Bacterial Blight of Soybeans: Seedling Infection During and After Emergence

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Approved for publication by the Associate Director of the Ohio Agricultural Research and Development Center as Journal Article No. 95-71.

The authors gratefully acknowledge the assistance of William B. Beery, H. A. J. Hoitink, and L. E. Williams.

Accepted for publication 2 March 1972.

ABSTRACT

When soybean seeds were planted in soil containing abrasive particles and dried leaves infected with *Pseudomonas glycinea*, the number and size of lesions on cotyledons of emerged seedlings were increased. Most disease was on the proximal end of the cotyledon, which is advanced through the soil as the hypocotyl elongates. Disease was most severe in those areas where the growing hypocotyl usually contacted the cotyledon. Unifoliolate leaves were rarely diseased. No disease occurred on trifoliolate leaves. Upward transfer of inoculum on emerged seedlings was indicated when seedlings that had diseased cotyledons, unifoliolate, or first trifoliolate leaves were subjected to simulated wind-rain storms and new lesions developed on leaves above the organ that was diseased prior to application of the "storm" treatment.

Phytopathology 62:1167-1170.

Additional key words: mechanical injury, storms, soil particle abrasiveness.

Bacterial blight of soybean (*Glycine max* [L.] Merr.), incited by *Pseudomonas glycinea* (Coerper) Stapp, apparently passes through two essential phases in Ohio. One phase is associated with an increase in the inoculum on the seed, or possibly in plant debris in the soil, that subsequently incites disease on seedling foliage. A second and more damaging phase involves outbreaks of the disease on upper parts of older plants, and apparently is dependent on transfer of inoculum from older to younger foliage by foliage contact and wind-blown rain drops, and on injury during a series of severe wind-rain storms (3).

Lesions on cotyledons of emerged seedlings are an obvious source of early inoculum (10). Our purpose in the work reported here was to investigate the initiation of these early lesions, the subsequent transfer of secondary inoculum, and the initiation of secondary lesions on the seedling foliage. A short account of the work is published (2).

MATERIALS AND METHODS.—*P. glycinea* isolate 040 (7) and the susceptible soybean cultivar Lindarin 63 were used for all studies. A steam-sterilized potting medium composed of peat moss, muck, and Wooster silt loam (2/5/5, v/v) was contained in 10-cm-diam plastic pots.

The effects of abrasives on the incidence of disease on cotyledons was determined by growing seedlings (5 seeds/pot) in the potting medium to which was added 60 mg of ground, dried, diseased leaf material and the abrasive. The leaf material that was added to the potting medium was obtained by inoculating leaves by the Q-stick method (7), drying the detached whole leaves when symptoms had developed, and grinding them in a Wiley mill. Sand which was used as an abrasive (8 g/pot) was obtained by screening crushed creek-bed granite through a 1-mm^2 grid. Carborundum (320-grit) was used at 0.6 g/pot. The diseased leaf material and abrasives were placed directly over and under the seed. Additional treatments consisted of additions to the soil of (i) diseased leaf material only; (ii) sand only; and (iii) Carborundum only. Controls did not contain either diseased leaf material or abrasives.

Pots were placed in a growth chamber that provided alternate periods of 8-hr darkness at 9-11 C and 16 hr of light at 19-21 C. Light (1,800-2,000 ft-c, 0.5 m above the bench) was provided by influorescent tubes augmented by incandescent bulbs. Water was supplied to the pots as a gentle spray from a 'Fogg-it' nozzle until seedlings emerged; then subirrigation was provided by placing the pots in a pan of water.

Lesions on cotyledons and unifoliolate leaves were recorded 25 days after planting. Nearly all lesions were incited by *P. glycinea*, as was shown in tests in which a needle was touched to lesions and then used to pierce leaves of greenhouse-grown seedlings. Leaf lesions were incited by inoculum taken from 162 of 163 lesions on 163 cotyledons.

Seedlings with lesions on cotyledons, on unifoliolate, or on trifoliolate leaves were subjected to simulated storms, and the subsequent distribution of lesions was noted. One to five seedlings/pot were used. In pots with more than one seedling, seedlings were 2.5-4 cm apart. We inoculated cotyledons of plants at the "crook" stage by puncturing them with a needle previously touched to 1- to 3-day-old colonies of *P. glycinea* on agar plates. We inoculated leaves of older seedlings by piercing them with a needle or rubbing them with a cotton swab previously touched to colonies of *P. glycinea*. The inoculated plants were placed on the greenhouse bench and watered by subirrigation until lesions developed.

Pots containing plants with diseased organs were placed in the mist stream of a "storm-simulator" which consisted of a 1-m^3 chamber of transparent polyethylene film into which a mist of water was directed by means of a nozzle (Spraying Systems Co., Type Pneu. Atom 1/4 J) that delivered mist and air during 6 of every 20 sec. Air-speed (24 km/hr) was

TABLE 1. The incidence and location of lesions on cotyledons of soybean seedlings germinated in a potting medium that contained dried *Pseudomonas glycinea*-infected leaves and the abrasives, sand or Carborundum

Treatment[a]	Plants emerged/ seeds planted	No. plants with lesions on cotyledons	No. cotyledons		No. lesions on cotyledons	Relative amount of disease[c]
			With lesions	With lesions in the trough[b]		
Experiment 1						
Control[d]	34/35	8	9	3	12	1.0
Sand	31/35	11	14	6	33	5.6
Carborundum	33/35	16	18	10	45	5.5
Experiment 2						
Control[d]	43/45	11	11	7	16	1.0
Sand	38/45	19	25	13	39	2.3
Carborundum	32/45	12	15	8	26	1.4
Experiment 3						
Control[d]	39/45	27	37	30	72	1.0
Sand	42/45	30	43	35	115	1.4
Carborundum	30/45	27	48	40	162	2.1

[a] Diseased leaf material and an abrasive were placed over and under the seeds at time of planting. In control treatments, only diseased leaf material was placed with seeds.

[b] Linear depression in cotyledon in which one-half of hypocotyl-radicle structure lies during dormancy (Fig. 1-B).

[c] An area approximately equal to the trough area of a cotyledon was used as the unit of measurement of diseased area. The total disease area on the control cotyledons in each experiment was taken as unity.

[d] In other control treatments; i.e., (i) sand only added, (ii) Carborundum only added, and (iii) diseased leaf material and abrasives not added, no lesions were present.

TABLE 2. Transfer during simulated wind-rain storms of *Pseudomonas glycinea* from diseased older organs to younger leaves of soybean seedlings as indicated by development of lesions[a]

Source of inoculum	No. experiments	Storm length (hr)	Seedlings/ pot	Disease[b] on leaves above the source of inoculum after simulated storms			
				On unifoliolate leaves	On 1st trifoliolate leaves	On 2nd trifoliolate leaves	On 3rd trifoliolate leaves
Cotyledons	7	11-18	4	15/56	c	c	c
Cotyledons	4	8-16	3	8/24	c	c	c
Cotyledons	3	15	1	3/6	c	c	c
Unifoliolate leaves	1	10	4		5/12	c	c
Unifoliolate leaves	2	8-16	3		7/18	c	c
1st trifoliolate leaves	1	16	5	0/10		8/15	0/15
1st trifoliolate leaves	2	15-16	4	0/16		14/24	10/24
1st trifoliolate leaves	1	16	3	0/6		9/9	0/9

[a] A simulated wind-rain storm consisted of an intermittent air-mist stream forcefully delivered from a nozzle into a 1-m^3 chamber.

[b] Number of diseased leaves or leaflets per number of leaves or leaflets.

[c] These leaves not open at time of the storm.

measured with a hand-held anemometer (18.5-cm-diam Climet 540, Climet Instruments, Inc.). Seedlings within the pot were thoroughly wetted, agitated, and blown against each other, as would be expected in a natural wind-rain storm. When the pot contained a single plant, the air-mist stream forced unifoliolate leaves into contact with cotyledons.

After the storm treatment, the pots were returned to the greenhouse bench and subirrigated. Lesions on leaves not inoculated prior to the storm treatment were counted after 8-15 days.

RESULTS.—*The influence of abrasive particles on the initiation of lesions on cotyledons during seed germination.*—The results of three experiments indicate that an increase in soil abrasiveness can result in an increased incidence of disease on cotyledons (Table 1). Abrasives increased disease, which was predominantly on the proximal portion of the cotyledons (Fig. 1-A). The "trough" areas (Fig. 1-B) were the site of many lesions, probably because of the injury caused by abrasives caught between the cotyledon and the growing hypocotyl. The importance of the trough area is indicated in Table 1. Of the 220 diseased cotyledons in the three

experiments, 152 had lesions in the troughs. Also, the number of lesions within the trough areas was greater than on other parts of the cotyledon. Thus, in Experiment 3 (Table 1), in which lesions in troughs were counted, about 44% of the lesions (152 of 349) were in troughs, even though the area of a trough comprised only an estimated 3-5% of the area of a cotyledon. The trough tissue may be more susceptible to *P. glycinea*, but we suspect that the increased mechanical injury in this area increases disease. Injury to the cotyledon also may result from abrasive contact between the hypocotyl and the cotyledon at locations outside the trough (Fig. 1-B); such contacts have been observed most often on the proximal end of the cotyledon.

Since 161 of 322 emerged seedlings had lesions on the cotyledon whereas only six seedlings had lesions on unifoliolate leaves and none had lesions on trifoliolate leaves, the cotyledons are indicated as the usual site of primary infection. Inoculation reduced emergence by about 14%. Some seedlings germinated but did not emerge because lesions extended from the cotyledon into the hypocotyl, which was destroyed. It is also probable that emergence was reduced by the growth-inhibiting toxin produced by *P. glycinea* in lesions on the cotyledons (4, 9).

Relationship of growth cracks to cotyledon infection.—Splitting of the cotyledon often occurs during germination. These growth cracks usually open centrally on the upper surface of the cotyledon. There is no evidence that they are caused by abrasion. Since we usually were unable to isolate microorganisms from newly opened cracks, they are not believed to be incited by pathogens. Sometimes, however, blight lesions were found at the edges of the cracks which indicates that *P. glycinea* may invade the cotyledon via this portal (Fig. 1-D).

Transfer of inoculum from cotyledons and lower leaves in simulated storms.—The pathogen was readily transferred during simulated wind-rain storms from lesions on cotyledons or on unifoliolate or first trifoliolate leaves to leaves immediately above these affected organs (Table 2). Presumably the transfer was effected in water droplets and by foliage contact after the pathogen became suspended in water on the plant surface. When the cotyledons bore lesions, 26% or more of the unifoliolate leaves developed symptoms after the storm treatments. In experiments in which one plant/pot was used, 50% of the unifoliolate leaves positioned above cotyledons with lesions subsequently developed symptoms. We have commonly observed contact between cotyledons and unifoliolate leaves on the same field plant. When the unifoliolate or first trifoliolate leaf bore lesions,

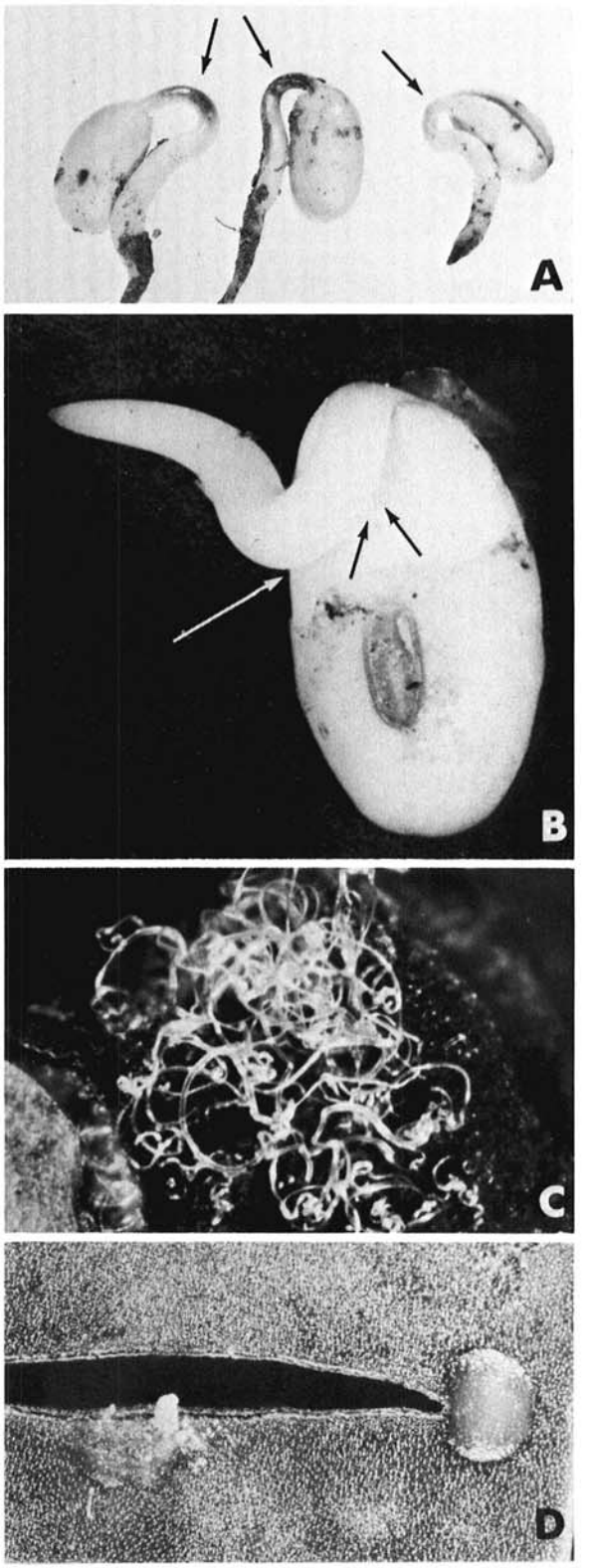

Fig. 1. A) Germinated field-grown soybean seeds. Hypocotyls elongate in the crook (arrows), dragging cotyledons through the overlay of soil (toward upper edge of the figure). B) A typical germinating seed showing the trough region (black arrows). Contact (white arrow) between cotyledon and radicle outside the trough. C, D) Exudates of *Pseudomonas glycinea* from lesions on cotyledons of germinating seeds. C) Filament form. D) Horn and blister forms at the edge of a growth crack in a cotyledon. *P. glycinea* was cultured readily from the exudates.

about one-half the leaflets immediately higher on the plant subsequently became diseased. No new lesions developed (i) on seedlings that were free of lesions at the time of the simulated storm treatment; (ii) on seedlings that had leaves with lesions but which were positioned in the storm chamber out of the direct path of the air-mist stream, but in a location where they were subjected to wetting as the mist settled; and (iii) on seedlings that were free of lesions and were positioned out of the direct path of the air-mist stream. New lesions did not develop on leaves that already bore lesions or on leaves of buds that were unexpanded at the time the simulated storm treatment was applied. In experiments in which only the first trifoliolate leaves bore lesions at the time the storm treatment was applied, the unifoliolate leaves failed to develop lesions. That lesions did not develop on leaves that were beneath leaves already infected with *P. glycinea* is puzzling, but parallel results were obtained in tests with field-grown plants (2).

Exudations from lesions.—In growth room and greenhouse tests described above, in which variously inoculated plants were protected from the deposition of water, an exudate found to consist of *P. glycinea* cells in a cementing substance often was extruded from lesions on cotyledons and leaves. These extrusions, which occurred as filaments (Fig. 1-C), blisters, or horns (Fig. 1-D), dissolved in water; on evaporation of the water, flakelike structures remained. These extrusions were not seen on lesions of field plants, presumably because they were dispersed in dew or rainwater. Filaments formed during the early stages of lesion development when the affected areas on cotyledons and leaves had a deep translucent green color and appeared water-soaked. On cotyledons, lesions several weeks old often turned brown but still retained a wet appearance. Filaments arising from dry necrotic areas also contained *P. glycinea*.

DISCUSSION.—This appears to be the first report in which an increase in soil abrasiveness has been linked to an increase in a seedling disease incited by a leaf-spotting bacterial pathogen. Our evidence indicates that the small injuries caused by abrasive soil particles predispose the cotyledons to *P. glycinea* prior to emergence. Under some conditions, bacterial blight may be so severe that the seedling does not emerge (5) or dies soon after emergence (10); this would appear to be of little epidemiological consequence. In this study, we were concerned with lesions on cotyledons on seedlings that continued to grow. It was demonstrated that these lesions can be a major source of inoculum that incites secondary lesions on seedling leaves. That initiation of these scattered secondary lesions on seedling leaves is dependent on the occurrence of storms was shown in simulated storm tests. These lesions on seedling leaves might readily provide sufficient inoculum to incite disease outbreaks on older plants (2).

Since abrasion and growth cracks predispose cotyledons to disease, other types of injury such as those inflicted during planting of the seed may also favor cotyledon infection.

While it is possible that overwintered *P. glycinea* can be transported from the soil to upper leaves via the colonized terminal bud (8) or via the vascular system (6), the sequence of events described in this paper indicates what probably happens most often under field conditions. There is little evidence to support the vascular hypothesis (1), and while the pathogen has been found in buds of field-grown plants (8), it may have arrived at that location by means other than bud colonization.

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