

**Negative Relationship of Stomatal Size
and Density with Resistance in Sugar Beet
to *Cercospora beticola***

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ABSTRACT

Resistance to *Cercospora beticola* of sugar beet heart leaves as compared to susceptibility of mature leaves on the same plant was not related to size of stomatal apertures, nor to density or stomatal movement. No association was found between degree of resistance and size of apertures or stomatal density in six sugar beet cultivars.

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Young heart leaves of sugar beet (*Beta vulgaris* L.) are highly resistant, if not immune, to infection by *Cercospora beticola* Sacc., whereas mature leaves are susceptible. Since the fungus can only penetrate through open stomata, differences in susceptibility among leaves on the same plant have been attributed to variations in stomatal aperture size and movement (5, 8). Pool & McKay (5) believed that resistance of young leaves could be explained by their smaller

stomatal openings that closed earlier in the day.

Wingard (8) stated that stomatal apertures of sugar beet heart leaves were too small for penetration by germ tubes of *C. beticola*. Results herein suggest that resistance of young leaves is not primarily due to size of stomatal apertures, nor to density or activity of stomata. Furthermore, examination of six sugar beet cultivars having varied degrees of resistance to leaf spot indicated no consistent association between resistance and size of stomatal apertures or density of stomata.

Three-month-old susceptible sugar beet plants were atomized with a conidial suspension of *C. beticola*. The plants were kept in a humidity chamber at 100% relative humidity and 30 to 32 C under constant light (ca. 520 ft-c). Severe leaf spot developed 10 to 12 days after inoculation in all but young leaves. Thus, resistance of young leaves was manifested under conditions that precluded stomatal periodicity (4, 5).

Heart and mature leaves were harvested from 4-month-old sugar beets growing in the field near Fort Collins, Colo. Leaves of comparable age were selected from five cultivars in three replications of a randomized block design. The cultivars are listed in Table 1. Strips of upper epidermis were removed from the middle of the blades adjacent to the midvein and mounted in lactophenol (equal parts phenol, lactic acid, glycerine, and distilled water) to assure turgidity of the stomatal guard cells (1). Comparisons of stomatal apertures in situ and in lactophenol revealed no significant differences in size. Measurements of aperture length and width were made on 50 stomata/leaf with the aid of a microscope and an ocular micrometer. Mean stomatal density of each cultivar was calculated from counts made from 10 random high-power (X 430) microscope fields per replication. All data were subjected to analyses of variance, and mean separations were performed using Duncan's multiple range test.

Significant differences were found in length and width of stomatal apertures and in stomatal density in heart leaves among the six cultivars (Table 1).

TABLE 1. Comparison of leaf spot disease ratings of six sugar beet cultivars with size of stomatal apertures and density of stomata in the upper epidermis of mature (M) and heart (H) leaves

Cultivar	Leaf spot rating ^{a,b}	Aperture measurements in μ ^{b,c}				Stomata/mm ² ^{b,d}	
		Length		Width		M	H
		M	H	M	H		
52-334	8.3 d	18.8 a	11.7 a	12.4 a	5.1 a	117 a	214 d
R & G Pioneer	6.8 c	13.6 d	6.4 bc	5.5 b	3.2 b	150 a	197 d
US H9B	6.5 c	16.9 b	7.6 b	6.4 b	3.6 b	138 a	236 cd
SP 5822-0	3.0 b	17.4 ab	7.0 c	6.0 b	3.4 b	110 a	310 b
US 201	2.3 ab	15.0 c	5.4 bc	5.8 b	2.9 b	153 a	287 bc
FC (504 x 502/2) x SP 6322-0	1.7 a	14.0 cd	6.4 bc	5.2 b	3.1 b	132 a	384 a

^a 1971 field ratings based on scale of 0 to 10, with 0 = no apparent infection and 10 = complete defoliation.

^b Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

^c Means of three replications, 50 measurements/replication per cultivar.

^d Mean counts from three replications, 10 random high-power (X 430) microscope fields/replication per cultivar.

Differences in stomatal density in mature leaves were not significant. With one exception, differences in aperture size could not be related to degree of field susceptibility. Only 52-334, the most susceptible cultivar, showed a trend toward larger apertures in both mature and heart leaves. Density counts of heart leaves indicated that the most resistant cultivars had more stomata than the highly susceptible lines.

Conidia of *C. beticola* were germinated in water on hanging-drop slides. Measurements of 100 germ tube diameters $15\ \mu$ from their apexes indicated a range from 1.3 to $2.1\ \mu$ (mean = 1.7). Stomatal aperture sizes of heart leaves (Table 1), therefore, would not be limiting to penetration by *C. beticola* in the cultivars examined. Measurements of stomatal apertures of other cultivars not reported herein always exceeded the mean diameter of *C. beticola* germ tubes.

The mechanism of resistance to infection of young sugar beet leaves by *C. beticola* is unexplained. Also, resistance of certain cultivars was not associated with physical characteristics of stomata. Stomatal tropism effects, as postulated by several authors (2, 3, 6, 7), might explain differences in resistance among leaves on the same plant or among cultivars; or, both manifestations of resistance might be governed by different mechanisms. The recent isolation of infection-induced phytoalexinlike compounds from inoculated resistant cultivars (D. D. Maag & G. Johnson, *personal communication*) would not explain

resistance in young leaves where infection does not occur, but could explain differences in resistance among cultivars.

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