Role of the Hypodermis and Secondary Cell Wall Thickening in Basal Stem Internodes in Resistance to Strawbreaker Foot Rot in Winter Wheat

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ABSTRACT

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Anatomical characteristics of the first elongated internode of 11 winter wheats were studied to determine their association with resistance to *Pseudocercosporella herpotrichoides*. Based on disease indices, cultivar VPM-1 was highly resistant; Cappelle-Desprez, Cerco, Golils, and Rubigus were resistant; Viking was intermediate; and Daws, Nugaines, Sprague, Stephens, and Selection 101 were susceptible to strawbreaker foot rot in the field. Resistance was correlated with hypodermis width and number of hypodermal cell layers, but not with epidermal cell wall thickness, lumen diameter, or stem diameter. Cell wall thickening and lignification occurred earlier in resistant than in susceptible wheats. The fungus invaded all wheats extensively, damaging parenchyma and vascular tissues, particularly phloem. Parenchyma cell walls were more susceptible to damage than lignified cell walls. Epidermal cell walls became thickened and lignified prior to contact with the fungus. Pith parenchyma cell walls became thickened and lignified in diseased tissue. Lignified cell wall appositions (lignitubers) were larger and formed in greater profusion at penetration sites in epidermal cells of stems of resistant than of susceptible wheats. We conclude that resistance to *P. herpotrichoides* is to a considerable extent correlated with structural attributes of the host and that these differences become more apparent as host maturity approaches.

Additional key words: eye spot.

Strawbreaker foot rot, caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton, is a serious disease of winter wheat (*Triticum aestivum* L.) in many parts of the world including the Pacific Northwest region of the United States (5,15,21,31). The pathogen infects leaf sheaths at or near the soil line and at the first two elongated stem internodes (5,15,28,31). Infection occurs from autumn through early spring (6,15,31). Dark-colored, elliptical, or eyeshaped lesions are formed on leaf sheaths and stems. On susceptible wheats, the stem area with lesions becomes sunken and shriveled, resulting in lodging and blighted heads (5,15,28,31).

In general, hard red winter wheats and wheats adapted to dryland areas are most susceptible (31), whereas wheats from northeastern Europe are most resistant (5). Resistance is relative in

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TABLE 1. Disease indices of winter wheats inoculated with *Pseudocercosporella herpotrichoides* at Puyallup, WA, in 1978

Wheat	Disease index[x]					Reaction
	14 Feb	13 Apr	16 May	12 June	Mean	
VPM-1124-R25-1	1.4 a[y]	1.6 a	1.9 a	1.9 a	1.7 a	HR[z]
Capelle-Desprez (PI 262223)	1.8 ab	2.4 bc	2.6 b	2.8 b	2.4 b	R
Cerco (CI 15922)	1.8 ab	2.3 b	3.1 bc	3.4 c	2.6 b	R
Golils (R37-NS738)	2.1 bc	2.3 b	3.1 bc	2.8 b	2.6 b	R
Rubigus	1.8 ab	2.5 bc	3.1 bc	3.3 bc	2.7 bc	R
Viking (PI 316424)	2.1 bc	2.6 bcd	3.5 cd	3.8 cd	3.0 cd	S
Daws (CI 17417)	2.2 bc	3.1 d	3.6 cd	3.5 cd	3.1 d	S
Nugaines (CI 13968)	2.8 d	2.7 bcd	3.8 d	3.8 cd	3.1 d	S
Sel 101 (CI 13438)	2.3 bcd	3.1 d	3.7 d	4.0 d	3.3 d	S
Sprague (CI 16376)	2.5 cd	2.9 cd	3.7 d	4.0 d	3.3 d	S
Growth stage	Leaf sheaths erect	Elongation	Boot heading	Ripening		

[x]Scale: 0 = healthy, 4 = all tillers with severe lesions.
[y]Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test, $P = 0.05$.
[z]Reactions: HR = highly resistant, R = resistant, and S = susceptible.

TABLE 2. Disease indices of winter wheats inoculated with *Pseudocercosporella herpotrichoides* at Pullman, WA, in 1979

Wheat	Disease index[x]				Reaction
	9 April	13 May	17 June	Mean	
VPM-1	1.3 a[y]	1.3 a	2.1 a	1.6 a	HR[z]
Cerco	1.5 a	1.8 b	3.0 b	2.1 b	R
Rubigus	1.3 a	2.3 c	2.8 b	2.2 b	R
Viking	1.5 a	2.3 c	3.0 b	2.3 b	R
Stephens	1.6 a	2.8 d	3.2 bc	2.6 c	S
Nugaines	1.4 a	2.8 d	3.6 cd	2.6 c	S
Daws	1.5 a	3.2 d	3.6 cd	2.8 c	S
Sprague	1.4 a	2.9 d	3.7 d	2.7 c	S
Selection 101	1.5 a	3.2 d	3.7 d	2.8 c	S
Growth stage	Rosette to leaf sheaths lengthened	Second node visible	Anthesis		

[x]Scale: 0 = healthy, 4 = all tillers severely diseased.
[y]Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test, $P = 0.05$.
[z]Reactions: HR = highly resistant, R = resistant, and S = susceptible.

common (hexaploid) wheat, ranging from highly susceptible to resistant; no wheat is immune. Sprague (30) reported that *Aegilops ventricosa* possessed a high level of resistance, and Ometz (in Maia [22]) introduced this resistance into *Triticum persicum*. Ecochard (in Maia [22]) used the amphiploid produced by Ometz and developed the line VPM-1 from a cross of (*A. ventricosa* × *T. persicum*) × *T. aestivum* 'Marne.' Maia (22) tested selections from this cross and demonstrated that VPM-1 possesses a high level of resistance.

Host characteristics apparently associated with resistance are shallow, open crowns, sparse tillering, coarse straw with tough leaf sheaths, broad leaves, short stature, late maturity, and anthocyanin pigmentation of stems (5,20,31). Some workers (15,31) stress that semidwarf nature and the ability to resist lodging are important characteristics of resistant wheats. The earlier lodging occurs, the more severe the yield loss will be (6,15,28); however, unlodged plants with severe lesions also lose yield (5,28). Moreover, Bruehl et al (5) have reported that semidwarf wheats can die standing up (dead stems with white heads).

Numerous workers have studied seedling infection and have attempted to explain resistance (1,2,7–12,16,21,27,28,31). Sprague and Fellows (31) reported that epidermal cells of outer leaf sheaths below fungal stromata developed epidermal cell walls thickened up to one-third the width of the cell cavity. Penetration of each inner leaf sheath and the stem was usually preceded by formation of a pseudoparenchymatous mat. They also observed that chlorenchyma within the outer leaf sheaths was easily penetrated, but the outer (innermost) epidermal cell wall of the inner epidermis retarded spread of the fungus.

Macer (21) developed a seedling test for resistance in which straw cylinders colonized by the fungus were placed over emerging coleoptiles. Leaf sheaths were scored for infection and penetration after 6–8 weeks. Lesions on resistant hosts were shallow and less extensive. The pathogen grew more slowly in tissues of resistant hosts, and a heavy deposition of tanninlike materials occurred in the lesions themselves. Results obtained in this manner were correlated with lodging and yield loss data from field plots. Macer concluded that seedling resistance and adult plant resistance were correlated.

Scott (27) observed that after an initial lag phase, the rate of fungal growth through leaf sheaths was independent of cultivar. He concluded that differences among cultivars were established during infection. Bateman and Taylor (1,2) showed that there was no significant difference between cultivars when leaf sheaths were inoculated, but when coleoptiles were inoculated, susceptible cultivars were penetrated more deeply. They concluded that leaf sheaths may afford a stronger mechanical barrier than coleoptiles. They also observed localized cell wall thickenings in leaf sheaths through which penetration hyphae passed, but found no difference among cultivars in incidence or form of the thickenings.

Defosse (8) and Defosse and DeKegel (9) reported thickening of epidermal cell walls in infected leaf sheaths; healthy outer epidermal cell walls of leaf sheaths were 1.2–1.4 μm thick while those of infected cells were 3.0–5.4 μm thick. Fehrmann and Mendgen (12) observed the deposition of an osmiophilic substance at the sites of attempted penetration in wheat coleoptiles. Chidambaram (7) found lignitubers in coleoptiles and leaf sheaths of wheat seedlings inoculated with *P. herpotrichoides*, but not in infected stems. Lignitubers were more abundant and well-developed in resistant cultivars than in susceptible varieties. Chidambaram concluded that lignitubers in leaf sheaths of resistant cultivars limit or delay the spread of the fungal hyphae.

Sprague and Fellows (31) found that epidermal cells of the culm were difficult to penetrate, but inner parenchyma tissues provided little resistance to spread of hyphae. They also found tissues throughout the diseased portion of the stem to be thick-walled, which they attributed to increased lignification.

Doussinault (11) studied the reaction of winter wheat cultivars, which varied in resistance to *P. herpotrichoides* and earliness of host maturity at both seedling and adult stages in the field. He suggested that three main factors were responsible for the resistance of wheats to *P. herpotrichoides*: the probability of plant infection, the resistance of leaf sheaths to penetration, and the resistance of stems to attack.

Due to the limited information on resistance to *P. herpotrichoides* in adult plants and the insufficient explanations for resistance in general, the present study was undertaken to determine if anatomical characteristics in the first elongated internode of winter wheat straw are associated with resistance. The term resistance as used in this paper refers to the restriction of lesion development. A preliminary report has been published (24).

MATERIALS AND METHODS

Field experiments were conducted during the 1978–1979 and 1979–1980 growing seasons. Hereafter, these will be called the 1978 and 1979 seasons, respectively.

Ten winter wheats (Table 1) varying in resistance to *P. herpotrichoides* were planted in four-row plots (1.83 m × 3.05 m) 7 September 1978 at Puyallup, WA, and 8 September 1978 at Pullman, WA, using a deep-furrow drill. A randomized complete block design with four inoculated and four uninoculated blocks was used.

Nine wheats (Table 2) were planted 6 September 1979 at Pullman, WA, in four-row plots. A randomized complete block, split plot design with four replications was used, with variety as the main plot factor and inoculation as the subplot factor. All cultural practices such as fertilization and weed control were consistent with local practices.

Isolates of the fungus were obtained from mature, diseased straws of the previous season and maintained on Difco potato-dextrose agar. The fungus was increased on sterilized oat kernels (4). Dry, infested oat kernels were scattered on the soil surface by hand 29 and 30 September 1978 at Pullman and Puyallup, respectively, and again on 14 December 1978 at Puyallup at the rate of 0.296 m^3/ha. Sporulation was profuse after 2 wk of cool, moist weather. Plants at both locations were inoculated when in the four-to-five leaf stage. In 1979, dry, infested oat kernels were spread on fiberglass screen and placed on outdoor sandbeds in October and allowed to sporulate naturally. Conidia were collected by washing the oat kernels in cold water and straining through cheesecloth. On 3 November 1979, two of the four rows in each plot were inoculated with a conidial suspension (2×10^5 conidia per milliliter) applied with a handsprayer near the crown to runoff. Plants were well-tillered and in the rosette stage at the time of inoculation.

In both years, uninoculated blocks were sprayed with benomyl (methyl-1-[butylcarbamoyl]-2-benzimidazolecarbamate) at the rate of 0.84 kg a.i./ha.

Samples of at least 100 tillers were collected monthly from each plot to determine disease severity. Disease severity was determined by grading individual tillers on the basis of 0 = healthy tiller, 1 = lesions restricted to outer, loose-fitting leaf sheaths, 2 = small lesions not extensively developed on tight-fitting leaf sheaths, 3 = moderately developed lesions with moderate to extensive discoloration or multiple lesions on tight-fitting leaf sheaths or stems, and 4 = severe lesions or dead tillers.

Disease indices were calculated by multiplying the number of tillers in each class by the class number, summing over classes and dividing by the total number of tillers (28).

Histology. Plants from the Pullman plots were removed at the following stages for anatomical study: after stem elongation, but before the boot stage (20 May 1979 and 21 May 1980), early kernel formation (29 June 1979 and 8 July 1980), and full maturity (16 August 1979 and 15 August 1980).

Representative stems were selected from each sample date and stem pieces 2–4 mm long were cut from the center of the first elongated internode. For samples of diseased material, stems with lesions in classes 2 and 3 were selected and a 2–4 mm stem piece, which contained the margin of the lesion, was used. Tissue pieces were fixed immediately in formalin-propiono-alcohol (5:5:90) for 1 wk or more. Vacuum was applied briefly to insure fixative penetration and air removal. Fixed tissue samples were dehydrated in a tertiary-butyl alcohol series (18). Samples from 1978 were embedded in Paraplast (melting point 56 C) (Lander Division of Sherwood Medical, St. Louis, MO 63103) and samples from 1979 were embedded in Tissue Prep (melting point 61 C) (Fisher Scientific Co., Fair Lawn, NJ 07410).

Samples were softened before sectioning by exposing embedded tissue and soaking in distilled water:glycerol:Tween-20 (polyoxyethylenesorbitanmonolaurate) (60:29:1) for 3 days. Transverse sections 10 and 7 μm thick were cut with a rotary microtome in 1978 and 1979, respectively. Sections were affixed with a modified Haupt's adhesive (3) to microscope slides cleaned with 95% ethanol. Sections were then stained with safranin and fast green (18). Staining reactions of anatomical structures were confirmed by staining duplicate slides with crystal violet and orange G, and randomly selected slides with phloroglucinol-HCl (18,19).

Outer epidermal cell wall thickness, hypodermis thickness, and number of hypodermal cell layers were measured at six different points of each stem section (23,25). Stem diameter was also measured. Stem lumen diameter was measured in 1978, and hypodermal cell wall thickness in 1979. The latter measurement was taken across two adjacent hypodermal cells in a plane tangential to the hypodermis.

Three stems of each wheat were examined at each of the three sampling dates. Data from the three sampling dates were used to calculate mean values for each wheat and variable. Correlation coefficients were calculated between mean values, as well as values from each of the three sampling dates and mean disease indices. Due to inadequate disease development in 1978 at Pullman, WA, disease indices were obtained at Puyallup, WA, and were used in the calculation of correlation coefficients.

RESULTS

Due to winter kill, data for Cappelle-Desprez and Golils are available only for 1978 while Stephens was studied only in 1979.

Disease indices were used to divide the wheats into three categories (Tables 1 and 2). Only VPM-1 was considered highly resistant, while Cappelle-Desprez, Cerco, Golils, and Rubigus were resistant. In 1978, Viking was considered susceptible, but it was resistant in 1979 (Tables 1 and 2). The rest of the wheats were rated susceptible in both years.

Histology of healthy, uninoculated stems, 1978. The most significant correlation between disease indices and anatomical features was obtained at full maturity (Table 3). At full maturity, hypodermis width and number of hypodermal cell layers were highly significantly correlated with mean disease indices ($r = -0.91$ and $r = -0.86$, respectively), as well as with each other ($r = 0.97$). Hypodermis width and number of hypodermal cell layers were significantly correlated with disease indices at all dates. VPM-1, the most resistant line, had the widest hypodermis (126.0 μm) with the most cell layers (8.9 layers). Selection 101, a highly susceptible line, had the narrowest hypodermis (51.3 μm) with the fewest cell layers (3.6). The remaining lines ranged from Cappelle-Desprez with a hypodermal width of 100.3 μm and 6.7 cell layers to Sprague with a hypodermal width of 53.4 μm and 4.1 cell layers (Table 3).

TABLE 3. Anatomical characteristics at full maturity (16 August 1979) and correlation coefficients with mean disease indices of winter wheats varying in resistance to strawbreaker foot rot, Pullman, WA, in 1978

Line	Stem diameter (mm)	Lumen diameter (mm)	Epidermal cell wall thickness (μm)	Hypodermis	
				Width (μm)	Cell layers (no.)
VPM-1	3.6 a[x]	0.15 a	7.0 c	126.0 a	8.9 a
Cappelle-Desprez	3.8 a	0.30 a	9.0 a	100.3 b	6.7 bc
Cerco	2.8 bc	0.20 a	5.7 ef	91.2 b	6.9 b
Rubigus	3.9 a	1.35 c	6.0 de	88.1 bc	6.8 bc
Viking	3.8 a	0.80 ab	6.2 d	73.0 d	6.2 bcd
Golils	3.9 a	0.23 a	5.1 f	67.8 d	5.3 bcde
Nugaines	3.4 ab	0.90 ab	7.9 b	75.5 cd	5.2 cde
Daws	3.8 a	1.15 c	6.0 de	68.4 d	4.8 def
Selection 101	2.6 c	0.66 ab	4.4 g	51.3 e	3.6 f
Sprague	2.3 c	0.35 a	5.2 f	53.4 e	4.1 ef
r[y]	0.44 ns	0.47 ns	−0.42 ns	−0.91**[z]	−0.86**

[x] Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test, $P = 0.05$.

[y] Correlation coefficients (r) between given variable and mean disease index for wheats obtained at Puyallup, Washington.

[z] Asterisks (**) indicate statistical significance at $P = 0.01$. ns = nonsignificant.

Stem diameter, lumen diameter, and outer epidermal cell wall thickness were not significantly correlated with mean disease indices at full maturity (r = 0.44, r = 0.47, and r = −0.42, respectively) or any other growth stage.

Stem diameter ranged from 2.3 mm in Sprague to 3.9 mm in Rubigus and Golils (Table 3). There was a tendency for stems of resistant wheats to have small lumens or no lumen in the first elongated internode, although this was not significantly correlated with disease indices. Neither stem diameter nor lumen diameter was significantly correlated with hypodermis width, number of hypodermal cell layers, or outer epidermal cell wall thickness.

Histology of healthy, uninoculated stems, 1979. Hypodermal width and number of hypodermal cell layers at full maturity were highly significantly correlated with mean disease indices (r = −0.95 and r = −0.92, respectively) and with each other (r = 0.98) (Table 4). Furthermore, hypodermal width and number of hypodermal cell layers was significantly correlated with mean disease indices at all other sampling dates.

VPM-1 had the widest hypodermis (110.6 μm) with the greatest number of hypodermal cell layers (8.4 layers). Sprague, a highly susceptible line with very weak straw, had the narrowest hypodermis (45.9 μm) with the fewest number of hypodermal cell layers (3.3 layers) (Table 4).

Stem diameter and outer epidermal cell wall thickness were not significantly correlated with mean disease indices at any sampling date.

Correlation was significant between hypodermal cell wall thickness and mean disease indices at the second (watery-ripe) sampling date (r = −0.68), but not at the first (preboot) (r = 0.25) or third (full maturity) (r = −0.08) sampling dates. Analysis of changes between sampling dates within wheats revealed that the resistant wheats, VPM-1, Cerco, Rubigus, and Viking, had significant increases in hypodermal cell wall thickness between first and second sampling dates but not between second and third sampling dates (Table 5). Conversely, the susceptible wheats, Nugaines, Stephens, Daws, Sprague, and Selection 101, had significant increases between the second and third sampling dates, but not between the first and second dates.

TABLE 4. Anatomical characteristics at full maturity (15 August 1980) and correlation coefficients with mean disease indices of winter wheats varying in resistance to strawbreaker foot rot, Pullman, WA, in 1979

Line	Stem diameter (mm)	Epidermal cell wall thickness (μm)	Hypodermis Width (μm)	Hypodermis Cell layers (no.)	Hypodermis Cell wall thickness (μm)
VPM-1	3.3 ab[x]	7.0 abc	110.6 a	8.4 a	8.2 abc
Cerco	3.3 ab	7.1 abc	75.2 b	5.2 bc	8.6 ab
Rubigus	3.8 a	6.0 bcd	77.6 b	6.2 b	6.0 c
Viking	3.6 a	7.5 ab	69.9 bcd	5.6 b	8.1 abc
Stephens	3.2 ab	7.5 ab	70.6 bc	5.4 b	9.3 a
Nugaines	2.8 b	6.1 cd	54.9 cde	3.7 d	7.9 abc
Daws	3.3 ab	5.9 cd	54.0 de	4.0 cd	6.9 bc
Selection 101	3.2 ab	8.0 a	53.3 e	3.8 d	8.6 ab
Sprague	2.8 b	5.6 d	45.9 e	3.3 d	6.6 c
Correlation coefficient[y] (r)	−42.0 ns	−0.12 ns	−0.95**	−0.92**[z]	−0.08 ns

[x] Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test, P = 0.05.

[y] Correlation coefficients between given variable and mean disease index for wheats obtained at Pullman, WA.

[z] Asterisks (**) indicate statistical significance at P = 0.01. ns = nonsignificant.

Correlation between 1978 and 1979 data was highly significant for stem diameter (r = 0.89), hypodermis width (r = 0.96), and number of hypodermal cell layers (r = 0.94). Outer epidermal cell wall thickness was not significantly correlated (r = −0.31) between years suggesting the possibility of large environmental influence on this variable.

Within the variables, outer epidermal cell wall thickness and hypodermal cell wall thickness were highly significantly correlated (r = 0.92) with each other.

In both years, significant changes in all anatomical features, except stem diameter, were apparent between the preboot and watery-ripe growth stages, but not between the watery-ripe and full maturity growth stages. This indicated that structural development in the first elongated internode was mostly complete by early kernel development. Stem diameter did not change significantly between sampling dates.

Development of the mature stem. The most apparent changes that took place between preboot and watery-ripe stages as revealed by changes in staining reactions were thickening and increased lignification of cell walls, especially in the hypodermis. In preboot samples the hypodermis and vascular bundles (xylem vessels and sclerenchyma sheath) were the most heavily lignified structures. There were no apparent differences in degree of lignification between resistant and susceptible lines at this stage (Fig. 1A and B). The inner wall layer of hypodermal cells appeared to be primarily cellulose with middle lamella areas beginning to be lignified. Epidermal, subepidermal parenchyma, intervascular parenchyma, and pith parenchyma cell walls were not yet lignified at this stage. Pith parenchyma cells had the thinnest cell walls.

By the watery-ripe stage, lignification had increased in epidermal, subepidermal parenchyma, hypodermis, and intervascular parenchyma cell walls (Fig. 1C and D). Pith parenchyma walls remained unlignified. Inner hypodermal cell walls no longer appeared to be primarily cellulose but had become lignified. Increased lignification of hypodermal and parenchyma cell walls was accompanied by increases in wall thickness (Fig. 1 C and D). Increases in wall thickening were greatest in resistant wheats (VPM-1, Cerco, Viking, and Rubigus), and least in susceptible wheats (Nugaines, Stephens, Daws, Sprague, and Selection 101). At the watery-ripe stage, only Stephens among the susceptible wheats had hypodermal cell walls thicker than those of resistant wheats (Table 5). In addition, hypodermal cells of resistant wheats showed less intercellular spaces and were more

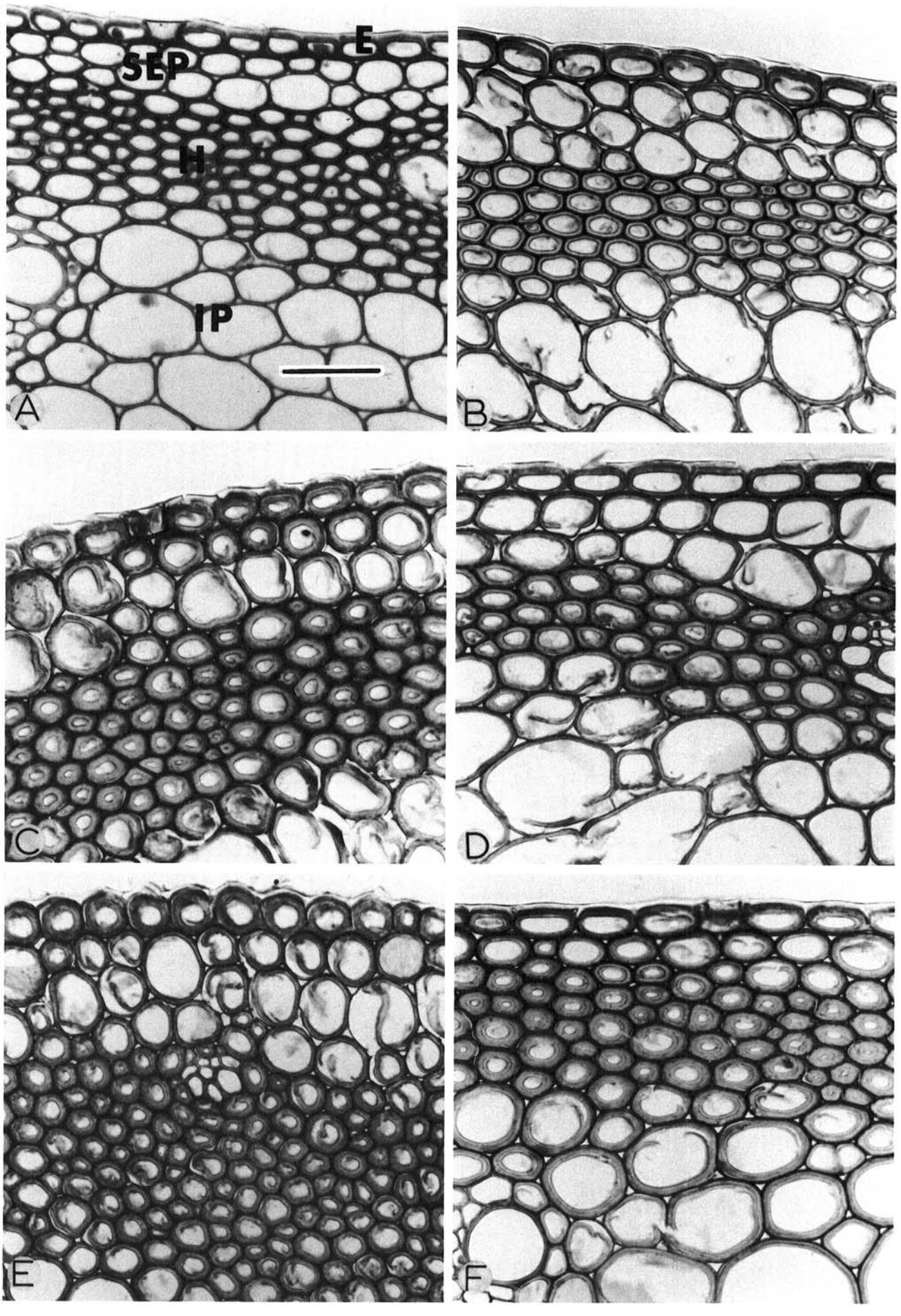
TABLE 5. Stem hypodermal cell wall thickness and susceptibility of nine winter wheats sampled at three growth stages at Pullman, WA, in 1979

Cultivar	Reaction[y]	Hypodermal cell wall thickness (μm) at growth stage: Stem elongation	Watery-ripe	Full maturity
VPM-1	HR	4.4 a[z]	7.9 b	8.2 b
Cerco	R	4.4 a	7.7 b	8.6 b
Rubigus	R	3.9 a	6.8 b	6.0 ab
Viking	R	4.5 a	8.4 b	8.1 b
Stephens	S	4.9 a	6.9 a	9.3 b
Nugaines	S	4.5 a	6.2 ab	7.9 b
Daws	S	4.2 a	4.5 a	6.9 b
Sprague	S	3.7 a	6.0 ab	6.7 b
Selection 101	S	5.8 a	6.8 ab	8.6 b

[y] Reaction: HR = highly resistant, R = resistant, and S = susceptible.

[z] Means within rows followed by the same letter are not significantly different according to Duncan's new multiple range test, P = 0.05.

Fig. 1. Transverse sections of the first elongated internode of healthy wheat stems. **A, C,** and **E,** VPM-1 (highly resistant); **B, D,** and **F,** cultivar Daws (susceptible). **A** and **B,** sampled at boot stage, the hypodermis is visible, but the cell walls are not lignified; **C** and **D,** sampled at watery-ripe stage, hypodermal cell walls of the resistant (**C**) wheat are thickened and lignified; **E** and **F,** sampled at full maturity, hypodermal cell walls of both wheats are lignified. Notice the extremely thick hypodermis in the resistant wheat (**E**). Scale bar = 50 μm. H = hypodermis, IP = intervascular parenchyma, SEP = subepidermal parenchyma, and E = epidermis.


E
SEP
H
IP
A
B
C
D
E
F

closely packed. At full maturity, hypodermal cell wall thickness and the degree of lignification of susceptible wheats had increased such that some susceptible wheats had wall thickness greater than resistant wheats (Table 4) (Fig. 1E and F).

Observations from diseased stems. The major effects of the fungus on host anatomy were decomposition of cell wall materials, presumably by extracellular pectinases and cellulases (7,17), and destruction of vascular tissues. The degree to which cell wall decomposition took place was related to resistance of the wheat. In resistant wheats only part of the stem section was usually affected, whereas in susceptible wheats the entire stem section was often degraded.

Parenchyma cells were more completely degraded than hypodermal cells (Fig. 2A). Hypodermal cell wall thickness was reduced and the hypodermis stained more densely on the lesion side of the stem. This was believed to be due to a failure of cell walls to thicken normally rather than a reduction in wall thickness due to degradation by the pathogen.

Phloem tissue was more severely damaged than xylem. Vascular bundles in the pith were more susceptible to damage than those embedded in the hypodermis. In general, the resistant wheats had apparently functional phloem in vascular bundles embedded within the hypodermis, but phloem tissue in the hypodermis of susceptible wheats was usually completely destroyed. Some plugging of xylem elements with hyphae or gelatinous substances had occurred, but all wheats had some apparently functional xylem at maturity.

There appeared to be two types of reactions to stem infection. One type resulted in increased thickening and lignification of pith cell walls adjacent to lesions. This reaction was not correlated with resistance and occurred to some extent in all lines. These modified pith cells did not stop the spread of the fungus within the stem, but they were less susceptible to degradation than unthickened pith cells. Thickened pith cell walls were not a characteristic of healthy

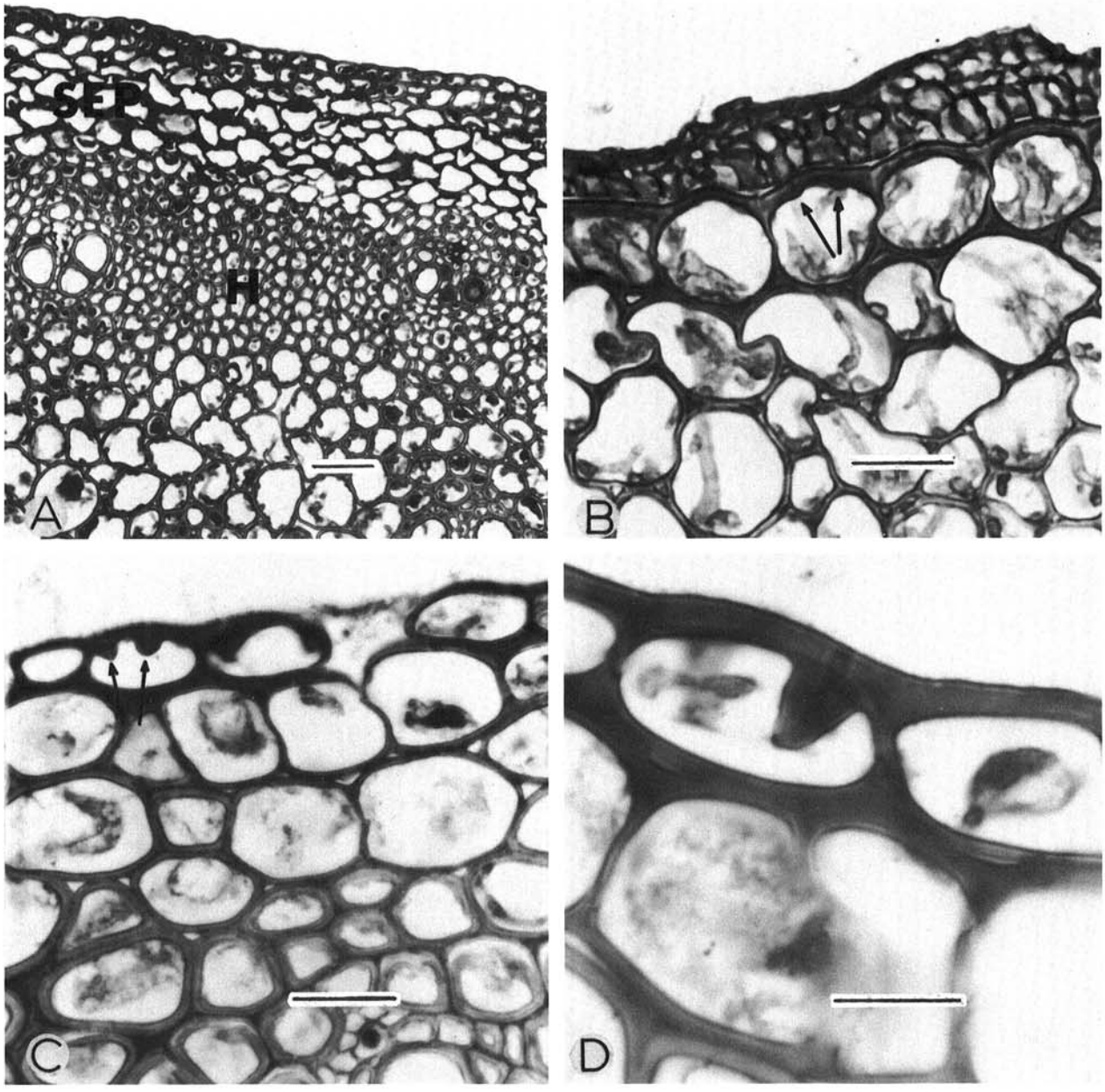


Fig. 2. Transverse sections of the first elongated internode of diseased wheat stems. **A,** Rubigus (resistant) at full maturity, showing collapse of subepidermal parenchyma (SEP) cell while the hypodermis (H) remains intact. **B,** Nugaines (susceptible) prior to boot stage, with pseudoparenchyma on the epidermis. Arrows indicate position of poorly developed lignitubers. **C,** Cappelle-Desprez (resistant) prior to boot stage, pseudoparenchyma has pulled away from the epidermis. Arrows indicate position of well-developed lignitubers. **D,** Cappelle-Desprez, well-developed lignituber enclosing penetration hypha. Scale bars: **A** = 50 μm, **B** and **C** = 20 μm, and **D** = 10 μm.

stems.

The second type of reaction to stem infection resulted in formation of localized appositions surrounding infection hyphae (Fig. 2C and D). These lignified appositions occurred on the inner side of outer epidermal cell walls and were associated with the presence of fungal pseudoparenchyma on the epidermis. Lignitubers purportedly function to retard the penetration of infection hyphae, and infection hyphae were visible.

Lignified appositions were observed in all lines studied but were most well-developed in stems of Cappelle-Desprez (Fig. 2C and D), where they appeared as distinct fingerlike projections in the cell lumen (cf, Fig. 2C and D). In the susceptible lines, appositions were small and not fingerlike (Fig. 2B). Appositions were more numerous in the resistant lines than in the susceptible lines.

DISCUSSION

Even though the winters are milder at Puyallup than at Pullman, and wheat reached a given stage of development earlier at Puyallup than at Pullman, the wheats reacted similarly at both locations and the correlation between disease indices for the two years was highly significant ($r = 0.95$).

Pathologists have developed seedling tests for resistance in the hope of accelerating progress in breeding programs. Macer (21); who pioneered the foot rot seedling tests, noted that the precision of his test increased with incubation periods extending to 6–8 wk. Delibes et al (10) distinguished three classes of resistance among 41 wheats in the seedling stage and 12 classes when the same wheats were evaluated in the adult stage. When Scott (27) evaluated Cappelle-Desprez (resistant) and Champlein (susceptible) in a seedling test he calculated that 13 replicates with four plants per replicate were required to distinguish differences between these wheats. We were unable to evaluate resistance of wheat grown in the field at Pullman when it was in the rosette stage (Table 2). Differences became apparent at later stages of development. The high correlation of resistance to the breadth of the hypodermis and the number of cell layers in it at or near maturity is all the more remarkable in that only three replicates entered the calculations. Frauenstein and Roskothen (14) reported a rag-doll test for resistance in the seedling stage that requires only 32 days. Our observations (*unpublished*) and the literature indicate that resistance is difficult to evaluate in early stages of host development.

The correlations between hypodermis width or the number of hypodermal cell layers and disease indices suggest that the hypodermis functions in resistance. VPM-1 and Cappelle-Desprez, the most resistant wheats in this study, had the widest hypodermis with the most cell layers (Tables 3 and 4). Selection 101 and Sprague, the most susceptible wheats, had the narrowest hypodermis with the fewest cell layers. Schaffnit (26) observed that the hypodermis of a resistant wheat was 71 μm wide and that of two susceptible wheats was 55 and 61 μm wide. The hypodermal cell walls of the resistant wheat were thicker (4.20 μm) than in the susceptible wheats (3.15 and 3.25 μm).

In the present study, hypodermal cell wall thickness was significantly correlated with mean disease indices only during the watery-ripe stage. In resistant wheats, hypodermal cell wall thickness increased significantly between preboot and watery-ripe stages, while susceptible wheats had significant increases between watery-ripe and full maturity (Table 5). Resistant wheats developed more heavily thickened and lignified subepidermal and intervascular parenchyma cell walls between preboot and watery-ripe stages than did susceptible wheats. We conclude that the early development of a lignified mechanical framework enables the host to reduce damage due to pathogenic action of the fungus.

Thickening of epidermal cell walls in leaf sheaths of seedlings beneath fungal pseudoparenchyma observed in this study has been reported by others (9,16,31). Sprague and Fellows (31) observed thickening of parenchyma cell walls within diseased stem tissue and found that this tissue was more brittle than normal. They attributed this brittleness to increased lignification. We too observed thickened, lignified pith parenchyma cell walls in diseased stem tissue. These were observed most frequently in cells adjacent to cells partially or wholly collapsed. Because lignified cell walls are not as susceptible to degradation as cellulose cell walls, they may have a structural function in diseased tissue. Increased lignification apparently occurs in advance of penetration of the tissues involved; however, it does not prevent spread of the pathogen within those tissues. The increased deposition of lignin probably reduces the rate of fungal progress and tissue degradation. In this respect, structural resistance is a rate-limiting factor operating throughout the life of the host plant. The rate and degree of lignification observed in cell walls of stem tissues probably occur in leaf sheath tissues also; however, we did not study leaf sheaths.

Localized deposition of cell wall materials in response to attempted penetrations by the fungus has been observed in seedlings (2,7,9,12). These appositions contain lignin (7) and were called lignitubers according to the terminology of Fellows (13). Lignitubers occurred in all wheats studied, but they were more numerous and more strongly developed in the resistant wheats (Fig. 2C and D). Chidambaram (7) and other workers (2,9,12) found lignitubers in epidermal cell walls of leaf sheaths of seedlings, and we observed them in the epidermis of the stem. Lignituber formation may be an accentuation of the epidermal cell wall thickening response. Both responses appear to be present in seedling and adult plants and function as general host responses to infection. A similar conclusion was made by Sherwood and Vance (29) who studied fungal penetration in 12 species of the Gramineae using incompatible leaf-infecting fungi. They concluded that apposition formation may be an inducible response that functions as a general resistance mechanism throughout the Gramineae.

Due to the complexity of morphological traits associated with resistance, it is unlikely that structural resistance is simply inherited as has been reported (10). These workers are probably following inheritance of a hypersensitive type of resistance (16) that is not necessarily associated with anatomical features. In support of this, VPM-1 and Cappelle-Desprez both have a well-developed anatomical framework; in addition, VPM-1 may possess some degree of hypersensitivity that confers an added degree of resistance above that of Cappelle-Desprez.

Results of this study indicate a close relationship between resistance to *P. herpotrichoides* and anatomical characteristics of wheat stems, specifically hypodermis width, number of hypodermal cell layers, and early development of a lignified mechanical framework. Together these factors have a rate-limiting effect on the pathogen and reduce damage to the stem. In resistant wheats, damage to the stem is delayed and reduced and the plant can produce a harvestable crop. We are not attempting to explain resistance completely based on anatomical characteristics, but anatomy is an important component of resistance. If anatomical features such as the hypodermis prove to be a major component of resistance, then resistance to *P. herpotrichoides* is not likely to be overcome by the development of specific races or strains of the pathogen.

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