experiments were described previously (25) with a few modifications described below. Wheat seeds pregerminated on moistened paper towels were planted (16/pot) 1 cm deep in a potting mix (55% peat, 35% pumice, and 10% sand; w/w/w) in 15-cm-diameter plastic pots. The pots were placed in the greenhouse (approximately 20 C) until the seedlings had reached the one- to two-leaf stage and then 24 hr before inoculation, the pots were moved to a walk-in growth chamber (2.7 × 2.7 m cooled by 0.76 metric horsepower refrigeration compressor). Unless stated otherwise, the experiments were conducted at 10 C (13 C with light) with diurnal lighting (10 hr of light) of approximately 150 $\mu E/cm^2$ supplied by fluorescent light tubes (Sylvania GRO-VHO-WS and Westinghouse CW-VHO-EW [1:2 mixture]) (25). The temperature and relative humidity in the plastic tent were recorded with a hygrothermograph; relative humidity fluctuated between 85 and 100% in all experiments. All experiments were designed as randomized complete blocks with subsampling and repeated once.

**Inoculation.** Inoculum consisted of a mixture of conidia from four isolates of *P. herpotrichoides* (PH 85-5-3, PH 85-7-8, PH 85-9-13, and PH 85-14-5). These wheat-type (w-type) (15,20), carbendazim-sensitive isolates were obtained from mature wheat straw with symptoms of foot rot and maintained as mycelial cultures on Difco potato-dextrose agar (25). Conidial inoculum was produced by growing the fungus on autoclaved oat kernels and then incubating the kernels outdoors on fiberglass screen through the autumn and winter (6,8). Conidial suspensions were prepared by washing the kernels, adjusting the concentration to $1 \times 10^6$ conidia per milliliter with a hemacytometer, and then mixing equal volumes from each isolate. Seedlings at the two-leaf stage (second leaf >1 cm) were sprayed with the suspension of conidia at the rate of 5 ml per pot and placed in plastic tents for the duration of the experiment.

**Preparation of leaf sheaths for evaluation.** Four weeks after inoculation, when plants were at the four- to six-leaf stage, the first-leaf sheaths were removed and fixed in glacial acetic acid and 50% ethanol (1:17, v/v) for at least 24 hr. The sheaths were then placed in tubes containing 0.01% (w/v) trypan blue in lactophenol, heated in a water bath at 85 C for 10 min to stain and clear the specimens (3), mounted in lactophenol on glass slides, rubbed gently to remove mycelial mats, and observed with a light microscope (25).

**Assessment of cell responses.** To ensure adequate infection and reduce variability, sheaths were considered ratable only if 20 or more infection sites (the area under a single mycelial mat with multiple penetration attempts) were present. Usually 50 infection sites were evaluated on each ratable sheath.

**Estimation of disease rating scores.** Disease rating scores (percent successful penetrations) were calculated from the number of unsuccessful penetrations determined by using five different disease rating systems based on epidermal cell responses. The epidermal cell responses used in the rating systems included attempted penetrations stopped by papillae or within epidermal cells in both hypersensitive (cells with brown, dense, granular cytoplasm) and nonhypersensitive cells. These responses were combined to establish the following five rating systems based on attempted penetrations stopped by: papillae or within epidermal cells in both hypersensitive and nonhypersensitive cells; papillae in both hypersensitive and nonhypersensitive cells; papillae or within epidermal cells in nonhypersensitive cells only; papillae in nonhypersensitive cells only; or, papillae or within epidermal cells in hypersensitive cells only (hypersensitive rating). Other possible combinations of epidermal cell responses were not used due to their low frequency of occurrence.

**Influence of isolates of *P. herpotrichoides* on disease development.** Daws (CI 17419), a susceptible winter wheat cultivar, was used to test the four isolates of *P. herpotrichoides* described above for differences in pathogenicity. The experiment was conducted at 15 C (18 C in light) and included four replications with five subsamples per replication.

**Comparison of lesion size and location on resistant and susceptible cultivars.** Lesion size was considered as a possible rating system to evaluate resistance. The lesion size and location were evaluated on three cultivars (Daws, Cappelle, and VPM-1) by using a dissecting microscope to measure the range and position of infection sites on the first-leaf sheath. The experiment included five replications with eight subsamples per replication. In the second experiment immediately after inoculation, the pots were individually covered with a plastic bag for the first 24 hr of incubation, as well as being placed in a plastic tent.

**Effect of temperature on disease development and ratings based on epidermal cell responses.** The effect of temperature on epidermal cell responses was studied by using the following cultivars or lines of wheat: Selection 101 (Sel 101; CI 13438) (susceptible), Daws, Cappelle-Desprez (Cappelle; PI 262223) (resistant), and VPM-1124-R25-1 (VPM-1; highly resistant). The experiment was conducted at 5, 10, 15, and 20 C (temperatures increased 3 C in light), and included eight replications with 10 subsamples per replication. After inoculation, the pots were individually covered with a plastic bag for the first 24 hr of incubation, as well as being placed in a plastic tent.

**Comparison of wheat cultivars.** Thirteen wheat lines or cultivars were evaluated for resistance to *P. herpotrichoides*: Lewjain (CI 17909; susceptible), Dusty (PI 486429; susceptible), Stephens (CI 17596; susceptible), Peck (CI 17298; susceptible), John (PI 494095; susceptible), Hill 81 (CI 17954; susceptible), GWB 80-112 (susceptible), Yamhill (CI 14563; susceptible), Daws, VPM-1, Cappelle, Cerco (CI 15922; resistant), and Roazon (PI 422330; resistant). The experiment was conducted at 13 C (16 C in light) and included four replications with 16 subsamples per replication. The spore suspension used to inoculate the seedlings contained the same four isolates described previously along with six additional isolates: PH 84-4-1, PH 84-10-1, PH 84-11-10, PH 85-3-1, PH 85-6-1, and PH 85-8-2. The sheaths were considered ratable if 10 or more infection sites (the area under a single mycelial mat with multiple penetration attempts) were present, while 20 or more sites were required in the other experiments. These parameters were established with disease ratings obtained from Daws, Cappelle-Desprez, and VPM-1.

**Coleoptile placement in relation to infection on the first-leaf sheath.** Daws was used to investigate the effect of coleoptile placement on infection by *P. herpotrichoides*. Coleoptile placement was determined when leaf sheaths were harvested, by establishing the position of the coleoptile in relation to the first-leaf sheath. If the coleoptile was touching the first-leaf sheath, the coleoptiles position was marked with india ink on the sheath. Then the sheaths were examined microscopically to determine where infection occurred on the first leaf sheath in relation to the position of the coleoptile. The experiment was conducted at 15 C (18 C in light).

## RESULTS

**Influence of different isolates of *P. herpotrichoides* on disease development.** Significant differences between the two experiments were not found; therefore, the data for the two experiments were combined for analysis. No significant differences in pathogenicity were found among the four isolates by using any of the five rating systems.

**Comparison of lesion size and location on resistant and susceptible cultivars.** The first-leaf sheaths of all cultivars were infected by *P. herpotrichoides* and lesions developed 0.9–2.3 cm from the point of sheath attachment to the crown (Table 1). Infection occurred in this localized area on all three cultivars even though Daws is more susceptible and has shorter leaf sheaths than Cappelle and VPM-1. Lesion length across all varieties averaged 0.4–1.2 cm, with little variation in size between experiments. Lesions on Daws were significantly larger than those on Cappelle or VPM-1. In the first experiment, lesions on VPM-1 were significantly smaller than those on Cappelle; however, the opposite was true in the second experiment. Lesions on the first-leaf sheath had more resistant reactions (attempted penetrations stopped by papillae, thickened cell walls, or hypersensitive responses) present at the periphery of the lesion, while susceptible reactions (direct penetration and penetrated papillae) were more common in the center of the lesion. The first experiment had less disease development than the second experiment, since the pots in the first experiment were not bagged for the first 24 hr of incubation.

**Effect of temperature on disease development and ratings based on epidermal cell responses.** Data for the two experiments were combined for analysis since significant differences between them were not found. The percentage of ratable sheaths at 5, 10, 15, and 20 C was 3, 67, 83, and 25%, respectively. Fungal growth on the surface and within the sheaths at 15 C was so prolific that the sheaths were difficult to rate since they tended to fall apart. Conversely, at 5 C most of the sheaths were not ratable because little or no growth of the pathogen occurred. At 10, 15, and 20 C, the susceptible (Sel 101 and Daws) and resistant (Cappelle and VPM-1) cultivars were significantly different under all five rating systems (Table 2). However, 10 C was the only temperature where Cappelle (resistant) could be differentiated from VPM-1 (highly resistant). The rating system based on penetrations stopped by papillae or within epidermal cells in both hypersensitive and nonhypersensitive cells at 10 C resulted in the greatest differences among highly resistant, resistant, and susceptible cultivars.

**Comparison of cultivars.** Roazon, VPM-1, Cerco, and Cappelle were the most resistant cultivars based on the average ratings (Table 3). In experiment 1, the disease ratings for Roazon, VPM-1, Cerco, and Cappelle indicated they were significantly more resistant than the other cultivars. In the second experiment, Roazon, VPM-1, Cerco, and Cappelle were not significantly different from some of the susceptible cultivars due to light disease pressure. However, there were no successful penetrations of the resistant cultivars, while there were successful penetrations on all of the susceptible cultivars. In Table 3, only one disease rating (PAP + CELL in both NHSR and HSR cells) is presented, although the other four rating systems gave similar results.

**Coleoptile placement in relation to infection on the first-leaf sheath.** Although coleoptile placement varied, most of the coleoptiles lay opposite the first-leaf sheath, and wrapped around the culm touching the first-leaf sheath on both sides (Table 4). Likewise, the greatest overall percentage of ratable sheaths occurred when the coleoptile was opposite the first-leaf sheath and touching on both sides. When the first-leaf sheath was ratable and the coleoptile touched both edges of the sheath, 96% of those sheaths were ratable on both sides where the sheath was contacted by the coleoptile. However, when the coleoptile touched only one side of the first-leaf sheath, the majority of the sheaths were ratable only on the side where the coleoptile touched the sheath. When the coleoptile fell away from the first-leaf sheath, none of the sheaths were ratable.

## DISCUSSION

Epidermal cell reactions in response to attempted penetration of wheat by *P. herpotrichoides* can provide the basis for a seedling test for resistance in which leaf sheaths of individual seedlings are evaluated. The greatest differentiation among highly resistant, resistant, and susceptible cultivars was obtained at 10 C using ratings based on fungal penetrations stopped by papillae or within epidermal cells in both hypersensitive and nonhypersensitive cells (PAP + CELL in HSR and NHSR cells) (Table 2). This rating system not only differentiated between susceptible and resistant cultivars, but was able to differentiate Cappelle (resistant) and VPM-1 (highly resistant). At 10, 15, and 20 C, resistant and susceptible plants could be reliably separated by using all five rating systems (Table 2). The rating based solely on the hypersensitive response (PAP + CELL in HSR cells) performed better than expected, since hypersensitivity was reportedly not related to cultivar resistance (3). However, Murray and Ye (25) found that the hypersensitive reaction differentiated the highly resistant from the susceptible cultivars, but could not differentiate the resistant from the moderately resistant or susceptible cultivars. Another rating system based on lesion size was considered, but

TABLE 1. Location of lesions on the first-leaf sheath of winter wheat 4 wk after inoculation with *Pseudocercosporella herpotrichoides*

| Cultivar | Sheath length (cm)[a] | Lesion midpoint (cm)[b] | Lesion length (cm) | Disease rating[c] |
|---|---|---|---|---|
| Experiment 1 | | | | |
| VPM-1 | 5.2 | 2.0 | 0.4 | 0 |
| Cappelle | 4.8 | 1.6 | 0.6 | 0 |
| Daws | 3.7 | 1.7 | 0.8 | 42 |
| | | LSD[d] | 0.1 | 14 |
| Experiment 2 | | | | |
| VPM-1 | 5.6 | 1.6 | 0.8 | 10 |
| Cappelle | 5.1 | 1.2 | 0.7 | 36 |
| Daws | 4.2 | 1.7 | 1.2 | 89 |
| | | LSD | 0.1 | 8 |

[a] Figures represent the mean of 20 plants in experiment 1 and 40 plants in experiment 2.
[b] Measured from the point of sheath attachment to the crown to the base and the top of the lesion and then averaged to get the midpoint.
[c] Disease ratings are the percentage of successful penetrations based on fungal penetration attempts stopped by papillae or within epidermal cells in both hypersensitive and nonhypersensitive cells.
[d] LSD = Fisher's least significant difference ($P = 0.05$).

TABLE 2. Percentage of successful penetrations in the first-leaf sheath of resistant and susceptible wheat cultivars 4 wk after inoculation with *Pseudocercosporella herpotrichoides* at three of four temperatures[x]

| | | Rating system[y] | | | | |
|---|---|---|---|---|---|---|
| | | HSR + NHSR | | NHSR | | HSR |
| Temperature | Cultivar | PAP + CELL | PAP | PAP + CELL | PAP | PAP + CELL |
| 10 C[x] | VPM-1 | 10 a[z] | 53 a | 30 a | 58 a | 80 a |
| | Cappelle | 28 b | 61 b | 40 b | 66 b | 88 b |
| | Daws | 86 c | 92 c | 87 c | 92 c | 98 c |
| | Sel 101 | 86 c | 93 c | 88 c | 93 c | 98 c |
| 15 C | VPM-1 | 18 a | 50 a | 32 a | 52 a | 86 a |
| | Cappelle | 20 a | 52 a | 35 a | 55 a | 85 a |
| | Daws | 86 b | 90 b | 88 b | 91 b | 98 b |
| | Sel 101 | 86 b | 90 b | 89 b | 91 b | 98 b |
| 20 C | VPM-1 | 31 a | 48 a | 44 a | 53 a | 87 a |
| | Cappelle | 18 a | 42 a | 32 a | 46 a | 87 a |
| | Daws | 87 b | 90 b | 89 b | 90 b | 98 b |
| | Sel 101 | 82 b | 88 b | 84 b | 89 b | 98 b |

[x] At 5 C only 3% of the plants were ratable; therefore, data from 5 C were not included in the table.
[y] Disease ratings to determine the percentage of successful penetrations based on various combinations of epidermal responses (HSR = hypersensitive cell, NHSR = nonhypersensitive cell, PAP = penetration stopped by papilla, and CELL = penetration stopped within epidermal cells).
[z] Fisher's least significant difference with $P = 0.05$; means within a column within a temperature followed by the same letter are not significantly different.

was found to be too variable for reliable estimates of resistance with individual plants (Table 1).

The optimum temperature for infection and disease development with this seedling test was near 15 C and decreased at 10, 20, and 5 C, respectively (Table 2). These data are consistent with previous work (11,18,34). Although 15 C was the best temperature for disease development, the sheaths were difficult to rate since they tended to fall apart. By shortening the incubation period at 15 C, it may be possible to maintain a high percentage of ratable sheaths and alleviate problems such as sheaths falling apart and not being able to differentiate between resistant and highly resistant cultivars.

Placing plastic bags over individual pots for the first 24 hr after inoculation resulted in greater disease development, presumably because relative humidity in individual pots was greater than in pots placed inside the plastic tent only. In both experiments comparing cultivars (Table 3) and the first experiment on lesion size (Table 1), pots were not individually bagged and the percentage of successful penetrations on Daws (susceptible) was only 36, 16, and 42%, respectively. However, in the second experiment on lesion size and in the temperature experiments (Table 2), pots were individually bagged and the percentage of successful penetrations on Daws was greater than 85% with all rating systems at 10 C. In experiments where individual pots were not bagged, especially larger experiments where relative humidity in the tent dropped during the inoculation of multiple pots, disease pressure was light and differences between resistant and susceptible plants were small and variability increased. By bagging individual pots immediately after inoculation, relative humidity in pots remained high even though it dropped inside the tent.

TABLE 3. Ratings of 13 winter wheat cultivars for resistance to *Pseudocercosporella herpotrichoides* based on epidermal responses to attempted penetration

| Cultivar[a] | Disease rating[b] | | |
|---|---|---|---|
| | Experiment 1 | Experiment 2 | Average |
| Roazon | 3 | 0 | 1.5 |
| VPM-1* | 13 | 0 | 6.5 |
| Cerco* | 5 | 0 | 2.5 |
| Cappelle | 9 | 0 | 4.5 |
| GWB 80-112* | 58 | 5 | 31.5 |
| Daws | 36 | 16 | 26.0 |
| Yamhill | 46 | 20 | 33.0 |
| Peck | 71 | 8 | 39.5 |
| Hill 81 | 61 | 16 | 38.5 |
| John | 62 | 69 | 65.5 |
| Dusty | 79 | 54 | 66.5 |
| Stephens | 75 | 36 | 55.5 |
| Lewjain | 97 | 22 | 59.5 |
| LSD[c] | 10 | 16 | |

[a] All cultivars are susceptible, except Cappelle, Cerco, and Roazon which are resistant, and VPM-1, which is highly resistant; * = breeding lines.
[b] Disease ratings are the percentage of successful penetrations based on fungal penetration attempts stopped by papillae or within epidermal cells in both hypersensitive and nonhypersensitive cells; 16 plants per cultivar were rated.
[c] LSD = Fisher's least significant difference ($P = 0.05$) for comparing means within a column.

TABLE 4. Coleoptile placement in relationship to the first-leaf sheath of Daws winter wheat and the importance to infection by *Pseudocercosporella herpotrichoides*

| Coleoptile placement[a] | Percent of total | No. of sheaths[b] | |
|---|---|---|---|
| | | Ratable | Non-ratable |
| Experiment 1 | | | |
| On top | 6 | 8 | 8 |
| Opposite, both edges | 74 | 147 | 37 |
| Opposite, one edge | 19 | 38 | 8 |
| Falls away | 1 | 0 | 2 |
| Experiment 2 | | | |
| On top | 1 | 1 | 1 |
| Opposite, both edges | 83 | 125 | 17 |
| Opposite, one edge | 10 | 14 | 4 |
| Falls away | 6 | 0 | 10 |

[a] The coleoptile position in relation to the first-leaf sheath just before harvest was marked with india ink. On top = coleoptile lies on top of the leaf sheath. Opposite, both edges = coleoptile lies opposite the sheath, with both edges overlapping and touching the first-leaf sheath. Opposite, one edge = same as with both edges, except only one edge overlaps and touches the sheath. Falls away = coleoptile falls away from the stem and does not touch the sheath.
[b] Ratable = 20 or more infection sites with successful or attempted penetrations per leaf sheath 4 wk after inoculation with *P. herpotrichoides*.

With this seedling test, and in the field, most lesions form at or near the soil line, which is likely due in part to the coleoptile and senescent tissue (coleoptile and leaf sheaths) being associated with infection at the seedling stage (4,17,22,25,27,28). The experiments on coleoptile placement (Table 4) showed that in this system, infection of the first-leaf sheath occurs primarily in areas where the leaf sheath is in contact with the coleoptile. Bateman and Taylor (4) also reported that the coleoptile was very important to establishing infection in seedlings since they were able to greatly reduce infection by removing the coleoptile. At least up to the four- to five-leaf stage, the coleoptile would appear to play an important role in infection. Infection in older plants may occur elsewhere, especially if senescent tissue is present (25). However, the association of the coleoptile with infection of seedlings makes rating penetrations and attempted penetrations by *P. herpotrichoides* much easier, since microscopic observations can be limited to the area on the first leaf sheath contacted by the coleoptile.

The coleoptiles and the first-leaf sheaths of the susceptible cultivars (Sel 101 and Daws) were found to senesce earlier than those of the more resistant Cappelle and VPM-1, supporting the hypothesis that *P. herpotrichoides* causes a disease of senescent tissue. Murray and Ye (25) reported that early senescence of coleoptiles and increasing host susceptibility resulted in the most rapid penetration, whereas delayed senescence and increased host resistance resulted in slower penetration. According to Higgins (17), the high susceptibility of the coleoptile does not appear to be due to the lack of chlorophyll. The initial restriction of colonization to the coleoptile is probably due to its rapid senescence by comparison with the other plant tissues (17). In the field, if the percentage of the yield limiting lesions due to infections other than those associated with the coleoptile were known, the importance of coleoptile-related infections could be determined. Reportedly, early infections, representing primary inoculum, contribute the most to yield loss, while late infections, representing the effect of secondary inoculum, contribute to inoculum for succeeding crops (7).

Isolates of *P. herpotrichoides* have been shown to vary in pathogenicity (2,5,10,15,19,20,21,23) on a specific host as well as different host genera (33). However, there is no evidence of physiological specialization. Scott and Hollins (32) found a significant interaction between cultivars and isolates in each experiment, but the pattern of interaction was found to differ from one experiment to another. They concluded that there was no consistent pattern of physiological specialization. Bruehl et al (9) found no difference in yield between plots inoculated with different isolates. With this seedling test, no significant differences were found among the four isolates inoculated on Daws, a susceptible winter wheat.

With this seedling test, epidermal cell responses in individual first-leaf sheaths of seedlings can be quickly and reliably evaluated for resistance to *P. herpotrichoides*. The results obtained correlate with field resistance (25) and have been useful in evaluating genetic material (38).

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