Soybean Seed Quality Losses Associated with Bean Leaf Beetles and *Alternaria tenuissima*

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ABSTRACT

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Abnormally high populations of the bean leaf beetle (*Cerotoma trifurcata*) in 1980 caused extensive injury to soybean (*Glycine max*) pods in some areas of Illinois. Pods and seeds of six soybean cultivars grown near Urbana were examined for injury and assayed for fungal infection and loss of seed germinability. Pod injury was most severe on late-maturing cultivars and least on Elf, the most pubescent cultivar tested. The incidence of seedborne *A. tenuissima* was correlated with pod injury ($r = 0.53$) and loss of seed germinability ($r = 0.63$). However, the correlations between pod injury and seed germinability losses varied among cultivars. *Alternaria alternata* was inconsistently observed on seeds infected with *A. tenuissima*, and was less pathogenic. Seed treatment with a mixture of captan plus benomyl increased the germination of seeds from injured pods. In a greenhouse study *A. tenuissima* consistently decayed seeds within artificially injured pods, but did not infect seeds within uninjured pods. *A. alternata*, *A. tenuissima*, and eight other species of fungi known to infect soybeans were isolated from adult bean leaf beetles.

Additional key words: Cercospora kikuchii, Fusarium spp., seed decay.

The bean leaf beetle (BLB) (*Cerotoma trifurcata* Forster) is an established pest of soybeans (*Glycine max* (L.) Merr.) in the United States. Feeding by adult beetles causes defoliation and pod injury and larvae destroy roots and Rhizobium nodules (6). In the southern United States BLB adults frequently are important defoliators; in the midwestern states, however, they normally occur in fewer numbers and cause little economic loss (6).

Mild weather conditions in the Midwest during the winters of 1979 and 1980 led to a low mortality of overwintering beetles and an abbreviated life cycle, resulting in populations reaching economically damaging levels in soybeans. In many parts of Illinois the populations were the highest ever recorded. High populations of the BLB and extensive pod injury were observed in soybean plots near Urbana. Uninjured and BLB-injured pods were collected and examined and their seeds were assayed.

Pod injury by the BLB usually is limited to the outer layers of the pod wall, leaving the thin membranous endocarp intact. Developing seeds are infrequently attacked, but the resultant exposure of seeds is believed to reduce their quality (6). The specific cause and nature of seed quality losses associated with BLB pod feeding have not been established.

We report here the BLB pod-feeding injury in six soybean cultivars, the effect of this injury on the mycoflora and germinability of seeds, and the fungi associated with BLB adults.

MATERIALS AND METHODS

Field sampling. Foundation seedlots of soybean cultivars Clark 63, Elf, Harcor, Union, Wayne, and Wells were planted on 30 May 1980 in Drummer silty clay loam soil at the University of Illinois at the Urbana-Champaign Plant Pathology Research Center near Urbana. Four replicated plots of each cultivar were arranged in a randomized complete block design. Each plot consisted of eight 6.1-m rows on 76-cm centers. Plant population was 27 to 32 plants per meter of row. The experiment was conducted simultaneously on two adjacent fields: one with a cropping history of 2 yr consecutively in corn and one with 2 yr consecutively in soybeans. The plots were monitored throughout the growing season to observe soybean diseases.

At harvest maturity 80 pods were sampled at random from each plot. Pod injury was most severe at the four upper nodes and

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sampling was limited to these nodes. Pods of nine cultivars from eight counties in Illinois also were sampled.

Pod and seed assays. The number of BLB feeding lesions per pod was more variable than lesion size and was easier to measure accurately than the percent injured area. Therefore, injury was assessed by determining the mean number of lesions per injured pod. Pods with one or more lesions were considered injured.

Seeds from injured and uninjured pods were separated and surface sterilized for 4 min in 0.5% NaOCl (10% Clorox; Clorox Co., Oakland, CA 94621), received three 1-min rinses in sterile distilled water, were placed in 9-cm-diameter culture plates (five seeds per plate) containing potato-dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI 48232), incubated for 6 days in the dark at 25 C, then were examined. Seeds were considered germinated if the radicle was 1 cm or longer.

Seeds (cultivar Wayne) from injured and uninjured pods were surface-dusted with 100 mg per 50 seeds of captan + benomyl (DPX115-B—(*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide, 50%, and [methyl 1-(butylcarbamoyl)]-2-benzimidazole carbamate, 10%, respectively; E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898). Excess fungicide was removed before the seeds were placed on water-saturated Kimpac blotter pads (Graham Paper Co., St. Louis, MO 63178) and incubated near 100% RH under constant fluorescent light and at 25 C. After 6 days, percent seed germination and incidence of fungi on the seeds were recorded.

Greenhouse pathogenicity tests. Greenhouse-grown soybeans (cultivar Harcor) were used to test the pathogenicity of a mixture of *Alternaria tenuissima* (Kunze ex Pers.) Wilt. isolates recovered from decayed seeds. At growth stage R_5 (3), BLB injury was simulated by excising a 4- to 5-mm-diameter portion of the pod wall adjacent to each of the seeds within a pod. Thirty injured and 30 uninjured pods were inoculated by using a camel's hair brush saturated either with water or a water suspension of macerated *A. tenuissima* mycelium. The inoculum was prepared by scraping mycelium from 5-day-old PDA cultures and macerating it with distilled water for 2 min in a Waring Blendor at slow speed. The mycelial concentration of 8 mg dry weight per milliliter of suspension was determined by dehydrating four 20-ml samples for 48 hr at 65 C. All plants were covered with plastic bags for 48 hr after inoculation. At growth stage R_7, pods were harvested and seeds assayed on PDA as described previously.

Beetle sampling. On 5 September BLB adults were collected from the same field plots. The beetles were washed for 3 hr in running tap water, wings were removed and the heads and abdomens separated. Forty beetles were surface sterilized for 4 min in 0.5% NaOCl, rinsed twice in sterile distilled water and placed in 9-cm-diameter culture plates containing PDA. Dishes were examined after incubation in the dark for 5 days at 25 C as described previously.

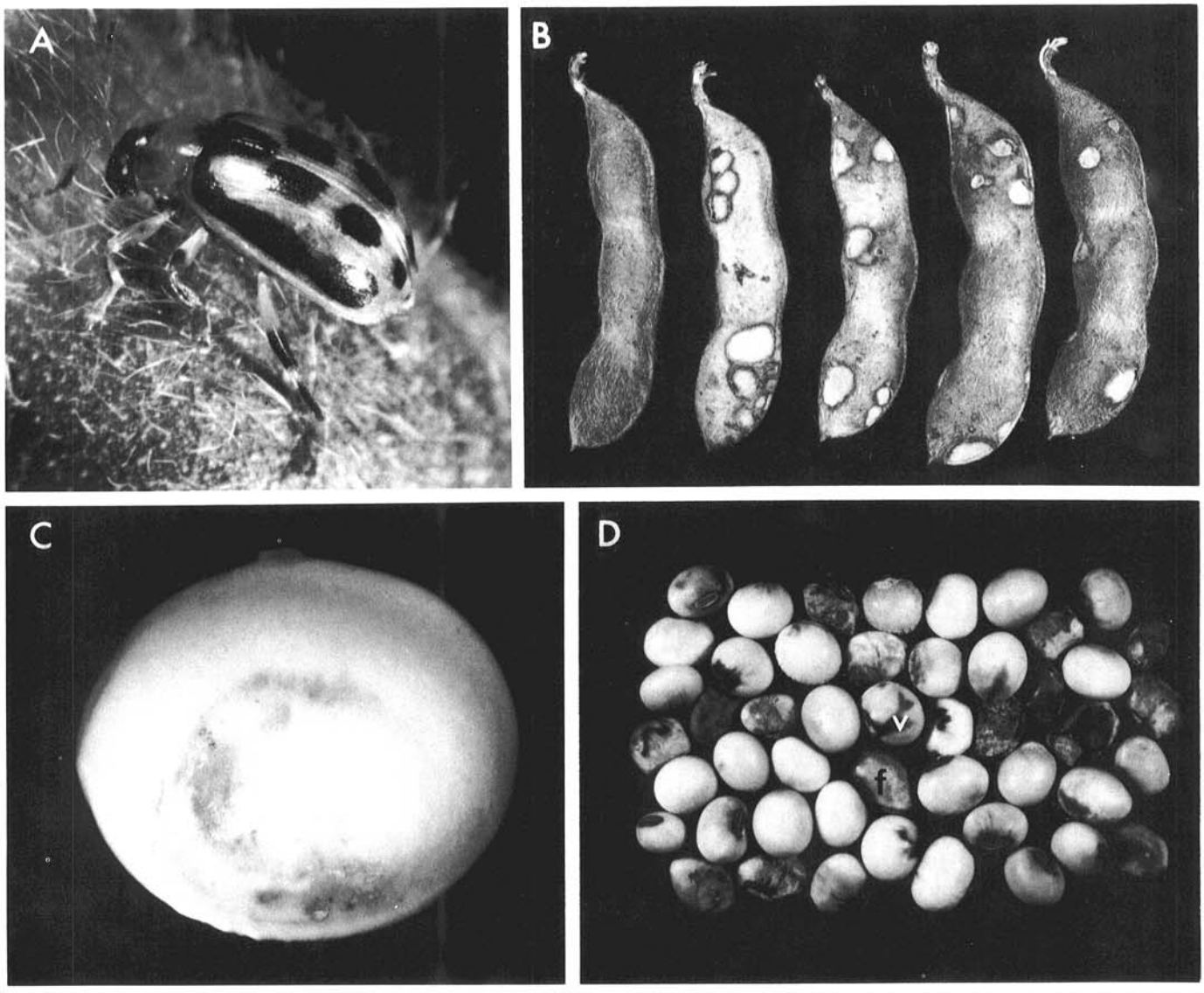

Fig. 1. Pod injury and seed symptoms associated with the bean leaf beetle, *Cerotoma trifurcata*. **A,** A bean leaf beetle. **B,** Soybean pods with feeding injury. **C,** Seed strain associated with pod lesion. **D,** Fungal decay (f) and viral mottling (v) of seeds from injured pods (cultivar Wayne).

TABLE 1. Soybean pod injury and seed germination losses associated with feeding by the bean leaf beetle, *Cerotoma trifurcata*

Previous crop history[w]	Cultivar and maturity group	Pods injured[x] (%)	Lesions per pod (mean no.)	Germination loss (%)[y]
Corn	Harcor (II)	46.0 a[z]	1.5 a	4.5 a
	Wells (II)	47.5 a	1.6 a	7.0 ab
	Elf (III)	17.8 b	1.4 a	4.0 a
	Wayne (III)	61.5 c	2.9 b	27.0 c
	Union (IV)	66.3 c	3.9 c	13.5 b
	Clark 63 (IV)	64.0 c	3.4 d	8.5 ab
Soybean	Harcor (II)	58.8 a	2.0 a	3.5 a
	Wells (II)	50.8 a	1.9 a	14.0 bc
	Elf (III)	25.0 b	1.2 b	1.0 a
	Wayne (III)	77.8 c	3.4 c	21.5 c
	Union (IV)	73.8 c	3.2 c	11.3 b
	Clark 63 (IV)	78.3 c	3.6 c	7.3 ab

[w] In the preceding 2 yr, the plots were planted to either corn or soybeans consecutively.
[x] Mean four replicates, each consisting of 80 randomly sampled pods from the upper four nodes.
[y] Calculated as percent germinated seeds from uninjured pods minus the percent germinated seeds from injured pods, after incubation on potato-dextrose agar for 6 days at 25 C.
[z] Within a crop-history group, means within a column with a letter in common are not significantly different according to Fisher's least significant difference test ($P = 0.05$).

RESULTS

Pod injury and seed quality. Feeding by adult BLB on pods caused roughly circular lesions ~1–4 mm in diameter (Fig. 1). Lesions were scattered over the pod but often were concentrated at either end when feeding was intensive. The number of injured pods and number of lesions per pod were highly correlated ($r = 0.796$) and were greatest in the late-maturing cultivars (maturity groups III and IV) (Table 1). However, cultivar Elf (maturity group III) had the lowest number of injured pods that were also the least severely injured. Decayed seeds from severely injured pods were shrunken, discolored green to brown, and occasionally had visible mold growth (Fig. 1). Less frequently, small circular stains were observed on seeds adjacent to feeding lesions on pods. The absence of punctures in the center of the stained areas indicated that stinkbugs (*Acrosternum hilare*) were not the cause.

The loss of seed germinability associated with pod injury was calculated as the percent germination of seeds from uninjured pods minus the percent germination of seeds from injured pods. Germination losses generally were greater in the late-maturing cultivars and were most severe in Wayne. Differences occurred within, as well as between, the maturity groups. The correlation coefficient (r) between number of lesions per pod and percent germination loss was 0.51 overall, and for each cultivar was: Harcor, 0.231; Wells, 0.763; Elf, 0.089; Wayne, 0.074; Union, 0.475; and Clark 63, 0.489. The variability of "r" among cultivars indicated that the seeds were not equally affected by pod injury and that factors other than pod injury severity may be important in the loss of seed germinability.

The number of injured pods was greater in the field planted to continuous soybeans than in the field previously planted to continuous corn, but the number of lesions per pod, germination losses, and seed mycoflora were similar. Because of these similarities only seed quality data from the continuous soybean field are reported (Table 2).

Pod injury was associated with an increased recovery of fungi from seeds of all cultivars (Table 2). *A. tenuissima* was the predominant fungus recovered from seeds from injured, but not from uninjured pods, comprising 86 and 34% of the total isolates, respectively. *Alternaria alternata* (Fr.) Keissler occasionally was observed as a mixed infection with *A. tenuissima,* but rarely was observed alone and was never observed sporulating on infected hypocotyl tissue as was *A. tenuissima.* Pod injury was correlated ($r = 0.525$) with an increased recovery of *A. tenuissima* from seeds. Seeds from injured pods of all cultivars, except Elf, exhibited significant germination losses correlated ($r = 0.633$) with an increased recovery of *A. tenuissima* compared to seeds from uninjured pods. This species was the only fungus consistently recovered from dead seeds.

TABLE 2. Germination of, and recovery of fungi from, soybean seeds grown in a field planted continuously to soybeans and harvested from pods either injured or uninjured by the bean leaf beetle, *Cerotoma trifurcata*

		Percent of seeds:[a]					
				Infected with:			
Cultivar and maturity group	Pod condition[b]	Germinated	Dead with Alt[c]	Alt[c]	Cer[c]	Pho[c]	Total fungi[d]
Harcor (II)	I	90.5[e]	7.0	54.0	10.5	2.5	70.5
	U	93.0*	0.5*	13.5*	29.5*	7.3	53.3
Wells (II)	I	82.0	17.5	55.0	7.5	0.5	65.5
	U	96.0*	1.5*	11.5*	22.5*	1.5	37.5*
Elf (III)	I	97.0	2.3	27.6	2.3	0.0	31.5
	U	98.0	1.0	7.5*	3.0	0.0	11.5*
Wayne (III)	I	73.0	22.5	54.0	1.5	2.0	60.0
	U	94.5*	1.8*	10.1*	5.8	2.8	18.5*
Union (IV)	I	86.0	13.5	47.0	2.0	0.0	53.5
	U	97.3*	0.0*	2.5*	4.5	0.0	9.8*
Clark 63 (IV)	I	88.0	10.0	39.0	0.0	0.5	43.0
	U	95.3*	0.0*	4.3*	4.0	5.0	13.3*

[a] Mean of four replicates of 50 seeds each after incubation on potato-dextrose agar for 6 days at 25 C.
[b] U = uninjured, I = injured.
[c] Alt = *Alternaria tenuissima,* Cer = *Cercospora kikuchii*, and Pho = *Phomopsis* spp.
[d] Includes those listed plus *Cladosporium* sp., *Epicoccum* sp., *Fusarium* spp., *Gliocladium* sp., *Macrophomina* sp., and *Nigrospora* sp.
[e] Within a cultivar group, means of I and U that are significantly different ($P = 0.05$) by a paired t-test are denoted by an asterisk.

Cercospora kikuchii (T. Matsu & Tomoyasu) Gardner, the causal agent of purple seed stain of soybean, was frequently recovered from seeds of cultivars Harcor and Wells (Table 2). *C. kikuchii* was recovered significantly more often from seeds harvested from uninjured than from injured pods. The recovery of *C. kikuchii* and *A. tenuissima* from seeds harvested from injured pods was inversely correlated ($r = -0.681$); however, the correlation between the recoveries of these fungi from seeds from uninjured pods was lower ($r = -0.130$). Bean pod mottle virus, which is transmitted by the BLB (7), was not observed in these field plots.

Seeds of cultivar Wayne harvested from injured pods and surface treated with captan + benomyl had a higher percent germination and lower incidence of *A. tenuissima* than untreated seeds (Table 3). However, fungicide treatment of seeds from injured pods did not increase germination to levels equivalent to seeds from uninjured pods. This suggests that seed death associated with BLB pod injury and *A. tenuissima* colonization occurs before as well as during germination.

TABLE 3. Effect of captan + benomyl seed treatment on soybean seed germination and the incidence of *Alternaria tenuissima* in Wayne seeds from pods either injured or uninjured by the bean leaf beetle, *Cerotoma trifurcata*

Seed		Seeds (%)[x] either:	
Source	Treated[y]	Germinated	Dead with *Alternaria*
Uninjured pods	Yes	84.5 a[z]	0.0 a
Uninjured pods	No	79.0 a	2.5 a
Injured pods	Yes	68.5 b	18.5 b
Injured pods	No	59.0 c	29.5 c

[x] Mean of four replicates of 50 seeds each.
[y] Each 50-seed sample was surface dusted with 100 mg of a 50% captan plus 10% benomyl mixture.
[z] Means in a column with a letter in common are not significantly different according to Fisher's least significant difference test ($P = 0.05$).

Isolation of fungi from adult beetles. Nine species of fungi known to infect soybean plants and seeds were isolated from surface-sterilized BLB heads and abdomens. The absence of common surface contaminants, such as *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp., suggests that the fungi isolated from the beetles may be internally borne. The predominant species isolated were *A. tenuissima* and, to a lesser extent, *A. alternata*. Both fungal species often were observed on the same BLB and were recovered from 85% of both the heads and the abdomens. *Epicoccum* sp., *Nematospora* sp., and *Phoma* sp. were recovered infrequently (less than 5%) from the heads; and *Fusarium equiseti* (Corda) Sacc., *Fusarium graminearum* Schwabe, *Fusarium tricinctum* (Corda) Sacc., and *Gliocladium roseum* Bainier were isolated infrequently from the abdomens.

Pathogenicity of *Alternaria tenuissima*. No seeds from artificially injured pods inoculated with *A. tenuissima* germinated and all were decayed and colonized by the fungus. Seventy-eight percent of the seeds from injured, water-inoculated pods germinated and none were infected by fungi. Some of the loss in seed germinability may be due to handling during pod treatment at growth stage R_5. None of the seeds from uninjured pods inoculated with *A. tenuissima* were infected, further suggesting that pod wounding is necessary for the fungus to parasitize seeds.

DISCUSSION

Our estimates of pod injury severity and seed quality losses were based on pods and seeds harvested from the upper plant nodes. Pod injury is more common in the upper third of the plant; therefore, the injury severity and seed germinability losses reported here probably are overestimates.

Pod injury and seed germinability losses varied among and within maturity groups. As group II cultivars matured, the beetles moved to the green, later-maturing cultivars, resulting in more pods being severely injured. Elf, which possesses dense, upright pubescence, had less injury caused by the BLB than the other cultivars, indicating the value of pubescence as a BLB feeding deterrent. High levels of pod injury were associated with a loss of seed germinability. However, the closeness of this association varied among cultivars. While BLB pod feeding may cause abortion of seeds, it is rarely directly responsible for death of developing seeds (1). Many factors may affect loss of seed germinability associated with pod injury. Among these are the integrity of the pod wall and seed coat, the stage of seed development, and the resistance of the seed to damage from exposure and infection and decay by fungi.

The most common cause of seed decay in Illinois, *Phomopsis* sp. (8), was not a factor in these experiments owing to dry weather during crop maturation and because sampling was limited to the upper plant parts. Phomopsis seed decay was 13% in one sample from Coles Co., but there was no difference in infection levels between seeds from injured and uninjured pods. *C. kikuchii* was recovered more frequently from seeds harvested from uninjured pods than from those from injured pods. Conversely, more *A. tenuissima* was recovered from seeds from injured pods. Thus, the competitive ability of these fungi may have been altered by the presence of pod injury.

The associations between BLB pod injury, seedborne *A. tenuissima,* and reduced seed germinability were consistent in the Urbana and statewide studies. *A. tenuissima* is a cosmopolitan weak pathogen of many plant species, including soybean (2,4,5,9). It has been isolated routinely from green soybean tissues throughout the growing season in Illinois. In an earlier assay of green pods from the same field plots, *A. tenuissima* was recovered from all sampled pods and was later recovered from 46% of seeds from injured pods and 9% of seeds from uninjured pods. High levels of seedborne *Alternaria* spp. have been associated with delayed harvest (11), frost injury (10), and stinkbug punctures (5). *A. tenuissima* causes leaf spot and wilt diseases in Kenya (9), but rarely damages soybeans in the midwestern USA. In this study, seeds were sometimes colonized by both *A. tenuissima* and *A. alternata*; however, the latter was less consistently isolated and was not as aggressive on diseased hypocotyls. Therefore, we believe that *A. tenuissima* is the primary pathogen involved in seed deterioration caused by *Alternaria* spp. The wounding and stressing of pods associated with BLB feeding predisposes seeds to infection by *A. tenuissima,* a common (but normally innocuous) fungus. Differential cultivar susceptibility to *A. tenuissima* may explain in part the variable levels of seed decay observed in cultivars with similar levels of pod injury. Infected seeds often decayed in the field before harvest; however, the increased seed germination associated with fungicide seed treatment indicates that infected viable seeds also may be killed by the fungus during germination.

All of the fungi isolated from adult BLB have been reported to infect soybeans and were isolated from seeds. *A. tenuissima* was the predominant fungus in both beetles and seeds. Because the mycoflora of insects commonly reflects their environment, the association of these fungi with beetles only suggests that transmission to plants is possible. Our data indicates that beetle injury is not required for any of these fungi to infect soybean seeds, but beetle feeding could increase the dispersion and inoculation of fungal pathogens to favorable sites such as wounds. Thus, facultative transmission of fungal pathogens may compound the effect of feeding injury.

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