Anatomy and Lignin Content in Relation to Resistance of *Dactylis glomerata* to Stagonospora Leaf Spot

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ABSTRACT

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Parallel veins of orchardgrass leaves were examined in relation to width of leaf spots incited by *Stagonospora arenaria*. Trials of 12 greenhouse-inoculated plants and 12 naturally infected, field-grown single-cross lines that ranged widely in mean leaf spot size gave similar results. Veins with large vascular bundles (enlarged metaxylem vessels present) alternated regularly with veins having small vascular bundles (enlarged metaxylem vessels absent). Vascular bundles usually were connected to the lower and/or upper epidermis by girders of lignified sclerenchyma and/or girders of thin-walled cells. Mean leaf spot size was not related to width of small or large vascular bundles, distance between vascular bundles, or percentage of veins with large vascular bundles. Edges of leaf spots usually stopped at parallel veins, but wider leaf spots crossed one or more veins. Leaf spots crossed veins having small vascular bundles more frequently than veins having large vascular bundles. Differences in vein crossing were related to the frequency of girders. About 86–88% of large vascular bundles had two girders (to the upper and lower epidermis), but only about 10–50% of small vascular bundles had two girders. The proportion of small vascular bundles with two girders was greater in resistant plants and lines than in susceptible plants and lines. Regression indicated that 40–55% of heritable variation in mean leaf spot width may be accounted for by frequency of small vascular bundles having two girders. Leaf spot size was not associated with constitutive lignin content of uninoculated leaves in two greenhouse trials of 14 single-cross F_1 lines. Inoculated leaves had higher lignin content than controls, indicating that ligninlike compounds were synthesized during infection.

Stagonospora arenaria Sacc. incites purple leaf spot of orchardgrass (*Dactylis glomerata* L.). The disease is widespread in hay and pasture stands of the northeastern United States and may reduce forage yield and quality (14,22). Cultivars of this open-pollinated polyploid species are heterogeneous for resistance. Most plants in each of 28 cultivars tested in greenhouse inoculations were rated moderately to highly susceptible on the basis of forming relatively large leaf spots (23). A few plants in each cultivar formed only small leaf spots and were considered to be resistant. Beginning with these apparently resistant plants, Berg et al (4) conducted five 2–3 yr cycles of phenotypic recurrent selection for small leaf spot size to develop resistant germplasm PL-OGDR1.

During the breeding program, Berg et al (3,6) studied inheritance of resistance in 82 single-cross F_1 lines from a diallel mating among five resistant and five susceptible parent plants from cycles 0 to 3. Mean leaf spot size of each F_1 single-cross line was similar to the mean of its two parents. Inheritance was primarily additive and multigenic. Leaves of some of the plants that were scored for disease reaction in the selection and inheritance studies were simultaneously sampled for the tests on leaf anatomy and lignin content reported here.

Lateral edges of Stagonospora leaf spots often coincide with parallel (longitudinal) veins of the host, and leaf spots are not as wide as they are long, indicating that veins may have a role in limiting leaf spot width (19). Certain cell types associated with grass leaf veins, including sclerenchyma fibers, inner bundle sheaths, and xylem elements, are lignified. Several authors have presented evidence that lignified cells are a barrier to growth of some pathogenic fungi (1,8). Early in the selection program we postulated that small leaf spot size might be due to increased size or lignification of veins or to closer spacing of veins.

The lignified components of orchardgrass leaves are highly resistant to digestion by rumen microflora (9), and high lignin content adversely affects forage feeding value (2,13). We were concerned that selection for small leaf spot size might lead to increases in poorly digested components. Accordingly, we undertook the present studies to determine whether resistance is associated with vein anatomy or lignin content. Leaf spot width was used as the measure of resistance, because appearances suggested that restriction of width is related to the parallel veins.

MATERIALS AND METHODS

Inoculation. Greenhouse plants were grown in peat/vermiculite mixture in pots. Inoculations were conducted by the methods of Zeiders et al (23). We used virulent isolates of *S. arenaria* isolated within the preceding 9 mo from naturally infected plants near State College, PA. Cultures for inoculum were grown on V8 juice agar for 10 days in incubators at 22 C with 12 h of fluorescent light daily. Conidia were scraped from the cultures and suspended in distilled water with 0.25 ml of Tween 20 surfactant l^{-1} to give a suspension of about 10,000 conidia ml^{-1}. The conidial suspension was sprayed on leaves until they were thoroughly wet. Inoculated plants were placed in a 3.3- × 3.3- × 2.1-m moist chamber at 20 ± 2 C for 48 h and then were returned to the greenhouse and maintained for 2 wk. Eight-centimeter-long samples from the middle of fully expanded leaf blades with >25 leaf spots per sample were cleared in ethanol/acetic acid (3:1, v/v) and stored in 50% ethanol.

Samples for anatomy trials. Twelve plants were selected for the anatomy study from a greenhouse experiment involving 12,000 plants of phenotypic recurrent selection cycles 0, 1, 2, and 3 described elsewhere (5). Four of the plants were representative of the most resistant plants among the 12,000, four were representative of the most susceptible plants, and four were intermediate, based on the size of the largest leaf spots on the plants.

Inoculation of regrowth of the plants confirmed the constancy of their reaction and provided samples for the anatomy study.

Twelve lines were selected for an anatomy study from 92 lines in a field experiment on the inheritance of resistance to *S. arenaria* (3). The selected lines included two resistant parent clones, two resistant F_1 lines, two susceptible parent clones, two susceptible F_1 lines, and four intermediate F_1 lines. Stagonospora leaf spot developed naturally in late summer 1989. Each of the lines was sampled in three replicate plots. Leaves that were fully expanded, nonsenescent, and showing the largest spots in the plot were chosen. There were >25 leaf spots on the single leaf selected from each plot. Samples were cleared and stored as in the greenhouse trial.

Measurement of anatomical traits. Video imagery was used to measure width, length, and area of 25 leaf spots on each of 12 cleared leaves from the greenhouse trial and 36 cleared leaves from the field trial. Figure 1A and B shows leaf spots in cleared resistant and susceptible samples. Images of the cleared leaves were projected on a video monitor at 25× and traced on transparent acetate sheets (Fig. 1C and D). Discrete leaf spots that appeared to originate from single infections were traced; touching or merged leaf spots were excluded. Width, length, and area of each leaf spot tracing was measured with a Bioquant MEG IV image analysis system (R & M Biometrics, Inc., Nashville, TN). Because leaf spots on an individual leaf differ in size at any one time, even when they are initiated synchronously in greenhouse inoculations (20), the mean of 25 leaf spots selected at random was used as the measure of susceptibility of the leaf.

A subsample of each leaf used to measure leaf spot size was sectioned to determine the vein anatomy. The subsample was taken at the midpoint of the sample and included the full width of the blade. The subsample was dehydrated with an ethanol/tertiary butanol series, embedded in paraffin, and sectioned transversely at 12 μm (12). Sections were mounted on slides and stained with safranin and fast green. All of the parallel veins in a cross section were classified for vascular bundle type and girder type according to Metcalfe (16). Distance between veins was measured from center to center of vascular bundles with an ocular micrometer (Fig. 2A). The width of each vascular bundle, including the inner bundle sheath, was measured transversely at its widest point (Fig. 2A). A diagram was prepared showing the types of vascular bundles and girders of each vein across the leaf. The locations of the 25 leaf spots measured in the sample were matched with veins on the diagram by examining the unembedded part of the leaf through a binocular microscope to determine where each leaf spot occurred with respect to each vein of the diagram. From this it was determined whether each leaf spot was confined between two veins or crossed one or more veins, and what type of vascular bundle occurred in veins that were crossed.

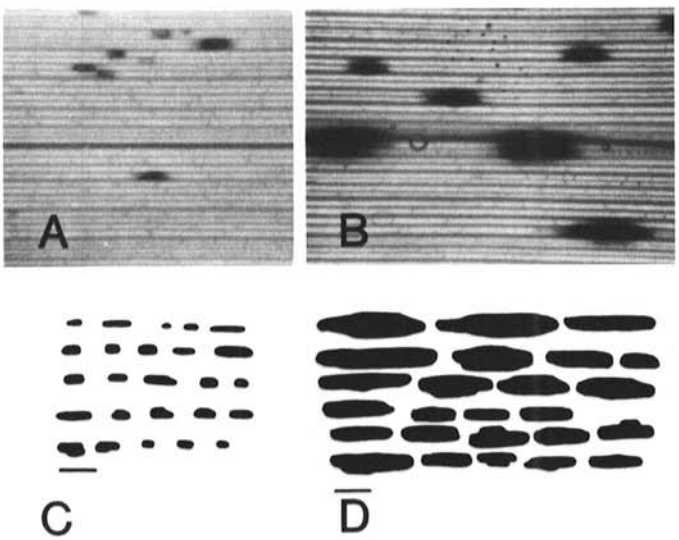

Fig. 1. Method for measuring leaf spot size (×5). **A,** Cleared leaf of a resistant plant. **B,** Cleared leaf of a susceptible plant. Note that some leaf spots cross parallel veins. **C,** Tracings of video images of 25 leaf spots from a cleared leaf of a resistant plant (×25). Length, width, and area were measured using computerized image analysis. **D,** Tracings of images from a susceptible plant. Bar = 1 mm.

Lignin content. Lignin content was measured in infected leaves and in uninoculated control leaves in two greenhouse trials. Fourteen F_1 single-cross lines from a diallel inheritance study (6) were used in both trials. This included four susceptible lines, four resistant lines, and six intermediate lines. Individual F_1 sibling plants were grown from seed in peat/vermiculite mixture in 4-cm-diameter × 21-cm-deep plastic pots.

Each trial had six replicate blocks. Each block was established on a separate bench. Two benches were located in each of three greenhouse sections that did not have supplemental lighting and that differed in natural light. Each block was divided into an inoculated subblock and a uninoculated subblock. The 14 F_1 lines were randomized in each subblock. The basic plot (experimental unit) for lignin determination and disease scoring consisted of seven sibling F_1 plants in seven adjacent pots. Different sets of siblings were used in each subblock and block. The second trial used a different set of seedlings and plot randomization plan than the first trial. The first trial was seeded 21 October 1986, inoculated 14 January 1987, and harvested 28 January 1987. The second trial was planted 24 February, inoculated 20 April, and harvested 6 May 1987. Plants were in a postjuvenile vegetative stage at inoculation. Inoculations were conducted as described above.

At harvest, leaves of inoculated plants were rated for leaf spot size on a scale of 1–9 where 1 = no visible leaf spots, 2 = flecks, and 9 = large leaf spots (about 1 mm wide × 5 mm long). Then all leaves from a seven-plant plot were pooled, freeze-dried, and ground to 1-mm particle size. Lignin content was determined using near infrared reflectance (NIR) spectroscopy (15). To develop calibration curves for the NIR program, subsamples of 50 plots were assayed for lignin by the permanganate method of Goering and Van Soest (11). The 50 subsamples included inoculated and uninoculated plots over the entire range of susceptibility in both trials.

Statistical analysis. In the anatomy trials, the responses of leaf spot width, as the dependent variable, to the various anatomical traits, as independent variables, were examined by regression analysis. Means of the 12 greenhouse plants or 12 field lines were analyzed using SAS PROC REG (18).

In the lignin trials, data on leaf spot size and lignin content were subjected to separate analyses of variance using SAS PROC ANOVA (18). Leaf spot size scores included only plants from the inoculated subblocks and were analyzed as a randomized complete block design with fixed effects for block (replication) and line. The lignin content data were analyzed as a randomized split block design with fixed effects. Comparisons of the error mean squares between the two trials indicated homogeneity of variances; therefore, combined analyses of variance over trials were conducted for spot size and for lignin content.

RESULTS

Vein anatomy. In agreement with earlier descriptions of *Dactylis* spp. (7,16), orchardgrass leaves showed the standard form of nonkranz anatomy characteristic of the subfamily Pooideae. Chlorenchyma was irregular with more than four cells between adjacent longitudinal veins (Fig. 2). The veins had a single central vascular bundle with inner and outer bundle sheaths, each one layer thick. Inner bundle sheath cells had thick inner and lateral walls; outer bundle sheath cells were round and thin-walled, with few chloroplasts (Fig. 3A). Vascular bundles were of two types distinguished by Metcalfe (16) as large vascular bundles, characterized by having prominent, enlarged metaxylem vessels, and small vascular bundles, which lack enlarged metaxylem vessels. Vascular bundles were sometimes covered on the top and/or bottom with chlorenchyma that was continuous with chloren-

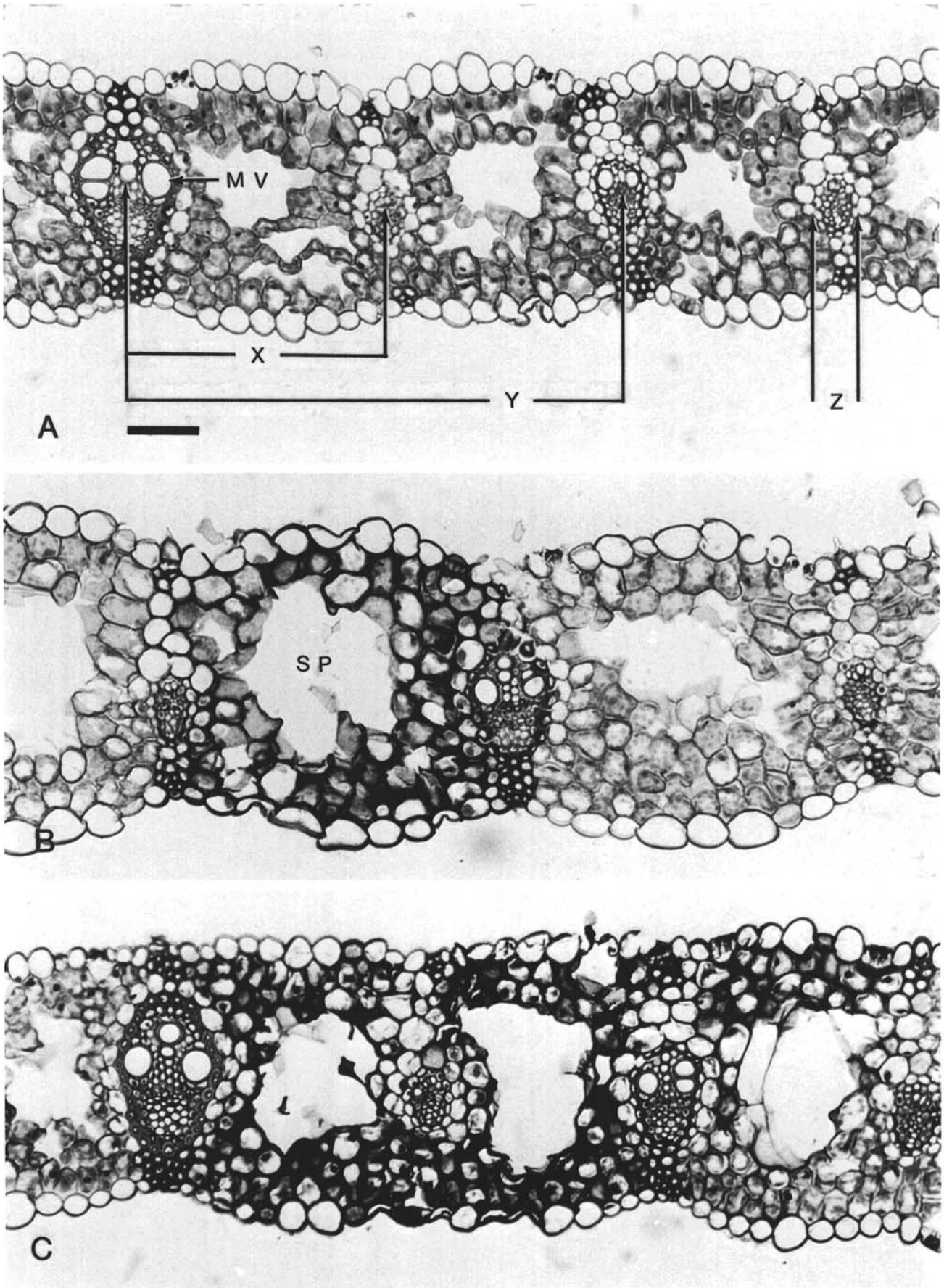

Fig. 2. Transverse sections of orchardgrass leaves (×275). Top (adaxial) surfaces are up. **A,** Repeated pattern of alternating large and small vascular bundles. Large vascular bundles are distinguished by enlarged metaxylem vessels (MV). X = distance between vascular bundles. Y = distance between large vascular bundles. Z = width of vascular bundle. **B,** A leaf spot (SP) in a resistant plant confined to the intercostal area between the small vascular bundle on the left and the large vascular bundle in the center. **C,** A leaf spot in a susceptible plant confined by a large vascular bundle on the left and crossing a small vascular bundle and a large vascular bundle at the center. Bar = 50 μm.

chyma of the intercostal area on each side of the vein (Fig. 3C). More frequently, however, specialized cells formed girders between the outer bundle sheath and the lower and/or upper epidermis. Girders were of two types: 1) sclerenchyma girders composed of sclerenchyma fibers, and 2) bundle sheath girders composed of thin-walled cells resembling outer bundle sheath cells, extending from the outer bundle sheath to the epidermis or to a subepidermal strand of sclerenchyma fibers (Fig. 3A).

Leaf spot width and vascular bundle traits. The mean width of leaf spots of the most resistant plant in the greenhouse was 198 μm; that of the most susceptible plant was 664 μm, and the average for the 12 plants was 424 μm. The respective values

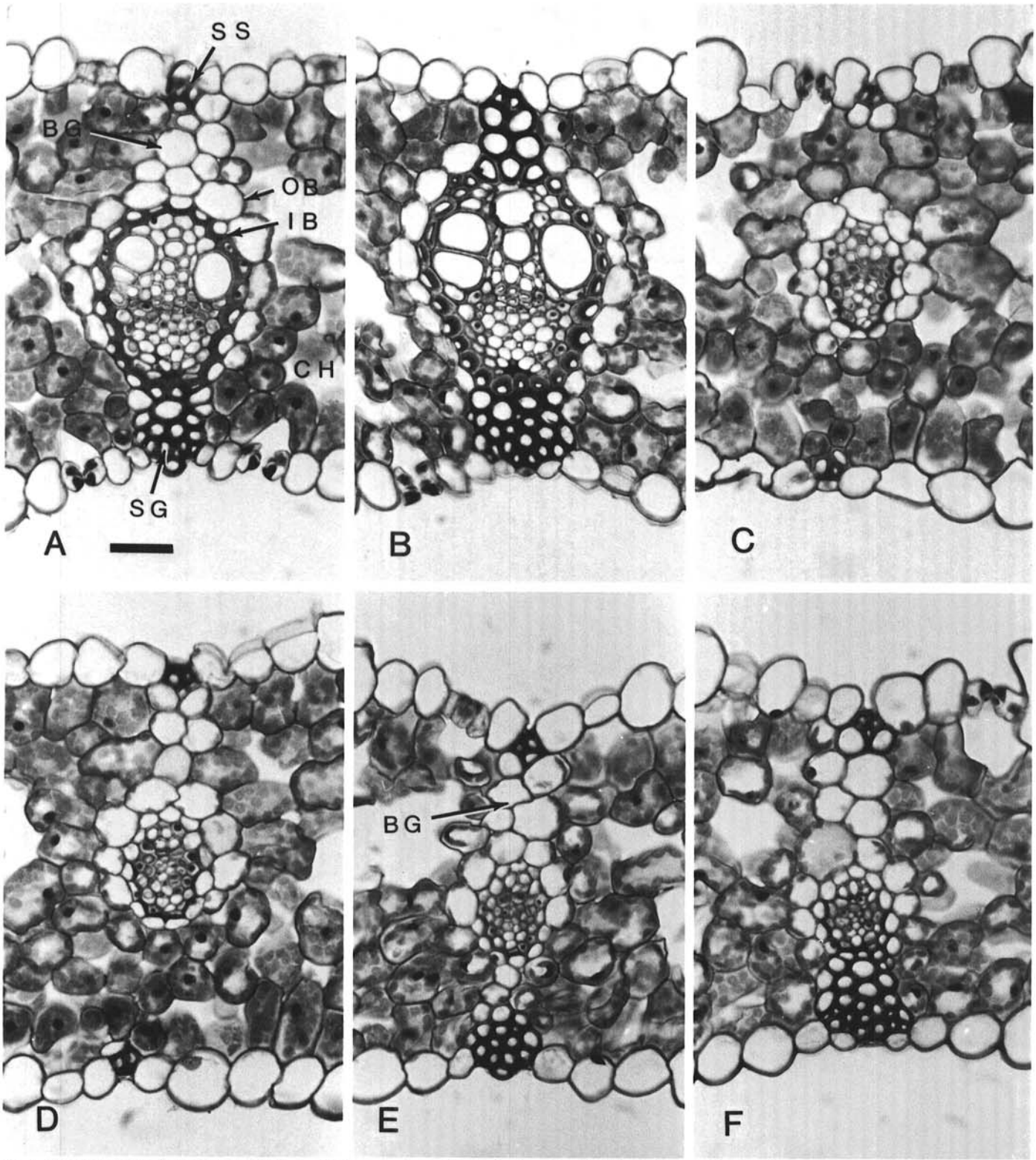

Fig. 3. Girder patterns frequently associated with large and small vascular bundles (×450). Both vascular bundle types have an inner bundle sheath (IB) of thick-walled cells and an outer bundle sheath (OB) of thin-walled cells with few chloroplasts. The two types of girder are sclerenchyma girders (SG) and outer bundle sheath girders (BG) that may connect with sclerenchyma strands (SS) in the epidermis. Note extensive intercellular spaces and chloroplasts in the mesophyll chlorenchyma (CH). **A,** Large vascular bundle with two girders, one of each type. **B,** Large vascular bundle with two sclerenchyma girders. **C,** Small vascular bundle with no girders. **D,** Small vascular bundle with one bundle sheath girder. **E,** Small vascular bundle with two bundle sheath girders. **F,** Small vascular bundle with two girders, one of each type. Bar = 25 μm.

for the 12 lines in the field were 256, 695, and 454 μm. Regressions of leaf spot width on leaf spot area or length showed highly significant coefficients of determination ($R^2 > 0.95$), indicating that measurements of width provide a meaningful indicator of susceptibility (data for length and area not given).

Large vascular bundles alternated with small vascular bundles in a quite regular pattern of large-small-large-small vascular bundles (Fig. 2A), although sometimes two or three adjacent vascular bundles were of one type. About 46% of the vascular bundles in the leaves from the greenhouse and 45% in leaves from the field were of the large type (Table 1). Leaf spot width was positively associated with frequency of large vascular bundles in the greenhouse trial but not in the field trial (Table 1). In either trial there was a narrow range among plants for width of large vascular bundles, width of small vascular bundles, and distance between vascular bundles; leaf spot width was not related to these traits (Table 1).

In resistant plants, leaf spots usually were confined between adjacent vascular bundles (Fig. 2B). Leaf spots occasionally crossed small vascular bundles but did not cross large vascular bundles of resistant plants. In susceptible plants, spots crossed some large vascular bundles (Fig. 2C) and crossed small vascular bundles more frequently (Table 1). Regressions of leaf spot width on frequency of small vascular bundles crossed were significant in both trials (Table 1).

Leaf spot width and girder traits. For convenience we used the designations *s, b,* and *o* for sclerenchyma girders, bundle sheath girders, and no girders, respectively. Vascular bundles had zero, one, or two girders. Thus there were six possible girder patterns for a given vascular bundle: *oo, bo, so, bb, bs,* and *ss*. All six patterns were associated with each type of vascular bundle (Table 2).

More than 85% of large vascular bundles had two girders (sum of patterns *bb, bs,* and *ss*) (Table 2). The *bs* and *ss* patterns (Fig. 3A and B) were the most frequent patterns for large vascular bundles. Fewer than 1% of large vascular bundles lacked girders (*oo*). Regression analysis was done for leaf spot width vs. each girder pattern and all possible combinations of girder patterns (*bo, so, bo + so,* etc.). The regressions indicated no relation between leaf spot width and girder pattern of large vascular bundles, with one exception. The exception was a positive association of frequency of the *bo* girder pattern and leaf spot width in the field trial (Table 2). The percentage and range of *bo* girders was small, indicating that this may not be an important trait in resistance.

Small vascular bundles had a lower frequency of girders than large vascular bundles. About 44 and 38% of small vascular bundles from the greenhouse and field, respectively, had no girders (Fig. 3C) and 32–40% had only one girder (Fig. 3D and Table 2). About 24 and 21% of small vascular bundles, respectively, had two girders (Fig. 3E and F). Plants varied in frequency of small vascular bundles with two girders from about 5% in the more susceptible plants to about 50% in the more resistant plants in the greenhouse. The corresponding range of small vascular bundles with two girders was 10–34% in the field. Regressions of leaf spot width vs. percentage of small vascular bundles with two girders were significant and negative ($R^2 = 0.409$ in the greenhouse; $R^2 = 0.548$ in the field). No other combination of girder patterns provided as strong an R^2 as the combination of *bb + bs + ss* (complete data not given).

TABLE 1. Vascular bundle traits of orchardgrass leaves and regressions of leaf spot width (μm) on the traits

Trait[c]	Greenhouse[a]					Field[b]				
	Mean	Range	R^2[d]	Intercept	Slope	Mean	Range	R^2	Intercept	Slope
Large bundles, percentage of all bundles	46.0	37.1–57.6	0.374*	−335	16.51	45.1	41.0–50.0	0.124		
Width of large bundles, μm	69.2	62.0–78.0	0.027			59.3	50.0–66.0	0.010		
Width of small bundles, μm	25.3	22.0–31.0	0.015			26.7	23.0–29.0	0.005		
Distance between large bundles, μm	390.0	329–469	0.122			339.0	309–379	0.014		
Distance beween any bundles, μm	187.0	163–207	0.001			155.0	139–166	0.050		
Large bundles crossed/leaf spot, number	0.07	0–0.23	0.262			0.14	0–0.47	0.898**	0.336	0.827
Small bundles crossed/leaf spot, number	1.04	0.19–2.08	0.739**	0.159	0.254	0.85	0.24–1.51	0.920**	0.145	0.362

[a] Mean of 12 plants; range gives means of the two extreme plants.
[b] Mean of three replications of 12 F_1 lines; range gives means of the two extreme lines.
[c] Large vascular bundles are characterized by prominent metaxylem vessels; small vascular bundles lack enlarged metaxylem vessels.
[d] Coefficient of determination of regression; intercept and slope given only if R^2 is significant at the 0.05 (*) or 0.01 (**) probability levels.

TABLE 2. Girder patterns associated with vascular bundles and regressions of leaf spot width (μm) on the girder pattern

Vascular bundle type[c]	Girder pattern[d]	Greenhouse[a]					Field[b]				
		Mean	Range	R^2[e]	Intercept	Slope	Mean	Range	R^2	Intercept	Slope
Large	*oo*	0.4	0–5.3	0.004			0.2	0–1.5	0.157		
	bo	11.0	5.3–23.8	0.100			8.1	4.9–12.7	0.598**	70	47.5
	so	2.4	0–11.8	0.001			3.4	0–8.0	0.016		
	bb	10.3	0–50.0	0.001			1.4	0–6.7	0.109		
	bs	35.7	20.0–52.6	0.013			36.2	24.4–54.2	0.092		
	ss	40.3	0–66.7	0.034			50.9	29.3–60.1	0.022		
	two girders[f]	86.2	71.4–94.7	0.064			88.4	81.1–93.3	0.327		
Small	*oo*	43.7	21.4–68.4	0.168			38.2	27.0–53.9	0.004		
	bo	29.5	4.3–61.9	0.176			32.9	16.0–55.0	0.245		
	so	2.8	0–17.4	0.259			7.6	2.2–13.9	0.004		
	bb	6.9	0–36.4	0.015			3.5	0–11.4	0.497**	557	−29.8
	bs	16.6	0–50.0	0.257			17.7	10.0–29.8	0.229		
	ss	0.5	0–5.9	0.004			0.2	0–1.5	0.087		
	two girders	24.0	5.3–50.0	0.409*	583	−6.63	21.4	10.0–34.2	0.548**	783	−15.5

[a] Mean of 12 plants; range gives means of the two extreme plants.
[b] Mean of three replications of 12 F_1 lines; range gives means of the two extreme lines.
[c] Large vascular bundles are characterized by prominent metaxylem vessels; small vascular bundles lack enlarged metaxylem vessels.
[d] Each bundle can have zero, one, or two girders. *o* = no girders, *b* = bundle sheath type of girder, *s* = sclerenchyma type of girder.
[e] Coefficient of determination of regression; intercept and slope given only if R^2 is significant of the 0.05 (*) or 0.01 (**) probability levels.
[f] Sum of patterns *bb*, *bs*, and *ss*.

Lignin content. Leaf spot size scores of the 14 lines ranged from 3.38 to 6.93 with a mean of 5.36 in the January trial. The scores ranged from 3.65 to 7.20 with a mean of 5.44 in May. Analysis of variance for data combined over trials indicated that the effect of line on leaf spot size was highly significant ($P = 0.01$), but the effects of trial and line × trial were not significant.

Trial was the major source of variation in lignin content (Table 3). The plants grown in winter had significantly lower lignin than the plants grown in spring. In either trial, inoculated leaves had a slightly higher lignin level than uninoculated controls, and the effect of inoculation was significant at $P = 0.05$. Differences among lines, although not great (note low standard deviations in Table 3), were significant and contributed more to variation than did the trial × line and inoculation × line interactions.

Regression analysis showed a weak, positive association of leaf spot size with lignin content of inoculated leaves in January ($R^2 = 0.292$; $P = 0.046$; intercept = −4.50; slope = 2.69). No significant association was found between leaf spot size and lignin content of uninoculated controls in January or between leaf spot size and lignin of inoculated or control plants in May.

DISCUSSION

When initiating a program of recurrent phenotypic selection for resistance to Stagonospora leaf spot, we were concerned that the basis for small leaf spot size might involve close spacing of veins and high amounts of lignified cells associated with veins. Earlier authors reported that growth of fungi in gramineous species was impeded by lignified sclerenchyma (1,8). Forage quality is inversely related to lignin content (13), and we postulated that resistant germplasm might have lower digestibility due to higher lignin content. Although the first three cycles of selection increased the level of resistance (22), Oberheim et al (17) found no difference in digestibility among the cycles. In the present study, regression indicated that genotypes with small leaf spot size do not have increased size or numbers of large vascular bundles, smaller distance between vascular bundles, or increased amounts of constitutive lignin. In the greenhouse trial, leaf spot width was positively associated with percentage of vascular bundles classified as large, a trend opposite to that expected if large vascular bundle size conferred resistance. The results indicate that resistant germplasm may be developed without increasing poorly digested components.

Because differences in leaf spot width among plants or lines were not related to distance between vascular bundles, and wide leaf spots were wider than vascular bundle spacing, it followed that the differences between narrow and wide leaf spots would be related to the number of vascular bundles that the spots crossed. Small vascular bundles were more vulnerable to crossing than large vascular bundles: compare 1.04 vs. 0.07 crossings per leaf spot for small and large vascular bundles, respectively, in the greenhouse trial, and compare 0.85 vs. 0.14 in the field trial (Table 1). The significant positive regression of leaf spot width vs. number of small vascular bundles crossed (Table 1) indicated that small vascular bundles of susceptible plants were crossed more frequently than small vascular bundles of resistant plants.

Crossing of vascular bundles was related to girder pattern in two respects. First, the greater vulnerability of small vascular bundles than large vascular bundles to crossing was related to the higher percentage of small vascular bundles than large vascular bundles having less than two girders; in the greenhouse trial about 76% of small vascular bundles lacked two girders, while only 14% of large vascular bundles did not have two girders (Table 2). Second, the decrease in crossing of small vascular bundles in plants with small leaf spots compared with susceptible plants was associated with an increase in frequency of small vascular bundles with two girders in resistant plants (Table 2). The coefficients of determination (R^2) indicated that about 40–55% of leaf spot width could be accounted for by absence of two girders on small vascular bundles. Apparently a leaf spot was less likely to cross a small vascular bundle girdered top and bottom than one with less girdering. Because the combination of *bb* + *bs* + *ss* girder patterns gave a higher R^2 than any other combination of girder pattern, it appeared that both the bundle sheath and the sclerenchyma types of girder were effective in contributing to decreased crossing of veins. No other anatomical feature was as strongly related to leaf spot width as was the girdering of small vascular bundles.

S. arenaria grows slowly in intercellular spaces of the chlorenchyma and shows no indication of degrading cell walls (19). A completely girdered vein presents a barrier of cell walls minimally interrupted by intercellular passageways between adjacent intercostal areas. Walls of girdered veins may impede growth of *S. arenaria* from one intercostal area to the next one. Or, as Akai and Fukutomi (1) suggested for other diseases, preformed physical barriers may slow the passage of fungi long enough to permit induced inhibitory mechanisms to act. The association of a barricadelike feature (the fully girdered vein) with resistance does not prove that the feature confers resistance. We are not aware of any other study showing a relation of leaf vein type and girder pattern to resistance.

Girder pattern could not entirely account for restriction of leaf spot size. Some fully girdered large vascular bundles of susceptible plants were crossed by leaf spots. Many small vascular bundles of resistant plants had no girder or one girder, but leaf spots stopped at these vascular bundles. Furthermore, longitudinal growth of leaf spots varied between resistant and susceptible plants, but there was no obvious anatomical feature limiting longitudinal growth. The nature of resistance components in addition to vein features is unknown.

Resistance was not related to constitutive lignin content of uninoculated plants. Infection may have induced formation of ligninlike compounds, as indicated by the higher mean lignin content of inoculated leaves. Induced lignification during penetration of cell walls has been implicated in resistance of grasses to fungi (21). Examination of lignin 2 wk after inoculation does not adequately test the role of induced lignin in resistance. Rapidity of localized response, rather than long-term synthesis of lignin, may be critical to resistance (10).

TABLE 3. Lignin content of orchardgrass leaves

Inoculated	January trial (%) Mean ± SD	May trial (%) Mean ± SD
No	3.49 ± 0.32	4.34 ± 0.30
Yes	3.66 ± 0.32	4.42 ± 0.31
Analysis of variance		
Source	df	MS
Trial	1	54.500**[a]
Block in trial, error a	10	0.259
Inoculation	1	1.300*
Trial × inoc	1	0.201
Block × inoc in trial, error b	10	0.219
Line	13	0.622**
Trial × line	13	0.159**
Inoc × line	13	0.154**
Trial × error × line	13	0.138**
Error c	260	0.053

[a] *, ** = F test significant at $P = 0.05$ and 0.01, respectively.

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