Powdery Mildew Development on Soybeans with Adult-Plant Resistance

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ABSTRACT

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Growth of *Microsphaera diffusa* on individual leaves of soybean, *Glycine max* (cultivars Clark, Cloud, Cutler, Hawkeye, Mukden, and Custer), at early seedling stage was followed by remission of fungal growth at later stages of plant development. Variable symptoms of green and yellow 'islands', interveinal necrosis, necrotic specks, and crinkling of the leaf blade appeared at time of remission of fungal growth. Mycelial and symptom development were affected by the cultivar, position and age of the leaf, and age of the plants at the time of inoculation. Leaf symptoms were almost absent on young seedlings when mycelia were abundant or on plants that were first inoculated at 50 days of age and which developed negligible infections. On plants inoculated at 8 days, remission of fungal growth occurred on four cultivars by 28 days after inoculation, and on two other cultivars only 18 days were required. When plants were first inoculated at 29 days of age, all six cultivars exhibited remission of fungal growth by 18 days after inoculation. Development of visible mycelia and symptoms were negligible if plants were inoculated at the fourth trifoliolate stage (50 days of age). Designation of adult plant resistance of *G. max* to *M. diffusa* requires several examinations of the interactions during the life span of the host.

The reactions of soybean *Glycine max* (L.) Merr. cultivars to inoculation with *Microsphaera diffusa,* Cke. & Pk., the pathogen that causes powdery mildew on soybeans (6,8), range from highly susceptible to highly resistant (1–3,5,7,9). Some soybean cultivars are resistant in the field but highly susceptible when grown in the greenhouse, and some appear immune in the field but exhibit mild susceptibility in the greenhouse (2,3,5). Cultivars susceptible at all growth stages may show leaf chlorosis, green islands, rusty patches under the fungal mat, and occasional defoliation; in other cultivars luxuriant growth of the fungus occurs without causing visible host symptoms (7,8). Temperature influences the rate of *M. diffusa* development on soybean cultivars. Mignucci et al (9) reported that powdery mildew developed luxuriantly at temperatures of 18 and 24 C but was arrested at 30 C in susceptible cultivars. Since disease development in resistant cultivars was not affected by the three temperatures studied, it was apparent that another major factor accounted for the variability of reactions observed in some cultivars when tested in the greenhouse and in the field. Therefore, this study of powdery mildew development on cultivars that have been reported to exhibit variable reactions (3) was undertaken by inoculating plants at three different growth stages and maintaining them in controlled environments.

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MATERIALS AND METHODS

Growing conditions. The soybean cultivars Clark, Cloud, Cutler, Hawkeye, Mukden, and Custer were selected for this study. Seeds of each cultivar were dusted with thiram and sown in pots (20-cm diameter). Later, plants were thinned to four per pot. Seeds were sown on 5 June, 26 June, and 17 July. Each sowing group was grown in three growth chambers programmed for 22 ± 1 C during the 14-hr day (flourescent bulbs, daylight-type, irradiance of 200 μEin/m^2/sec), 18 ± 1 C during the 10-hr dark period and 50-65% relative humidity. Control plants of each sowing group were kept isolated in another chamber under similar conditions.

Inoculations. All plants, except those used as controls, were simultaneously inoculated by shaking heavily infected Harosoy soybean plants over them. At inoculation (25 July), the three sowing groups were at growth stages, V1, V3, and V5, (ie, one, three, and five leaf stages, respectively) (4). After inoculation, the heavily infected Harosoy plants (four pots containing four plants each)

that served as the source of inoculum for each sowing group were kept inside the growth chambers for the duration of the experiments. In this manner, the inoculation of developing leaves came from the movement of *M. diffusa* conidia in air currents within the growth chamber.

Disease assessment. Percent leaf area covered by the fungus was estimated and symptoms were described on each leaf at the first, second, third, and fourth node of each plant 8, 14, 18, 28, and 38 days after the first date of exposure to the inoculum Pathogen development was estimated according to a 0 – 5 scale in which 0 = absence of mycelia, and 1, 2, 3, 4, and 5 = 12, 30, 50, 75, and 100% leaf area covered by the fungus. Leaves were marked to permit disease assessments on the same leaves each time.

RESULTS AND DISCUSSION

Two major events occurred during *M. diffusa* infections on the six cultivars tested. First, the fungus successfully grew on leaves at the early seedling stage; and, second, fungus remission occurred on the same leaves (Tables 1 and 2) at laater growth stages. Symptoms (Table 3) on individual leaves were variable and their appearance coincided with fungus remission. Most common symptoms were green and yellow 'islands,' interveinal necrosis, necrotic specks, and crinkling of the leaf blade. Pathogen and symptom development appeared to be affected by the cultivar, age and position of the leaf, and age of the plant at the time of inoculation. Leaf symptoms were almost absent on young seedlings when mycelia were abundant and in the oldest group of plants that showed negligible infections. As symptoms became visible, fungal colonies declined. The plants exposed to inoculum from the time they were 8 days old (Table 1) exhibited a drastic reduction of the leaf area covered by the fungus by the time they were 36 days old, even on cultivars that initially were heavily colonized. Only 18 days were required for plants inoculated at 29 days (Table 2) to have remission of fungal growth. The unifoliolate leaf appeared to be the most susceptible leaf on the plants inoculated at 8 days (Table 1). At 14 and 18 days after beginning of inoculation, there was more fungal growth on unifoliolate leaves of plants inoculated at 8 days than on the same leaf position of plants inoculated at 29 days. In general, the lower leaves of young seedlings (Table 1) seemed to be more susceptible than did leaves at higher positions. In addition, there were fewer infections on successively higher leaves. Some leaves on some cultivars (Cloud, Custer, and Cutler, Tables 1 and 2) never supported visible fungal growth. Colonization of leaf tissue was greatest on Custer followed by Clark.

On plants inoculated at 29 days (Table 2), visible fungal growth never developed on primary leaves of cultivars Cloud, Cutler, and Hawkeye. In this group, the first trifoliolate was the most susceptible leaf on Clark, Cloud, Cutler, Custer, and Mukden. On Hawkeye, the second trifoliolate leaf was the most susceptible to infection by *M. diffusa.* Infection of unifoliolate leaves was delayed on Clark soybeans compared to Custer and Mukden (at 8 days after inoculation they already were showing fungal growth on that leaf). Moreover, 8 days after inoculation, the primary leaves of Clark were susceptible when plants were inoculated at the V1 stage (Table 1) but less susceptible when inoculated at the V3 stage (Table 2). Clark, Custer, and Hawkeye maintained low levels of fungal growth (2 – 4%) on the second and third trifoliolate leaves, while Cutler showed no visible fungus growth at any leaf after 38 days of continuous exposure to the fungus (plants 67 days old). Cloud supported low levels of fungal growth only on the first trifoliolate leaves for the same period. Only Hawkeye had visible mycelia on leaves at the fourth nodes (23, 9, 2, and 2% at 43, 47, 57, and 67 days old, respectively).

The development of visible mycelia and of symptoms was negligible when plants were inoculated at the V5 stage (50 days old). At 8 days after inoculation, Clark had a few scattered fungal mycelia on the second trifoliolate leaves. All cultivars in this group, except Custer and Mukden (less than 2%), showed no visible fungal colonies at 14 days after exposure to the inoculum. At 28 days after inoculation, a few colonies (less than 1% leaf area covered by mycelia) had appeared on Clark soybeans.

The general tendency when evaluating cultivars for disease development in the greenhouse is to inoculate seedlings to speed up results and to increase the number of tests in the space available. On the other hand, when evaluating cultivars in the field, most workers

TABLE 1. Percentage of leaf area covered by *Microsphaera diffusa* on individual leaves of soybean cultivars exposed continuously to inoculum since the plants were at the unifoliolate stage (8 days old)

Cultivar	Leaf	Leaf area covered by the fungus (%) on plants of indicated age (days)				
		16	22	26	36	46
Clark	unifoliolate	8[a]	55	70	0	–
	1st trifoliolate	–[b]	0	46	6	0
	2nd trifoliolate	–	–	0	7	0
Cloud	unifoliolate	0	44	46	6	–
	1st trifoliolate	–	3	15	14	5
	2nd trifoliolate	–	–	0	0	0
Custer	unifoliolate	1	84	76	7	–
	1st trifoliolate	–	0	70	1	0
	2nd trifoliolate	–	–	0	0	0
Cutler	unifoliolate	0	34	12	2	–
	1st trifoliolate	–	3	12	6	2
	2nd trifoliolate	–	–	0	0	0
Hawkeye	unifoliolate	0	36	30	14	–
	1st trifoliolate	–	0	48	32	40
	2nd trifoliolate	–	–	0	17	12
Mukden	unifoliolate	0	36	53	1	–
	1st trifoliolate	–	0	32	32	27
	2nd trifoliolate	–	–	0	17	6

[a] Mean percentage of leaf area covered by the fungus based on 16 plants.
[b] Represents the absence of a leaf because either the leaf had not formed or had abscised.

TABLE 2. Percentage of leaf area covered by *Microsphaera diffusa* on individual leaves on soybean cultivars exposed continuously to inoculum since the plants were at the second trifoliolate leaf stage (29 days old)

Cultivar	Leaf	Leaf area covered by the fungus (%) on plants of indicated age (days)				
		37	43	47	57	67
Clark	unifoliolate	0[a]	15	3	1	–[b]
	1st trifoliolate	34	36	30	0	0
	2nd trifoliolate	11	17	30	7	3
Cloud	unifoliolate	0	0	0	0	–
	1st trifoliolate	0	4	1	1	3
	2nd trifoliolate	0	0	0	0	0
Custer	unifoliolate	9	3	14	0	–
	1st trifoliolate	40	47	45	0	2
	2nd trifoliolate	15	32	15	1	3
Cutler	unifoliolate	0	0	0	0	0
	1st trifoliolate	26	26	4	1	0
	2nd trifoliolate	3	7	1	0	0
Hawkeye	unifoliolate	0	0	0	0	0
	1st trifoliolate	30	15	14	4	4
	2nd trifoliolate	38	54	55	15	3
Mukden	unifoliolate	15	24	12	0	0
	1st trifoliolate	45	34	27	4	3
	2nd trifoliolate	24	36	38	0	0

[a] Mean percentage of leaf area covered by the fungus based on observation of 16 plants.
[b] Represents the absence of a leaf because either the leaf had not formed or had abscised.

TABLE 3. Hosts reactions of six soybean cultivars with adult resistance after 28 days of continuous exposure to *Microsphaera diffusa* inoculum, beginning in the V1, V3, or V5 stage of plant development

Plant growth stage[a]	Leaf symptoms			
	Unifoliolate	First trifoliolate	Second trifoliolate	Third trifoliolate
V6	Yellow 'islands' (Cutler and Hawkeye)	Green 'islands' (Cloud, Cutler, Custer, and Clark)	Crinkling of leaf blade (Hawkeye, Cloud, Cutler, and Clark)	Green and yellow 'islands', rusty patches on underside (Cloud and Clark)
R3	Yellow 'islands' (Clark, Custer, Cutler, Hawkeye, Mukden). Rusty patches on underside (Mukden, Cloud, and Cutler). Vein necrosis and dark greening (Cloud). Interveinal necrosis (Mukden and Hawkeye).	Necrotic specks (Mukden, Cloud, and Clark). Yellow 'islands' (Cutler and Custer). Interveinal necrosis and green 'islands' (Cutler).	Yellow 'islands' (Mukden, Cutler, Custer, and Clark). Rusty patches on underside (Mukden, Cutler, and Custer). Crinkling (Mukden).	Crinkling (Hawkeye, Cutler, and Clark). Yellow island' (Hawkeye, Cutler, Clark, and Custer). Rusty patches on the underside (Cutler and Clark).
R5	No leaf symptoms	No leaf symptoms	No leaf symptoms	No leaf symptoms

[a] The growth stage at 28 days after continuous exposure to the inoculum: V6 = plants with completely unrolled leaves at the sixth node, beginning with the unifoliolate node; R3 = plants with pods 0.5 cm (1/4 inch) long at one of the four uppermost nodes with a completely unrolled leaf; R5 = plants with beans beginning to develop at one of the four uppermost nodes in a completely unrolled leaf. Plants were first exposed to the fungus when at the V1, V3, and V5 stages; ie, with one, three, and five leaves respectively on the main stem.

have depended on natural infections that build up on susceptible hosts to provide inoculum for the test plants. With this procedure, disease will usually reach its maximum when test plants are in adulthood. It is very likely, that reactions of cultivars in these tests have been compared between seedling and adult stages of development (2,3). If a cultivar exhibits adult-plant resistance, variable reactions will be found with that system. Adult-plant resistance may not be evident when viewed at a single point in time, as shown by this study. Screening soybean cultivars for resistance to *M. diffusa* could be done in the greenhouse provided that plants are continuously exposed to conidia beginning at seedling stage until near flowering when the expression of adult-plant resistance will become apparent. Since *M. diffusa* development is sensitive to high temperatures (9), temperatures should not exceed 24 C during the screening tests. Soybean cultivars that sustain low populations of *M. diffusa* at the seedling stage, but arrest the development of the pathogen at later growth stages, are as valuable as cultivars that are resistant at all growth stages, especially in areas where the fungus arrives late in the growing season.

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