

## Incidence of Microorganisms in Soybean Seeds Damaged by Stink Bug Feeding

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### ABSTRACT

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Stink bug feeding damage, seed viability (7-day germination), seed vigor (4-day germination), and microorganism incidence were evaluated in seeds from upper and lower halves of soybean (*Glycine max*) plants damaged by four stink bug population levels in 1985 (cv. Forrest) and by two levels in 1986 (cv. Centennial). The stink bug complex included *Acrosternum hilare*, *Euschistus* spp., and primarily *Nezara viridula*. In both years, increased stink bug populations resulted in increased percentages of seeds damaged by feeding. Decreases in seed viability and vigor occurred primarily in upper plant halves, where feeding damage was more severe. Incidence of seedborne *Fusarium* spp. increased with increased amounts of stink bug

damage in seeds from both upper and lower plant halves. Seedborne bacteria increased in incidence only in seeds from upper halves. Incidence of seedborne *Cercospora kikuchii* decreased in response to stink bug damage in upper plant halves. Incidence of seedborne *Alternaria* spp., *Colletotrichum truncatum*, and *Phomopsis* spp. were not influenced by stink bug damage. Studies in 1986 on effects of delayed harvest on stink bug-damaged and nondamaged seeds indicated that delays of up to 6 wk reduced seed viability and vigor and modified microorganism incidence so that the effects of stink bug damage were not detectable.

*Additional key words:* insect-disease interactions, integrated pest management, soybean seed microflora.

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Soybean (*Glycine max* (L.) Merr.) in the United States is damaged by a complex of phytophagous stink bugs (Hemiptera: Pentatomidae) that includes the southern green stink bug, *Nezara viridula* (L.); the green stink bug, *Acrosternum hilare* (Say); and several species in the genus *Euschistus*, including the brown stink bug, *E. servus* (Say) (21). Damage occurs when adults and nymphs puncture seeds and feed on contents liquified by salivary enzymes. Visible evidence of stink bug feeding can vary from single punctures with little or no seed coat shriveling to multiple punctures with extensive shriveling. Feeding damage has been associated with decreased seed germination and seedling emergence; changes in seed protein, oil, and fatty acid content; and decreased yield (13,19).

Losses in seed quality and yield also can be caused by microorganisms that infect soybean seeds. Numerous studies have examined seedborne microorganisms in response to such abiotic and biotic factors as rainfall (14), harvest date (22), and presence of other microorganisms (1,11). However, few investigators have addressed the effect of damage caused by insects on soybean seed microflora. Stink bugs are known to vector *Nematospora coryli*, the causal fungus of yeast spot (2). Shortt et al (15) reported that wounding of soybean pods associated with feeding by the bean leaf beetle, *Cerotoma trifurcata*, predisposed seeds to infection by *Alternaria tenuissima*, which is a weak pathogen of soybeans. Kilpatrick and Hartwig (6) found differences in incidence of several fungi in seeds damaged by *N. viridula*. We report the effects of different levels of stink bug feeding damage on incidence of seedborne microorganisms under conditions of properly timed and delayed harvests.

### MATERIALS AND METHODS

**1985 Studies.** 'Forrest' soybeans (maturity group V) were planted in Sharkey clay soil (6.1% sand, 60.9% silt, and 33.0% clay) at the Louisiana Agricultural Experiment Station, St. Gabriel, on 18 June 1985. Forty small plots, which consisted of three rows (1.83 m in length) planted on 0.91-m centers, were established in an 8 × 5 pattern in the field. Each plot was surrounded by a 1.83-m plantfree border. Because stink bugs normally exhibit a clumped distribution within soybean fields (20), the experiment was designed in this manner to encourage naturally occurring clusters of immigrating stink bug adults to colonize and be confined within small plots. Plantfree borders discouraged interplot movement of nymphs that resulted from eggs laid by immigrating adults. This technique has proven successful for enhancing buildup of different stink bug population levels in small plots without the confounding effects normally encountered when using caged populations (13).

All plots were left untreated until the R<sub>4</sub> growth stage (3), at which time sampling for stink bugs was begun. Development of stink bug populations was monitored by sampling 1.83 m of row from each plot using a ground cloth (7) on a weekly basis through R<sub>7</sub>. Counts were made of adults and nymphs of *A. hilare*, *Euschistus* spp., and *N. viridula*; only nymphs ≥ 6 mm in length were counted, because this size represents the lower limit at which nymphs would be included in economic threshold determinations in Louisiana. Insects were returned to plots after sampling. For control of stink bugs, at R<sub>4</sub>, 16 of the 40 plots were chosen at random to receive applications of methyl parathion (0.5% active ingredient) in alternate weeks. Using a hand-held pump sprayer, insecticide was applied to runoff immediately after sampling. This was done to provide plots that would serve as stink bug-free controls. The remaining 24 plots received no treatment and were

left to be colonized by immigrating stink bug adults. Seasonlong means and peaks for stink bug populations were determined at harvest maturity for each of the 40 plots. From these, 16 plots were selected for further study and separated into four groups of four plots each whose seasonlong population means represented populations that were controlled using insecticide (0.3) or that increased naturally to low (1.5), moderate (3.0), or high (3.8 stink bugs per row-m) levels (Fig. 1A). Thus, the experiment was conducted in a completely random design, with four treatments and four replicates per treatment.

At harvest maturity (25 October), all plants in six randomly selected sections of row (0.30 m in length) were removed from each plot and pooled. Fourteen individual plants were chosen at random from each pooled sample and divided into upper and lower halves based on the number of main stem nodes. A 14-plant sample comprised approximately 10% of the total plant population in each plot and provided sufficient seed to conduct all tests. Seeds from all samples were shelled by hand. Samples of 100 seeds from each plant half then were examined to determine the percentage of seeds damaged by stink bug feeding (5).

Tests for seed viability (7-day germination) and seed vigor (4-day germination) were conducted on two 50-seed samples selected at random from both upper and lower plant halves of each plot according to procedures described by TeKrony and Egli (17). Incidence of seedborne microorganisms was determined from two 50-seed samples from upper and lower plant halves of each plot. Seeds were surface-disinfested in 1.5% sodium hypochlorite for 2 min, rinsed twice for 4 min in sterile deionized water, and then transferred aseptically to culture plates (five seeds per plate) that contained 20 ml of Difco potato-dextrose agar acidified with lactic acid (pH = 4.7). Plates were incubated under fluorescent light ( $25 \mu\text{E m}^{-2} \text{s}^{-1}$ ) (16 hr light:8 hr dark) in the laboratory at 19–22 C. All microorganisms growing from seeds were identified after 7 days based on cultural characteristics and, in the case of fungi, spore morphology. Microorganisms identified were *Alternaria* spp., *Aspergillus* spp., *Botryodiplodia* spp., *Cercospora kikuchii* (T. Matsu. & Tomoyasu) Gardner, *Colletotrichum truncatum* (Schw.) Andrus & W. D. Moore, *Fusarium* spp., *Penicillium* spp., *Phomopsis* spp., and bacteria.

The effects of stink bug population levels on feeding damage, viability and vigor, and microorganism incidence in seeds from upper and lower halves of plants were analyzed using ANOVA. Mean separations were made using Duncan's new multiple range test. Statistical significance is reported herein at the  $P \leq 0.05$  level.

**1986 Studies.** Two separate studies were conducted during 1986. The first was a replicate of the 1985 small plot study, with one modification. Plot size was increased to five rows, each 3.05 m in length. The second study, a large field experiment, was conducted using 'Centennial' soybeans (maturity group VI) planted in

Commerce silty clay loam soil (19.8% sand, 61.2% silt, and 19.0% clay) on 23 May 1986. 'Centennial' was used rather than 'Forrest' because of ready availability of suitable fields on experiment station land. The experimental design was a randomized complete block with two treatments and four replicates per treatment. Individual plots were 12 rows wide (row width = 0.91 m) and 18.29 m long. Larger plots enabled us to sample for stink bugs using the sweep net technique (7), which is preferred for the ease with which large areas can be sampled. Stink bug populations in all plots were monitored weekly from R<sub>5</sub>–R<sub>7</sub> by taking 50 sweeps per plot using a 38-cm diameter sweep net. In treatment 1, stink bug populations were allowed to increase uncontrolled; in treatment 2, stink bugs were controlled by applications of methyl parathion at early R<sub>5</sub>, late R<sub>5</sub>, and R<sub>6</sub>, as dictated by stink bug populations (Fig. 1B). Insecticide was applied at a rate of 0.56 kg active ingredient per hectare using a Hi-Boy sprayer (Hahn, Inc., Evansville, IN).

At harvest maturity (29 October) and again at 3 wk (19 November) and 6 wk (11 December) after maturity, all plants in three randomly selected row-m were harvested from each plot and pooled. At each harvest date, stink bugs were sampled from three row-m in each plot using a ground cloth to reduce damage done to mature plants. Fourteen-plant samples from each plot were processed as described for 1985. Seed viability and vigor and microorganism incidence were determined and statistical analyses conducted as described previously.

## RESULTS

**Stink bug populations and damage.** Stink bug populations in small field plots developed to four levels during 1985 (Fig. 1A). Population means for all sample dates combined were 0.3, 1.5, 3.0, and 3.8 stink bugs per row-m for controlled-, low-, moderate-, and high-population plots, respectively. Peaks for these same levels reached 1.0, 3.0, 4.9, and 9.5 stink bugs per row-m at R<sub>5</sub>, R<sub>5</sub>, R<sub>6</sub>, and R<sub>6</sub>, respectively. *A. hilare*, *Euschistus* spp., and *N. viridula* comprised 29.4, 9.1, and 61.5%, respectively, of the total number of stink bugs sampled during the season.

In 1985, feeding damage to soybean seeds increased significantly as stink bug populations increased in both upper and lower halves of plants (Table 1); however, damage was more severe in upper halves. The percentage of damaged seeds in upper plant halves increased to 52.3% at low population levels, whereas damage approaching this level was not observed in lower plant halves until populations reached high levels. A similar pattern was observed for seed viability (7-day germination) and seed vigor (4-day germination) (Table 2). Both parameters decreased steadily in seeds from upper plant halves; however, significant reductions in these parameters were detected in lower plant halves only when populations reached high levels.

In 1986, we were unable to complete the small plot study because of low levels of stink bug infestation. Therefore, efforts concentrated on the large plot study, in which two stink bug

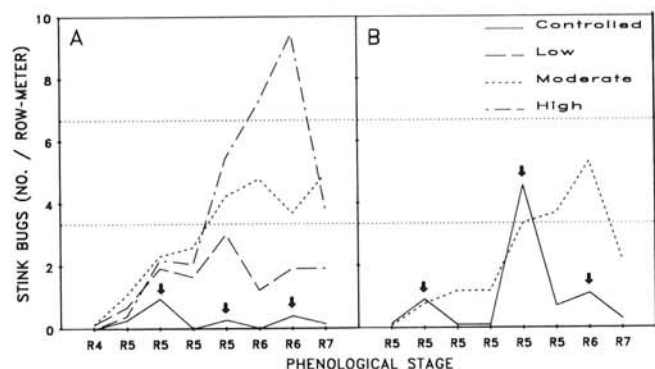


Fig. 1. A, Trends for four stink bug population levels that developed in experimental plots in 1985 (cv. Forrest). B, Trends for two stink bug population levels that developed in plots in 1986 (cv. Centennial). Horizontal lines represent levels of one- and twofold the economic threshold for stink bugs. The current economic threshold for stink bugs in Louisiana is 3.3/row-m. Arrows indicate applications of methyl parathion for stink bug control.

TABLE 1. Soybean seeds damaged by stink bug feeding in upper and lower halves of soybean plants in St. Gabriel, LA, during 1985 and 1986

Year	Cultivar	Stink bug population level <sup>a</sup>	Damaged seeds (%) <sup>b</sup>	
			Upper half	Lower half
1985	Forrest	Controlled	22.9c	18.1b
		Low	52.3b	22.5b
		Moderate	70.2ba	35.9ba
		High	77.7a	46.2a
1986	Centennial	Controlled	20.5b	12.0b
		Moderate	38.5a	22.8a

<sup>a</sup> Means for all sample dates combined. In 1985, controlled = 0.3, low = 1.5, moderate = 3.0, and high = 3.8 stink bugs per row-m. In 1986, controlled = 1.0 and moderate = 2.2 stink bugs per row-m.

<sup>b</sup> Values for each cultivar in the same column followed by the same letter did not differ significantly ( $P \geq 0.05$ ).

populations were compared (Fig. 1b). Numbers of stink bugs sampled were converted from number per 50 sweeps to number per row-m (12) to allow ready comparison with 1985 results. Population means were 1.0 and 2.2 stink bugs per row-m for controlled and moderate populations, respectively. Peaks for these same levels reached 4.5 and 5.3 stink bugs per row-m at R<sub>5</sub> and R<sub>6</sub>, respectively. The high peak in controlled-population plots resulted from an immigration of adult stink bugs at mid-R<sub>5</sub> (Fig. 1b). This infestation was treated with methyl parathion as soon as detected, thus limiting the feeding duration of these stink bugs to fewer than seven days. *A. hilare*, *Euschistus* spp., and *N. viridula* made up 5.5, 16.6, and 77.9%, respectively, of the total stink bug population. Ground cloth samples of stink bug populations taken at and following R<sub>8</sub> showed that numbers of stink bugs decreased to near zero by harvest maturity and remained as such, indicating that stink bug damage after R<sub>7</sub> was minimal.

Moderate stink bug populations in 1986 caused significant increases in percentages of damaged seeds from both plant halves (Table 1). As in 1985, feeding damage was more severe in seeds from upper halves of plants. This corresponded to significant decreases in seed viability and vigor (Table 2). The percentage of damaged seeds in lower plant halves was not sufficiently high to cause significant reductions in these parameters.

Delaying harvest 3 or 6 wk caused overall reductions in both seed viability and seed vigor (Table 2). Significant reductions in these parameters induced by stink bug feeding that were observed at the time of optimal harvest still were detected in damaged seeds after a harvest delay of 3 wk. However, a harvest delay of 6 wk decreased viability and vigor sufficiently so that effects of stink bug feeding were not detected.

**Seedborne microorganisms.** *Alternaria* spp., *C. kikuchii*, *C. truncatum*, *Fusarium* spp., *Phomopsis* spp., and bacteria were isolated frequently from soybean seeds. *Aspergillus* spp., *Botryodiplodia* spp., and *Penicillium* spp. were isolated infrequently and therefore were omitted from statistical analyses. Approximately 90% of all bacterial isolates demonstrated a white, fluidal colony morphology similar to that exhibited by *Bacillus*. In both study years, incidence of each microorganism did not differ significantly between upper and lower plant halves in plots where stink bugs were controlled and feeding damage was minimal.

In 1985, incidence of *Fusarium* spp. increased significantly as stink bug damage increased in both upper and lower halves of plants (Table 3). Incidence of *C. kikuchii* decreased significantly and that for bacteria increased significantly only in seeds from upper halves of plants, where feeding damage was greater. Incidence of seedborne *C. truncatum* and *Phomopsis* spp. was not affected significantly by stink bug feeding (Table 3). Patterns of seedborne microorganism recovery in 1986 were similar to those in 1985 (Table 3). However, the incidence of seedborne *Fusarium* spp. increased significantly only in seeds from upper halves of plants. Incidence of *C. kikuchii* again decreased as feeding damage in upper halves of plants increased. Incidence of *Alternaria* spp., *C. truncatum*, and *Phomopsis* spp. was unaffected by stink bug damage.

Incidence of seedborne microorganisms at three harvest dates in 1986 is presented in Figure 2. Data from upper plant halves only are shown, because differences in microorganism incidence in response to stink bug damage were confined to seeds from upper halves of plants in 1986. Increases in incidence of seedborne *Fusarium* spp. in moderate-population plots that were evident at

TABLE 2. Viability (7-day) and vigor (4-day germination) of soybean seeds from upper and lower halves of soybean plants in St. Gabriel, LA, during 1985 and 1986

Year	Cultivar	Harvest date	Stink bug population level <sup>a</sup>	7-day germination (%) <sup>b</sup>		4-day germination (%)	
				Upper half	Lower half	Upper half	Lower half
1985	Forrest	25 October	Controlled	63.0a	60.1a	60.0a	59.7a
			Low	45.3b	60.0a	44.0b	58.3a
			Moderate	43.0b	63.3a	37.3b	58.3a
			High	31.3c	49.0b	25.3c	43.5b
1986	Centennial	29 October	Controlled	64.8a	66.5a	56.0a	55.5a
			Moderate	49.8b	67.0a	34.5b	54.8a
		19 November	Controlled	34.8a	39.0a	28.3a	31.5a
			Moderate	20.3b	35.8a	14.5b	27.5a
		11 December	Controlled	6.3a	7.8a	2.5a	3.8a
			Moderate	4.0a	4.8a	2.5a	1.0a

<sup>a</sup> Means for all sample dates combined. In 1985, controlled = 0.3, low = 1.5, moderate = 3.0, and high = 3.8 stink bugs per row-m. In 1986, controlled = 1.0 and moderate = 2.2 stink bugs per row-m.

<sup>b</sup> Values for each harvest date in the same column followed by the same letter did not differ significantly ( $P \geq 0.05$ ).

TABLE 3. Microorganisms (number isolated per 50 seeds) from soybean seeds at harvest maturity from upper and lower halves of soybean plants in St. Gabriel, LA, during 1985 and 1986

Year	Cultivar	Stink bug population level <sup>a</sup>	<i>Fusarium</i> spp. <sup>b</sup>		<i>Cercospora kikuchii</i>		<i>Phomopsis</i> spp.		<i>Colletotrichum truncatum</i>		<i>Alternaria</i> spp.		Bacteria	
			Upper half	Lower half	Upper half	Lower half	Upper half	Lower half	Upper half	Lower half	Upper half	Lower half		
1985	Forrest	Controlled	7.4c	9.1b	24.9a	10.5a	23.7a	18.5a	4.8a	3.2a	...	...	3.0b	5.1a
		Low	19.3b	6.8b	14.3ab	8.9a	27.8a	29.2a	7.4a	2.1a	...	...	9.4a	3.6a
		Moderate	23.6b	15.9ab	11.3b	9.5a	17.9a	25.8a	3.7a	3.8a	...	...	9.6a	6.5a
		High	41.5a	25.6a	6.0b	12.2a	24.9a	32.9a	3.0a	4.0a	...	...	11.9a	7.3a
1986	Centennial	Controlled	14.5b	13.6a	3.4a	1.8a	28.1a	27.8a	4.9a	6.0a	7.9a	7.7a	...	...
		Moderate	25.2a	12.4a	1.5b	2.2a	31.0a	30.6a	4.5a	6.6a	8.2a	7.5a	...	...

<sup>a</sup> Means for all sample dates combined. In 1985, controlled = 0.3, low = 1.5, moderate = 3.0, and high = 3.8 stink bugs per row-m. In 1986, controlled = 1.0 and moderate = 2.2 stink bugs per row-m.

<sup>b</sup> Values for each cultivar in the same column followed by the same letter did not differ significantly ( $P \geq 0.05$ ).

<sup>c</sup> Microorganisms not isolated sufficiently frequently from seeds to be included in statistical analyses.

optimal harvest time were not detected in seeds harvested 3 or 6 wk later. Incidence of *Fusarium* spp. in seeds from controlled-population plots increased steadily with delayed harvest; however, seeds from moderate-population plots had a high initial incidence of this fungus that did not increase further after harvest was delayed. Similarly, incidence of *C. kikuchii* differed between population levels at time of optimal harvest, but this difference also was not evident when harvest was delayed. Incidence of seedborne *Phomopsis* spp. increased slightly with delayed harvest; a significant difference in incidence of this fungus between treatments was observed only for seeds harvested after 3 wk. A similar pattern was observed for *C. truncatum*. Seedborne *Alternaria* spp. declined slightly with delayed harvest and did not reflect any effect due to stink bugs. Bacterial incidence in seeds increased with harvest delay and approached significance ( $P = 0.06$ ) for seeds harvested 6 wk late.

## DISCUSSION

Incidence of seedborne microorganisms examined in both years did not differ significantly between upper and lower portions of plants in plots where stink bugs were controlled and feeding damage was minimal. In addition, our use of determinate cultivars removed sequential seed maturation as a confounding factor. Thus, the observed differences in microorganism incidence likely were in response to different levels of stink bug feeding damage in upper and lower plant halves rather than to normal within-plant distribution patterns of these microorganisms. In other studies, incidence of *C. kikuchii* was greater in seeds from the upper half of plants (10), whereas seedborne *Phomopsis* was most severe in the bottom third of plants (4). Differences in results between our study and previous studies (4,10) may have resulted from the use by other workers of indeterminate cultivars or from the abundant rainfall and high relative humidity in southern Louisiana, which may have encouraged more uniform distribution of microorganisms within the soybean canopy.

Stink bug feeding damage influenced several seedborne

microorganisms examined in this study. Incidence of *Fusarium* spp. was consistently increased in damaged seeds. Similar results were reported by Kilpatrick and Hartwig (6), who found this fungus more frequently in stink bug-damaged seeds than in nondamaged seeds. Furthermore, McGee et al (8) presented evidence that *Fusarium* may be a factor in reduced seedling emergence. Therefore, the increased incidence of *Fusarium* spp. in damaged seeds may compound problems with germination and stand establishment already associated with stink bug feeding (5). Incidence of seedborne *C. kikuchii* was reduced in damaged seeds, which is in agreement with a previous report (6). Injury to soybean pods caused by the bean leaf beetle also resulted in reductions in seedborne *C. kikuchii* (15). In the current study, the response of *C. kikuchii* only in seeds from upper plant halves, where stink bug damage was greater, suggests that certain minimal levels of stink bug damage may be necessary before effects on this fungus can be observed. Such also could be true for incidence of seedborne bacteria in 1985. In addition, *N. viridula*, which was the most abundant stink bug species in our study, was reported to transfer 13 genera of bacteria during feeding (9). Included in these were *Bacillus*, *Corynebacterium*, *Pseudomonas*, and *Xanthomonas*, each of which contains species that have been recovered from soybean seeds (16). Thus, increased incidence of bacteria in damaged seed also may have resulted from introduction of bacteria during feeding by stink bugs.

Harvest delays caused sharp reductions in seed viability and vigor and generally acted to lessen effects of stink bug feeding on soybean seeds. Other reports have described reduced viability and vigor (18) and increased incidence of seedborne fungi (22) as a result of delayed harvest. The present study also revealed some interesting recovery patterns for seedborne *C. truncatum* and *Phomopsis* spp. with delayed harvest. For both fungi, significant differences in incidence between treatments were observed only in seeds harvested after a 3-wk delay. Incidence of these fungi in damaged seeds after 3 wk was the same as in nondamaged seeds after 6 wk. Thus, the general effect of harvest delay on these fungi was accelerated in seeds damaged by stink bugs.

Our results show that incidence of microorganisms in stink bug-damaged soybean seeds was similar in two cultivars planted in different years, during which a wide range of stink bug populations was encountered. Because all microorganisms examined in this study are part of the normal soybean seed microflora and can infect seeds in the absence of stink bug feeding (16), our results suggest that the primary effect of stink bug feeding damage was to modify the incidence of these microorganisms. Such modifications may have been due to changes in nutritional quality of soybean seeds (19) or to extensive physical disruptions in seeds that result from stink bug feeding.

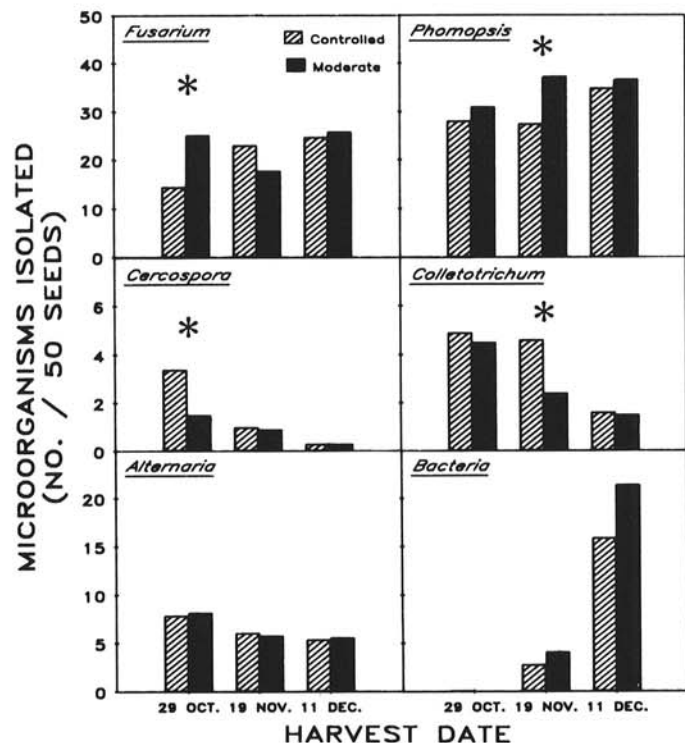


Fig. 2. Microorganism incidence in seeds harvested from upper halves of 'Centennial' soybean plants at three harvest dates in 1986 from experimental plots with controlled and moderate stink bug population levels. Asterisks indicate significant ( $P < 0.05$ ) differences between treatments.

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