Influence of Phaseolus vulgaris Blossoming Characteristics and Canopy Structure upon Reaction to Sclerotinia sclerotiorum

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ABSTRACT

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Bean (*Phaseolus vulgaris*) lines or cultivars with indeterminate growth habit produced more blossoms, and had more colonized senescent blossoms than did those with determinate growth habits, but senescent blossoms accumulated within and around both types; thus, many potential sites for colonization by *Sclerotinia sclerotiorum* were present in both. However, disease severity was not always correlated with frequency of colonizable sites. The structure of a dry edible bean canopy affected white mold disease incidence and severity. The most susceptible entries produced a canopy that was significantly more dense in terms of leaf area × dry weight/height than was that of resistant types. The growth habit of the plant; i.e., determinate or indeterminate, did not exclusively influence the incidence of infection. The more critical determinant of disease severity was conferred by the distribution of the leaf area, especially near the ground. Bean germplasm can be screened in the greenhouse for reaction to *S. sclerotiorum* if suitable methodology is utilized.

Additional key words: *Whetzelinia sclerotiorum*, epidemiology, infection, disease avoidance.

Sclerotinia sclerotiorum (Lib.) de Bary = *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont (18) has a wide host range which includes 374 species of 237 genera in 62 plant families (23) and causes white mold disease of dry edible beans. This disease is the major production problem of commercial Great Northern (G.N.) and Pinto cultivars in the North Platte Valley in western Nebraska. Chemical control (29) and cultural practices such as crop rotation (10) have not effectively controlled this disease. Field resistance or tolerance has recently been identified in *Phaseolus* germplasm (3, 4, 7, 12, 28).

Row spacing, plant growth habit, and planting density influence bean canopy development and white mold disease incidence and severity (5, 6, 9, 11, 13, 16, 27, 30). Some cultivars are not severely infected by *S. sclerotiorum* by virtue of a disease avoidance mechanism(s), due to the plant structure. An open plant canopy facilitates air circulation and light penetration and results in a more rapid drying of dew-covered leaf and moist soil surfaces (11), thereby reducing microclimatic conditions favorable for fungal development. Since senescent blossoms are sites for the primary colonization and subsequent plant infection by *S. sclerotiorum* (1, 2, 10, 15, 17, 19), cultivars that differ with respect to their time and quantity of production could exhibit differences in disease incidence. These observations prompted a study of specific bean blossom production and canopy structure characteristics in relation to white mold disease.

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MATERIALS AND METHODS

Experimental design.—Dry edible bean breeding lines such as near-isogenic G.N. Code P #82 (vine) and G.N. Code P #92 (compact bush) and cultivars G. N. Tara (vigorous vine), small white Aurora (upright semi-vine), and dark red kidney Charlevoix (tall open bush) were planted on 2 June 1975, and 1 June 1976. All entries were planted in a randomized complete block design, and one of the five (6 rows wide, 4.5 m in length) and two (12 rows wide, 10 m in length) blocks planted in 1975 and 1976, respectively, were chosen randomly for determination of canopy characteristics. Distance between rows was 56 cm and the field was furrow irrigated in alternate rows every 7-10 days.

Granular applications of the insecticide Disyston {0,0-diethyl S-[2-(ethylthio) ethyl] phosphorodithioate} and the herbicide Eptam (S-ethyl n,n-dipropylthiocarbamate) were incorporated during planting. Liquid foliar applications of Sevin (1-naphthyl N-methylcarbamate) were applied every 7-10 days during late July to early August to prevent infestation by the Mexican bean beetle.

Blossom production.—Blossom production was estimated by counting all newly-opened blossoms at 3-to 4-day intervals on an average of 25-30 randomly-selected plants per entry during 17 July to 30 July 1975, and 21 July to 4 August 1976. The blossoms which had senesced or aborted and fallen directly below these plants onto the soil surface also were counted every 3-4 days during 17

July to 19 August 1976, and 25 July to 4 August 1976. The number of senescent or aborted blossoms observed to be colonized by *S. sclerotiorum* on the soil surface on 12 August 1976, also was recorded.

Canopy density.—Canopy density of between-row, within-row, or total plant canopy was calculated as follows: Canopy density (cm/g) = total leaf area (cm^2)/average plant height (cm) × total dry weight (g). Canopy samples were collected from two randomly-selected 48-cm row lengths within each entry on four dates in 1975 (30 July; 6, 13, and 19 August) and in 1976 (26 July; 2, 9, and 16 August). Samples were divided into two components, one within the plant row and the other between plant rows and over the irrigated row. The within-row component measured 28 cm in width and was that portion of the canopy centered on the main vertical stem. The between-row component measured 28 cm in width and was that portion of the plant canopy which extended laterally from the within-row site into the irrigated furrow. The within-and between-row samples were divided into 10-cm levels from the ground to the top of the plant using a thin wire grid as a guide. Canopy leaf samples were cut with scissors, hand picked from each component, and their total area was measured directly with a Model LI-3000 leaf-area meter (Lambda Inc., Lincoln, NE 68503). The weight of stem, vine, and leaves after drying at 50-60 C for 2-3 days was used for the total dry weight per sample component.

Disease reaction.—Disease incidence and severity represent the average percentage of the above-ground plant showing signs of *S. sclerotiorum* infection. The data were collected on 1 September 1975, from 25 plants in five replicates and 31 August 1976, from all plants in 3-m row length in three replicates.

RESULTS

Blossom production.—Variation in blossom production occurred within and between entries in 1975 and 1976 (Table 1). The indeterminate (vine) types generally produced significantly greater total numbers of blossoms although the determinate (bush) types produced comparable numbers of blossoms in 1976. Blossom production was not monitored closely after the 1st wk of August because of the dense vine growth. However, periodic observations revealed that many blossoms still were being produced during mid- to late August, especially by those plants with an indeterminate growth habit.

Although many senescent blossoms had fallen onto the soil surface, not all were colonized by *S. sclerotiorum*. A significantly higher number of senescent blossoms were present on the soil surface beneath Tara than the other lines or cultivars in 1976, and many of these blossoms became colonized by the white mold fungus. There were very few colonized blossoms beneath Aurora or Charlevoix in 1976 (Table 1), or beneath any of the entries in 1975 (less than 0.02 per plant). To determine if these noncolonized blossoms were escapes or resistant to *S. sclerotiorum*, a greenhouse test was conducted. A *S. sclerotiorum*-infested oat kernel or a drop of ascospore suspension (10^4/ml) was placed on each senescent

TABLE 1. Blossom production by plants of dry edible bean breeding lines and cultivars, and blossom colonization and severity of infection by *Sclerotinia sclerotiorum* in western Nebraska

Breeding line or cultivar	Growth habit	Accumulative number blossoms per plant[u]				Colonized blossoms[v] on soil	Disease[w] severity (%)	
		Recently opened		Senescent on soil				
		1975	1976	1975	1976	1976	1975[x]	1976[y]
G.N. Code P #82	vine	20.8 a	13.4 b	11.8 a	6.9 b	0.90 b	18.8 b	20.7 b
G.N. Code P #92	compact bush	4.6 c	12.6 b	3.5 bc	4.9 b	0.82 b	34.6 a	51.3 a
G.N. Tara	vigorous vine	10.5 b	23.1 a	6.7 b	15.7 a	2.30 a	46.0 a	56.1 a
Aurora (small white)	upright semi-vine	9.8 b	8.4 c	6.6 b	5.8 b	0.02 b	11.4 bc	0.5 c
Charlevoix (dark red kidney)	open bush	6.8 bc	13.6 b	1.7 c	6.8 b	0.05 b	3.8 c	0.0 c

[u]An overall cumulative plant average was calculated by adding the average production of blossoms per plant (17 July to 30 July 1975, and 21 July to 4 August 1976) or senescent blossoms on the soil surface (17 July to 19 August 1975, and 21 July to 4 August 1976) at each observation date.

[v]The number of blossoms colonized by *S. sclerotiorum* in 0.42 m^2 of irrigated row on 12 August 1976. Data converted to colonized blossoms beneath an individual plant from plant density data.

[w]Sclerotium populations after planting were determined to be 0.17 ± 0.10 in 1975 and 0.91 ± 0.17 in 1976 by previous methods (24).

[x]Average individual plant infection determined from five replicate groups each of 25 plants on 1 September.

[y]Average individual plant infection determined for all plants in three replicate 3-m sections of row on 31 August.

[z]Mean separation within columns by Duncan's multiple range test, $P = 0.05$.

blossom obtained from each of the different bean entries. After incubation in 90-100% relative humidity at 18-22 C, all blossoms were colonized, irrespective of color or genotype. The cultivars and lines used were as follows: G. N. UI #59, G. N. 1140, Pinto UI 111, Valentine, a Black Turtle Soup selection (SP-1), and some *P. vulgaris* and *P. coccineus* Plant Introduction lines.

Senescent blossoms frequently adhered to plant organs such as leaves, pods, or in branch and stem axils within the plant canopy, especially in indeterminate entries and Code P #92. Many of these blossoms subsequently became colonized and provided initial sites for above-ground infection during mid-to late August in both years.

Canopy density.—Only data collected on the last two dates in either year were considered pertinent to infection during the white mold epidemic. Although there was a 2- to 3-day delay in determining the leaf area of some samples, no measurable loss in leaf turgor or size occurred except for a few leaves which were accidentally damaged during harvesting.

Bean cultivars or breeding lines develop various types of canopy structure, which although distinctive, can change during the growing season. Representative canopy density data are shown in Fig. 1. The canopy architecture produced by the indeterminate growth habit of G.N.Tara and Code P #82 changed appreciably from 13 to 19 August 1975. This change coincided with an observed shift from vegetative plant development (increase in leaf area) into a reproductive stage of pod set and seed development. Also, the increasing weight of newly-formed pods appears to pull the canopy closer to the soil surface, especially during late August. The canopy structure of the other entries was relatively constant and appeared to be influenced less by morphological changes during plant development.

Most of the leaf area of the indeterminate G.N. Code P #82 and Tara was present in the lower canopy levels near the soil (Fig. 1). In comparison, the leaf area of the determinate G.N. Code P #92 and Charlevoix was more evenly distributed throughout the canopy profile. However, Charlevoix was different in that much of its canopy was produced above the 40-cm level, and the lower levels were noticeably more open than those of the other entries. The upright semi-vine Aurora had the next most open canopy structure which was distributed throughout the 0- to 30-cm levels.

Canopy data at each level used for Fig. 1 were combined to give within-plant-row, between-plant-rows

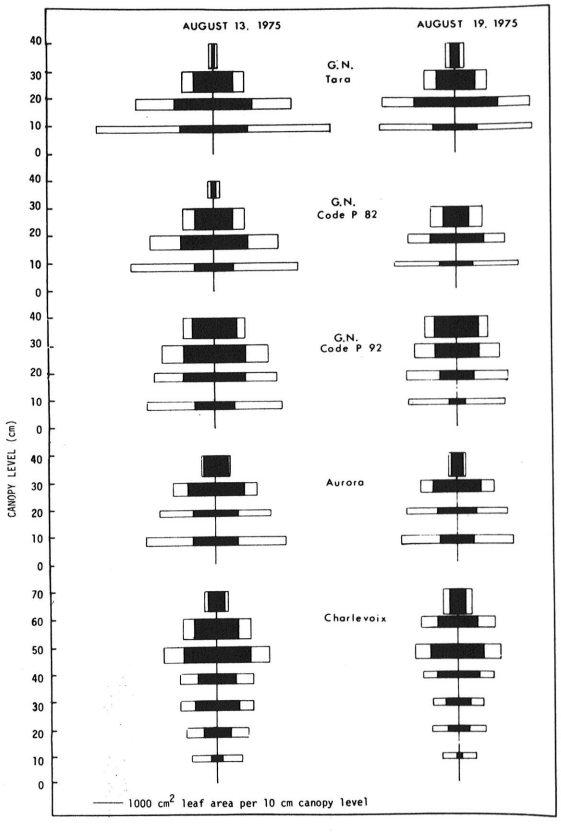

Fig. 1. Diagrammatic representation of dry edible bean canopy structure observed in western Nebraska on two dates in 1975. Between-row component (□) and within-row component (■). The relative thickness of each canopy level was determined by the percentage of leaf fraction contributing to the dry weight of that level. Length of bars was determined from leaf area.

TABLE 2. Plant canopy structure of dry edible bean breeding lines and cultivars grown in western Nebraska on 9 August 1976

Breeding line or cultivar	Between-row component[x]			Within-row component[x]			Canopy total			Plant height (cm)
	Leaf area (cm^2)	Dry wt. (g)	Canopy[y] density	Leaf area (cm^2)	Dry wt. (g)	Canopy density	Leaf area (cm^2)	Dry wt. (g)	Canopy density	
Code P #82	5,467 ab	36.3 a	4.86 a	6,442 a	69.3 a	2.82 a	11,909 ab	105.6 a	3.43 a	33 c
Code P #92	5,231 ab	29.8 a	4.88 a	7,224 a	82.1 a	2.36 a	12,455 ab	111.8 a	2.98 ab	38 b
Tara	8,271 a	48.4 a	4.55 a	7,689 a	77.5 a	2.67 a	15,960 a	125.8 a	3.39 ab	38 b
Aurora	4,925 ab	27.1 a	4.71 a	6,957 a	80.3 a	2.26 a	11,882 ab	107.4 a	2.87 ab	38 b
Charlevoix	3,604 b	21.6 a	3.71 a	6,505 a	73.5 a	1.84 a	10,109 b	95.1 a	2.22 b	48 a

[x]Within-row = portion of canopy centered on the main vertical stem and 14 cm on either side; between-row = portion of canopy that extended into the irrigated furrow (14 cm) on each side of the plant row.

[y]Canopy density (cm/g) = total leaf area (cm^2)/average plant height (cm) × total dry weight (g).

[z]Mean separation by date within columns by Duncan's multiple range test, $P = 0.05$.

(irrigated furrow), and total-plant comparisons for leaf area, plant dry weight, and canopy density. Representative sampling data at one date in 1975 are presented in Table 2. The between-row component of Tara had the greatest leaf area on most dates and the greatest dry weight on all dates. There were few significant differences in between-row canopy density when all the breeding lines or cultivars were compared. On the other hand, most entries had comparable leaf area and plant-dry-weight values for the within-row component, although the canopy density values differed. Aurora and Charlevoix always had a total canopy density less than that of Tara or Code P #92, but the differences were not usually significant. Tara usually had a greater total leaf area and total canopy density than the other entries. The compact bush (Code P #92) had a slightly greater total leaf area than its near-isogenic vine counterpart (Code P #82), but was similar in other respects.

Disease reaction.—The severity of *S. sclerotiorum* infection was determined on 1 September in 1975 and 1976, and was similar in both years (Table 1). Tara and Code P #92 were most severely infected, and Code P #82 also was infected moderately. Aurora and Charlevoix had low white-mold severity in 1975 and were essentially disease-free in 1976.

Yield data were collected in both years. However, because of differences in plant maturity, yielding ability, and adaptability the yield differences attributable to white mold disease are unknown. Thus, these data are not presented.

A series of growth-room experiments was designed to determine which environmental factors were critical for the selection of resistant cultivars under controlled conditions. Detached plant organs such as leaves, stems, pods, or cotyledons and intact plants of different ages were inoculated with infested oat kernels, colonized bean tissue, or colonized senescent blossoms (blossoms drenched 2-3 days earlier with an ascospore suspension, 10^4/ml). Inoculated plants or plant organs were incubated for 5-10 days at different temperatures in 80-100% relative humidity with 8-12 hr of fluorescent lighting (G.E. cool-white, 400-W). The mature-plant (post-bloom) reactions of susceptible G.N. Tara and resistant Charlevoix, Valentine, and a Black Turtle Soup selection (SP-1) were distinguishable following inoculation of the plants with colonized bean blossoms and incubation at 20-22 C. Resistant reactions included fungal localization within a 10 to 40 mm diameter water-soaked lesion, occasionally accompanied by a light-brown or reddish-brown border. Susceptible reactions usually consisted of a 30- to 100-mm diameter water-soaked lesion followed by branch or complete plant wilt within 7-10 days after inoculation. All cultivars were susceptible at temperatures of 16-19 C, and the fungus caused no infection at temperatures of 28-32 C.

DISCUSSION

Successful infection by *S. sclerotiorum* requires prior colonization of senescent plant organs such as blossoms, cotyledons, leaves, seeds, seed capsules, pollen, or injured plant tissue (1, 2, 9, 10, 15, 17, 19, 20, 21, 25). The abundance of senescent bean blossoms and leaves which accumulated within and around plants of all entries indicates that many potential sites for *S. sclerotiorum* colonization existed, irrespective of growth habit. However, plants of entries with an indeterminate growth habit produced more blossoms and had more colonized senescent blossoms than did those with a determinate growth habit. However, disease severity on determinate Code P #92 was significantly greater than on indeterminate Aurora in both years. Thus, despite the greater numbers of colonizable blossoms on Aurora, disease severity was not greater.

Many blossoms were not colonized even though they were beneath the canopy of a susceptible indeterminate entry which was severely infected. Since blossoms of all entries were colonized in greenhouse trials, these field escapes must be attributable to either unfavorable microclimatic conditions or lack of inoculum (ascospores). Microclimatic conditions had to be favorable for some blossom colonization and plant infection; therefore, field escapes probably reflected lack of a sufficient number of ascospores at the blossom site. However, inoculum sources both local and from outside the bean field still caused enough blossom colonizations to incite a detectable incidence of white mold infection on Aurora in both 1975 and 1976 and also on Charlevoix in 1975.

In general, canopy growth and development were greater in 1975 and may be due to the effect of a nearby windbreak (22). Thus, data for the same breeding line or cultivar in the 2 yr may not be comparable.

White mold disease severity is correlated with dry bean canopy structure. The more susceptible entries, G.N. Tara and Code P #92, produced a canopy that was significantly more dense, especially within the plant row, than that of resistant types. In addition to a more dense canopy, Tara's disease reaction also may be influenced by genetic sensitivity conferred by one of its parents, Tepary *(Phaseolus acutifolius)*, which appears to be highly sensitive to infection by *S. sclerotiorum* (12). Greater irrigation frequency and amount also has been shown to increase inoculum production and plant infection in Tara (8, 24, 26). The field-resistant cultivars, Aurora and Charlevoix, have a more open canopy and an upright type of growth habit. In addition, Charlevoix appears to have some physiological resistance whereby the fungus is localized if infection occurs (Steadman and Schwartz, *unpublished*). A similar localization of infection was reported in soybean cultivars resistant to *S. sclerotiorum* infection (14).

Indeterminate plant growth habit commonly has been associated with increased disease incidence and severity. It is noteworthy that G.N. Code P #92 (bush) was more susceptible than the near isogenic G.N. Code P #82 (vine). Thus, the bush growth habit per se does not decrease the incidence of infection. Instead, the plant canopy structure and canopy density associated with the respective growth habits determine whether microclimatic conditions created within the plant canopy are more or less favorable for colonization and infection.

Haas and Bolwyn (16) reported that high individual plant weight was correlated with white mold infection in Canada and that high plant weight implies a foliage-dense

plant with a high leaf-area index. Analysis of our data for total plant weight and leaf area on an individual plant basis revealed that the canopy of Charlevoix was similar to that of Tara. However, Charlevoix was significantly less prone to infection due to its tall open plant structure, low canopy density, and/or physiological resistance. Therefore, canopy analysis should consider the plant canopy structure in addition to plant weight and leaf area. A cultivar which is designed to maximize disease avoidance should possess an upright growth habit, open plant structure, and low canopy density to ensure that microclimatic conditions within the canopy are unfavorable for local inoculum production (23), blossom colonization, and plant infection by local or outside inoculum.

Abawi et al. (2), Adams et al. (4), and Weihing (*unpublished*) developed greenhouse procedures to screen for resistance to *S. sclerotiorum*. However, these tests select for a very high degree of resistance which is present in *Phaseolus coccineus* or *P. coccineus* × *P. vulgaris* hybrids but found only rarely in *P. vulgaris*. A more sensitive and critical test was needed to detect levels of field resistance or tolerance observed in *P. vulgaris* lines and cultivars (7, 12). In the field, cultivars with physiological resistance are exposed to fluctuating temperature and moisture regimes favorable (by night) and unfavorable (by day) to *S. sclerotiorum* (Blad, Steadman, and Weiss, *unpublished*). Our results have shown that temperature can have a decisive effect on the disease reaction. Thus, it appears that resistant cultivars can limit fungal development during the day and that the fungus may be unable to overcome this limitation during nights when conditions are favorable for infection. Therefore, greenhouse screening programs to select cultivars resistant to *S. sclerotiorum* should utilize environmental conditions comparable to field conditions.

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