Poor Seed Quality and Rugosity of Leaves of Virus-Infected Hood Soybeans

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ABSTRACT

Mean percentage of germination of 17 seed lots of Hood soybeans collected from 11 states was 64, as opposed to 90 for a control seed lot selected for good quality and high germination. All Hood seed lots contained some cracked seed, but the control seed lot contained no cracked seed. The mean percentage of cracked seed for all Hood seed lots was 22. A correlation coefficient of −0.931 was calculated for electrical resistance of leachates from all Hood seed lots with a percentage of seeds with cracks.

All field-grown Hood soybean plants grown from all seed lots showed symptoms similar to soybean mosaic, whereas no control plants showed symptoms. A virus was transmitted from field-grown Hood plants to greenhouse-grown Bansei soybean plants and to *Phaseolus vulgaris, Dolichos lablab,* and *Cyamopsis tetragonoloba.*

Leaf cells of Bansei soybean plants infected with the virus from Hood plants contained densely stained circular inclusions, membrane-bound inclusions, and pinwheel inclusions in areas characterized by the occurrence of numerous vesicles. These structures were not observed in healthy leaf tissue. Phytopathology 60:883-886.

Reports of erratic performance of the soybean *Glycine max* (L.) Merr. 'Hood' promoted this study. While stands were generally good and seed yields high, occasionally germination and plant stands were low, and plants grew poorly. Research workers familiar with the variety suggested that disease might be influencing its performance.

Materials and Methods.—Soybean seed lots harvested from cooperative soybean test plots were obtained from agronomists in several states. The seed originally sown in these test plots was distributed through the U.S. Regional Soybean Laboratory, and was of high varietal purity. All soybean seed used in field or greenhouse tests was approximately 7 months old. Because of its good seed quality and high germination, a soybean line UM-4 was selected as a control for comparison with Hood seed lots. Seed was sown 2.5 cm deep on 15 May at the rate of 200 seeds per 5.5-m row for field observations. Rows were 158 cm apart, and each seed lot was replicated three times.

For greenhouse germination tests, seed was sown 2.5 cm deep in washed river sand contained in metal flats. Germination of seed both in the greenhouse and in the field was determined 2 weeks after emergence.

All seed lots were examined before staining and germination tests. Seed in all lots was considered sound and of good quality, and no lot had more than 1% split seeds.

Seeds were examined for cracks by the tetrazolium chloride method (3). Seeds were placed in a moist paper towel overnight at 30 C, then in a 1% tetrazolium chloride solution for 4 hr at 35 C. Seed coats were removed after staining, and the seeds were examined.

The electrical resistance of the leachate from 25 soybean seeds was measured with a Wheatstone bridge (9). A dip conductivity cell, with a constant of one, was used. The seed from each seed lot was placed in a corked 35-ml test tube containing 25 ml of deionized water at room temp. All resistance measurements were corrected to 25 C. Tubes were shaken to facilitate diffusion of electrolytes in the leachates before resistance was determined. Resistance was determined 1 hr after water was added to the soybeans.

Virus inoculum was prepared by expressing crude sap from leaves of virus infected plants. Sap was diluted 1/10 with 0.01 M phosphate buffer at pH 7.2. Leaves of plants to be inoculated were first dusted lightly with 600-mesh Carborundum. Plants were inoculated with cheesecloth pads saturated with sap that was applied to young leaves. After inoculation, leaves were rinsed with distilled water.

Seedlings, 2-3 weeks old, of *Dolichos lablab* L., *Cyamopsis tetragonoloba* L. (guar), and *Phaseolus vulgaris* L. 'Kentucky Wonder' (wax pole bean), were used as local lesion hosts for a virus thought to be soybean mosaic virus (SMV).

For electron microscopic observation, punches 1 mm in diam were taken from healthy and from virus-infected leaves of Bansei soybeans. The samples were fixed overnight in a 2% solution of glutaraldehyde in 0.05 M phosphate buffer, pH 7.3, at 4 C. The tissue was rinsed with several changes of phosphate buffer and postfixed for 1 hr in a 1% solution of osmium tetroxide in buffer. The tissue was dehydrated in an ethanol-propylene oxide series and embedded in Epon 812 according to the procedures of Luft (8). Sections 60 mμ thick were cut with a diamond knife on an LKB ultramicrotome, and were picked up on Formvar-covered grids. The sections were stained 10 min in a methanol solution of uranyl acetate (14) and examined in an RCA EMU-3f microscope.

Results.—*Seed tests.*—Mean field germination of 17 seed lots of Hood soybeans was 64%, and ranged from 36 to 84% (Table 1). Germination of control seed of the soybean line UM-4 was 90%. Germination in sand in the greenhouse was higher than in the field in all cases but one. Germination in sand was at least 10% greater than in field soil in nine (53%) of the seed lots, and at least 20% greater in five (29%) of the seed lots. The range of germination in sand was 61-93%. Control seed germinated only 3% higher in sand than in field soil.

All Hood seed lots had some cracked seed. Six seed lots (35%) had more than 25% cracked seed. The percentage of cracked seeds ranged from 7-68, and the mean was 22. None of the control seed was cracked.

A correlation coefficient of −0.931 was calculated for the correlation of electrical resistance of leachates from all Hood seed lots with percentage of seeds with cracks. Electrical resistance of the leachates ranged from 107 $\times 10^3$ to 675 $\times 10^3$ ohms (Table 1). Mean resistance of all Hood seed lots was 498 $\times 10^3$ ohms. The resistance of the uncracked control seed lot was 749 $\times 10^3$ ohms.

Symptoms.—Hood seedlings appeared normal, but the fifth and succeeding trifoliolate leaves were stiff, leathery, and slightly rugose. These symptoms increased and were severe throughout August and September (Table 2). Upper leaves were dark blue-green, with small areas of lighter green scattered over the surface

TABLE 1. Comparison of field and greenhouse germination, seed cracking, and electrical resistance of seed leachates of 17 seed lots of Hood soybean to UM-4 control seed

Location	Field germination[a]	Greenhouse germination[a] (sand)	Seed with cracks[b]	Resistance of leachate[b]
	%	%	%	*ohms* $\times 10^3$
		UM-4 seed (control)		
Guelph, Ont.	90	93	0	749
		Hood seed		
Portageville, Mo.	84	66	7	675
Plymouth, N.C.	79	88	9	651
Georgetown, Del.	73	93	10	638
Hartsville, S.C.	72	84	8	643
Jay, Fla.	72	77	10	657
Clayton, N.C.	71	77	12	627
Willard, N.C.	69	87	15	604
Warsaw, Va.	69	93	15	615
Keiser, Ark.	61	76	18	583
Stuttgart, Ark.	61	70	17	551
Experiment, Ga.	61	68	17	594
Stoneville, Miss.[c]	61	90	36	301
Petersburg, Va.	60	68	37	321
Linkwood, Md.	57	81	26	215
Marianna, Ark.	56	71	38	308
Stoneville, Miss.[d]	54	61	32	373
Stillwater, Okla.	36	68	68	107
Mean for Hood seed	64	78	22	498

[a] Mean of three replications, 200 seed/replication.
[b] Mean of four replications, 25 seed/replication.
[c] Loam field soil.
[d] Clay field soil.

TABLE 2. Mean leaf rugosity score of various leaves of Hood soybeans planted on three dates

Trifoliolate leaf no.[a]	Mean leaf rugosity score[b] — Date seed was sown: 20 May	1 July	1 August
1	1	1	1
2	1	1	2
3	1	2	2
4	1	3	4
5	2	3	4
6	2	4	
7	2	4	
10	3		
15	4		
18	5		

[a] Numbered from the base of the plant to the apex.
[b] Mean of four replications, 10 leaves/replication. 1 = Healthy leaf; 2 = slightly rugose leaf; 3 = moderately rugose leaf; 4 = severely rugose leaf; 5 = very severely rugose leaf.

of leaflets. Leaflet margins were turned down, and leaf blades were extremely rugose and distorted (Fig. 1). Leaves less severely affected were occasionally quite asymmetric, with one side of a leaflet being nearly twice the size of the other. Such leaves would sometimes have unusual outgrowths of tissue along the margin of one side of the blade, forming semicircular to sinuate lobes.

Individual plants were examined in each row representing the 17 original seed lots from different locations. All plants had the same severe symptoms characteristic of soybean mosaic.

Two additional plantings of a single seed lot of Hood were made on 1 July and 1 August. Symptoms appeared sooner after the July plantings than after the May planting. Moderately severe symptoms occurred on the fourth trifoliolate leaves of plants from the August sowing (Table 2).

Control plants and plants of 20 additional soybean varieties grown in the vicinity of the test were observed throughout the growing season. None of these plants developed symptoms similar to those observed on Hood plants.

The variety Hood is grown in the South. It is not adapted to growth in the North, and will not mature. Field plantings of Hood at Stoneville, Mississippi, were examined in late September, and leaves were only slightly rugose, whereas, at Ames, Iowa, all young

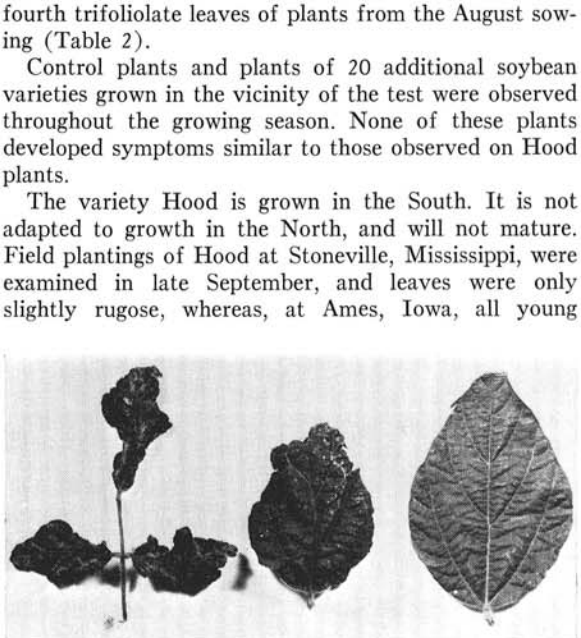

Fig. 1. Fourth trifoliolate leaf and leaflets of Hood soybeans planted (left to right) 1 August, 1 July, and 15 May.

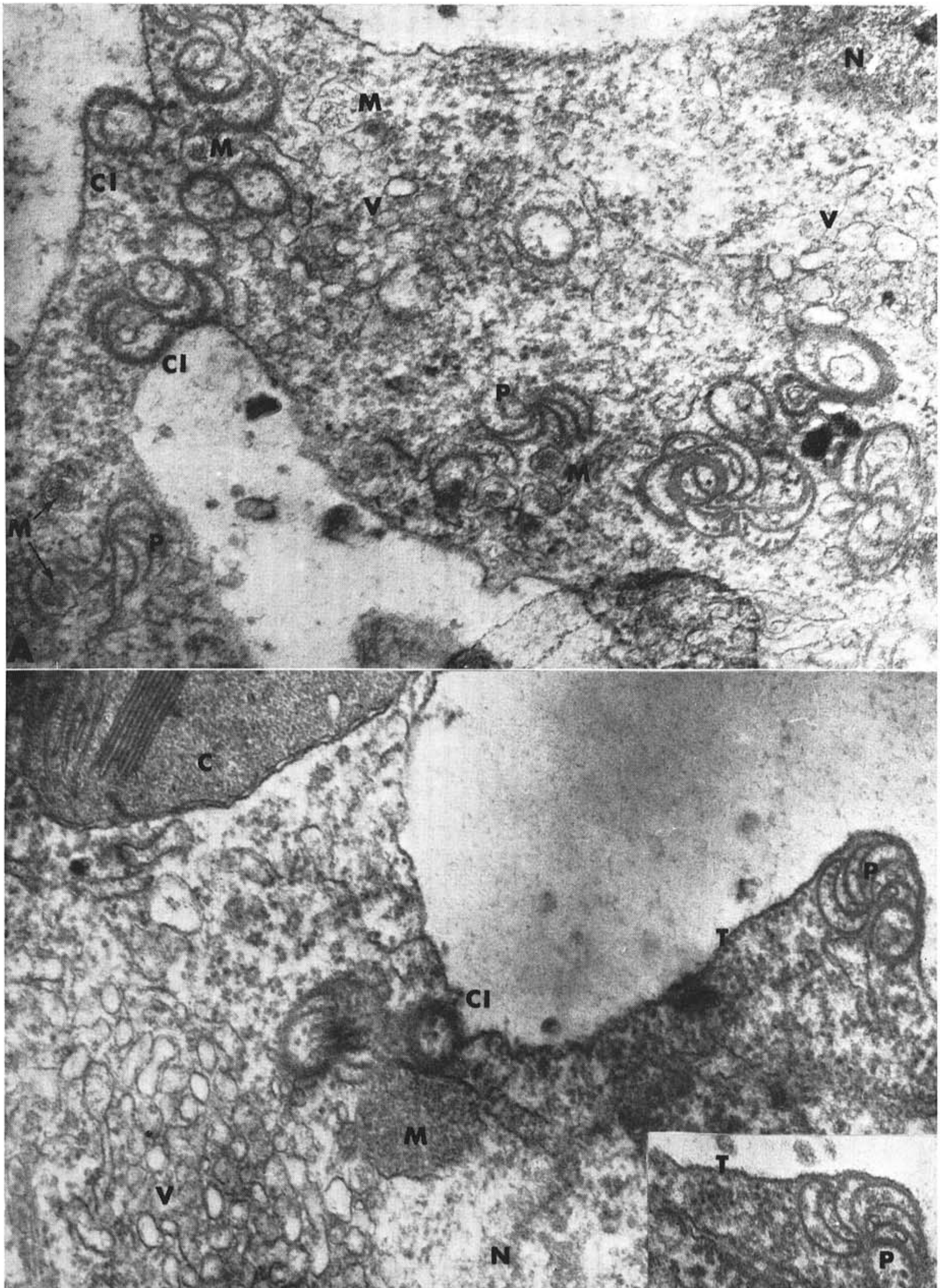

Fig. 2. Sections of leaf cells from SMV-infected soybeans. (×50,000) **A)** Cell inclusion bodies in a vesicular area near the nucleus. **B)** Cell inclusion bodies in a vesicular area bounded by the nucleus, tonoplast, and a chloroplast. (Inset) A pinwheel inclusion bounded by the tonoplast. C = chloroplast; CI = circular inclusion; M = membrane-bound inclusion; N = nucleus; P = pinwheel inclusion; T = tonoplast; V = vesicles.

leaves on Hood plants were severely rugose. The varieties Lee and Jackson, also adapted to growth in the North, produced no rugose leaves when grown at Ames in the same field plot with Hood.

Fourteen different Hood seed lots were grown the next year. All plants again developed severe symptoms of soybean mosaic. No control plants showed symptoms.

Virus transmission.—Sap collected from leaves of 10 field-grown Hood plants was used to inoculate 10 plants each of Bansei soybeans (2), *Cyamopsis tetragonoloba* (12), *Dolichos lablab* (5), and Kentucky Wonder wax pole beans (*Phaseolus vulgaris*) (12). Symptoms similar to soybean mosaic developed on all inoculated soybean plants in 2 to 3 weeks. Discrete local lesions appeared on inoculated leaves of *D. lablab* and wax pole beans in 5-7 days. Noninoculated plants developed no symptoms.

Inclusion bodies.—Leaf cells of infected Bansei soybean plants were characterized by groups of numerous vesicles in various areas of the cytoplasm (Fig. 2). Several types of inclusion bodies were observed in these areas.

The most obvious inclusions were pinwheel-shaped (Fig. 2). When observed in transverse section, these inclusions appeared as symmetric, curved arms or septa radiating out from a central core. There were usually six to eight septa/body. Circular inclusions were observed in conjunction with the pinwheels, and appeared to consist of one or more coiled septa.

Membrane-bound inclusions with densely-stained central areas (Fig. 2) were observed near pinwheels. Although the cores of some of these bodies suggested a reticulum, close examination revealed no organized structure that might be associated with virus rods. Vesicular areas and inclusion bodies were not observed in healthy leaf tissue.

DISCUSSION.—Presley (9) observed that seed with different degrees of injury or deterioration may be detected by differences in conductivity of distilled water leachings. He noted that growth of miscellaneous fungi was absent from vigorously germinating cotton seed with high resistance readings, but was progressively more abundant and earlier in appearance on seed progressively more deteriorated and with relatively low-resistance readings. These observations suggest that the poor field germination of some Hood seed lots was due to invasion of the germinating seedlings by microorganisms. The relationship, if any, of the virus which is presumed to be SMV in Hood seeds to cracking remains obscure. At minimum, the extent of cracking within a single variety of soybeans is unusual, and was not previously reported.

Inclusion bodies similar to those seen in infected Bansei soybean leaves have been observed in plant leaves infected by several viruses, including turnip mosaic, tobacco etch, potato Y, watermelon mosaic, bean yellow mosaic, bean common mosaic, sugarcane mosaic, lettuce mosaic, sunflower mosaic, and maize dwarf mosaic (1, 4, 6, 7). In every case, the viruses were long, flexuous rods 730-750 mμ in length. The same holds true for SMV, which has been reported to be a long, flexuous rod 748 × 12 mμ (10).

The virus isolated from Hood was supplied to J. P. Ross, who utilized it in a study of pathogenic variation among isolates of SMV (13). Ross reported that the virus gave a positive serological reaction in microprecipitin tests with antiserum produced to an identified isolate of SMV, and also produced local lesions on Kentucky Wonder wax pole beans. The soybean variety, Ogden, was resistant to the Hood virus isolate as well as to six SMV isolates.

The severe rugose symptoms observed on leaves of Hood from August until October may be due to the cooler temperatures during vegetative growth. Conover (2) reported that rugose symptoms of SMV-infected soybean plants were severe at 18.5 C and largely masked at 29.5 C.

Because we were unable to find symptomless Hood plants among thousands of field-grown plants examined, we believe that plants of the soybean variety Hood may be infected with SMV or a related virus. Detailed data supporting our contention that the virus in Hood soybeans is indeed SMV will appear elsewhere (11).

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