Fungi and insect damage to soybean seeds harvested at immature stages in tropical environments[1, 2]

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ABSTRACT

Cultivars 'BSR 101' (Maturity Group II) and 'Cumberland' (Maturity Group III) of soybean were planted in three environments in Puerto Rico in 1981 and 1982 for a study of whether the damage caused by pests is lessened by seeds being harvested at different stages of development in tropical field conditions. Harvest of seeds began 24 days after flowering (DAF) and continued at weekly intervals until plants reached maturity at 59 DAF. Pest symptoms were identified, and their incidence was recorded on the basis of two 50-seed samples for each cultivar, harvest date, and environment. Immature soybean seeds harvested as early as 24 DAF had symptoms of purple seed stain [*Cercospora kikuchii* (T. Matsu. & Tomoyasu) Gardner], downy mildew [*Peronospora manshurica* (Naum.) Syd. ex Gaum.], anthracnose [*Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncatum* (Schw.) Arx.], and pod blight [*Phomopsis* spp.), insect damage from lima bean pod borer [*Etiella zinckenella* (Treitschke)], and southern green stink bug [*Nezara viridula* (L.)]. Maximum levels of pest damage were observed on seed harvested 38 DAF or later, depending on the organism and the environment. Results indicate that the harvest of immature soybean seeds at 31 DAF could reduce the incidence of pest damage identified in this study.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] breeding programs of the northern United States use tropical locations to advance generations, obtain hybridizations, increase seed, and make selections during the winter when the crop cannot be planted in its area of adaptation (1). At these nursery locations, it is possible to plant two generations during the 6-month

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period of winter. In this planting schedule, where the experimental material is exchanged between the adapted and the nursery locations, timing of harvest becomes very important. Ortiz et al. (8) have found that one way to shorten the generation length of the crop at tropical locations is to harvest viable immature seed. In their work, they concluded that it is possible to harvest immature seed with adequate germination as early as 31 days after flowering (DAF). This harvest procedure reduces the generation time by 10 to 14 days.

Soybean seeds may be infested by pests throughout their development. Pod blight (*Phomopsis* spp.), and purple seed stain [*Cercospora kikuchii* (T. Matsu. & Tomoyasu) Gardner] fungi have been isolated from immature soybean seeds of cultivars grown at temperate locations (6, 7). In Puerto Rico, purple seed stain, pod blight (*Phomopsis sojae* Leh.), anthracnose [*Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncatum* (Schw.) Arx.], and seed mold (*Fusarium semitectum* Berk. & Rav.) have been reported to be the most common fungi associated with soybean seeds harvested at maturity (4, 5). There is no information as to whether these or other pathogens could damage immature soybean seeds harvested at tropical locations. Reports of insects feeding on immature soybean seeds at temperate and subtropical locations have been published (2, 9, 10, 11). Southern green stink bug [*Nezara viridula* (L.)] and the lima bean pod borer [*Etiella zinckenella* (Treitschke)] have been observed feeding on soybean seeds at early stages of seed development, thus reducing their yield and quality (2, 9, 10, 11). The purpose of the study was to determine whether the damage caused by pests is lessened by seeds being harvested at different stages of development in soybeans grown in tropical field conditions.

MATERIALS AND METHODS

Two soybean cultivars, BSR 201 of Maturity Group II and Cumberland of Maturity Group III, were planted in three environments at the Iowa State University-University of Puerto Rico Soybean Breeding Nursery at the Isabela Substation, Isabela, Puerto Rico, in 1981 and 1982. Two of the environments were plantings under the natural day length conditions of Puerto Rico (unlighted environments) 18 November 1981 and 8 February 1982. Natural average day length during the growing season was 11 hr for the November planting and 12 hr for the February planting. The third environment was planted 18 November 1981 under lighted conditions (lighted environment). In the lighted environment, plants were exposed to continuous light for 15 days after emergence, a 14.5-hour day for an additional 35 days, and natural day length thereafter. Lighting consisted of 240-V, 1,500-W quartz-iodide bulbs installed on poles approximately 7 m high. To determine age of developing seeds, flowers were identified with a tag the day they opened. An average of

two flowers per plant were identified. The harvest of seeds began at 24 DAF and continued at weekly intervals until plants reached maturity, 59 DAF, stage R8 (3). Pods harvested from each cultivar, harvest date, and environment were allowed to dry at ambient conditions for 3 weeks before they were hulled by hand.

Seed damage on the basis of visible symptoms and signs of infection by fungi and infestation by insects was identified and recorded on two 50-seed samples for each combination of cultivar, harvest date, and environment. We obtained the 50 seeds by harvesting one three-seeded or two-seeded pod from each of approximately 20 to 30 plants within each of the treatments. Visible symptoms for each pest were 1) purple seed stain [*Cercospora kikuchii* (T. Matsu. & Tomoyasu) Gardner], seed purple discoloration; 2) downy mildew [*Peronospora manshurica* (Naum.) Syd. ex Gaum.], grey-white crust of oospores; 3) anthracnose [*Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncatum* (Schw.) Arx.], brown seed discoloration; 4) pod blight (*Phomopsis* spp.), grey discoloration and seed cracking and shrivelling; 5) lima bean pod borer [*Etiella zinckenella* (Reitschke)], eaten seed and exit holes; and 6) southern green stink bug [*Nezara viridula* (L.)], punctures and shrunken craters on the seed coat.

Seeds showing fungal symptoms and insect larvae damage by different species were counted. No significant differences were observed between cultivars; therefore, data presented are averages of two samples and two cultivars at each harvest date and environment.

A germination test with two replications was conducted under laboratory conditions. Seeds were placed on top of cellulose cloth on aluminum trays and watered with distilled water. After 5 days at 27° C and 90% relative humidity, the germination percentage was determined. A seed was considered germinated when the radicle pierced the seed coat and grew twice the length of the cotyledons. Before the germination test, seed quality scores were assigned to every sample on the basis of percentage of seeds showing fungal and insect damage according to the scale: 1=<2%, 2=3%, 3=3 to 5%, 4=5 to 8%, 5=>8%.

RESULTS AND DISCUSSION

Symptoms of purple seed stain on immature soybean seed were identified as early as 31, 24, and 38 DAF in the November- and February-unlighted, and November-lighted environments (table 1). Maximum level of incidence in the respective environments were reached at 45, 59, and 38 DAF. The first symptoms of anthracnose occurred 24 and 52 DAF in the February-unlighted and the November-lighted environments. The greatest amount of anthracnose in seeds was observed at 59 DAF in the February-unlighted environment when plants reached maturity. Downy mildew was first identified at 38, 52, and 31 DAF in the November- and

TABLE 1.—*Incidence of fungal infestation and insect damage on soybean seeds harvested at different stages in three environments in Puerto Rico averaged over replications and cultivars*

Days after flowering	Fungi											
	Purple seed stain (*Cercospora kikuchii*)			Pod blight (*phomopsis* sp.)			Anthracnose (*Colletotrichum dematium*)			Downy mildew (*Peronospora manshurica*)		
	NU[1]	FU[1]	NL[1]	NU	FU	NL	NU	FU	NL	NU	FU	NL
	%											
24		0.5						0.5				
31	0.5	3.0										3.0
38	1.0	5.0	5.0							1.0		4.0
45	2.0	5.0	1.0					0.5		1.0		3.0
52	2.0	7.0	5.0		2.0				0.5		1.0	2.0
59	0.5	8.0	4.0		2.0			5.0				4.0
$\bar{x}$	1.0	4.8	2.5		0.7			1.0	0.1	0.3	0.2	2.7
Sd[2]	0.7	1.4	1.2		0.8			1.6	0.2	0.4	0.4	1.2
	Insects											
	Southern green stink bug (*Nezara viridula*)									Lima bean pod borer (*Etiella zinckenella*)		
24		1.0									6.0	
31		4.0									15.0	0.5
38		5.0									10.0	
45		2.0									27.0	
52		5.0									28.0	
59		2.0									25.0	
$\bar{x}$		3.2									18.5	0.1
Sd[2]		1.4									7.6	0.2

[1] NU = November unlighted; FU = February unlighted; NL = November lighted.

[2] Sd = Standard error of the difference of two means for comparisons among harvest dates.

February-unlighted and November-lighted environments. Its maximum incidence was observed at 38 DAF in the lighted environment. Pod blight was first identified at 52 DAF in the February-unlighted environment, when some of the pods were already turning yellow (table 1). *C. kikuchii* was the fungus most prevalent, and its symptoms were observed at all environments. *P. manshurica* was also found on seeds harvested in all the environments, but the percentage of infestation was lower than that for *C. kikuchii*, except in the November-lighted environment, where these fungi had similar levels. *C. dematium* was identified in two of the three environments, but at a lower incidence observed for either purple seed stain or pod blight. *Phomopsis* sp. was present only in the February environment.

Damage by larvae of two insect species, *Etiella zinckenella* and *Nezara viridula*, was identified on immature soybean seeds as early as 24 DAF in the February-unlighted environment (table 1). *Etiella zinckenella* was the most common insect feeding on seeds, particularly in the February-unlighted environment. *E. zinckenella* was also found on a few seeds harvested from plants in the November-lighted environment, but its incidence was negligible compared with that in the February environment. Damage from larvae of southern green stink bug (*Nezara viridula*) was identified only in the February-unlighted environment and at a lower rate than for *E. zinckenella*. Maximum damage by the southern green stink bug appeared at 38 DAF, and maximum damage by the lima bean pod borer at 45 DAF. Greater infestations of lima bean pod borer in the February-unlighted environment may be due to increases in the natural photoperiod and the rainy season onset in Puerto Rico that normally occurs in April and May. However, the relative importance of day length and precipitation in determining the population levels of the lima bean pod borer has not been established. Southern green stink bug, a sucking insect, favors succulent tissue for feeding. Lima bean pod borer, a chewing insect, attacks at later stages of seed development. Damage by both insects decreased close to and at seed maturity, probably reflecting the more difficult feeding from mature tissue or the preference for other sources of younger plant tissue.

Seed harvested from the February-unlighted environment had the lowest average germination percentage and the poorest seed quality score (table 2). The highest average germination percentage and intermediate seed quality scores were observed for the seed harvested from the November-lighted environment. Highest germination percentages were observed on seeds harvested at 38 DAF. Differences in germination percentages between the seeds harvested at 31 and at 38 DAF were not significant. For the two unlighted environments, seed quality deteriorated for seed harvested at 45 DAF. For the lighted environment, the poorest seed quality was observed on seed harvested at 38 DAF.

TABLE 2.—*Germination percentages and seed quality scores of soybean seeds harvested at different stages in three environments in Puerto Rico averaged over replications and cultivars*

Days after flowering	Germination			Seed quality[2]		
	NU[1]	FU[1]	NL[1]	NU	FU	NL
	%			Score		
24	33	20	86	1.0	4.0	1.0
31	66	50	95	1.0	4.5	3.0
38	97	62	100	1.5	4.0	4.0
45	95	38	98	2.0	5.0	2.5
52	92	35	98	1.5	5.0	4.0
59	94	36	100	1.0	5.0	4.5
$\bar{x}$	80	40	96	1.3	4.6	3.2
LSD[3] (0.05)	38	29	3	0.2	0.2	0.4

[1] NU = November unlighted; FU = February unlighted; NL = November lighted.

[2] Scores ranged from 1 (excellent) to 5 (poor).

[3] LSD is the least significant difference with which to compare consecutive germination percentages and seed quality scores.

Results indicate that immature soybean seeds may be attacked by pests as early as 24 DAF (table 1). For pests identified in this study, maximum levels of damage were observed at 38 DAF or later, depending on the organism and the environment. On the basis of these observations, harvest of soybean seeds at 31 DAF, as proposed by Ortiz et al. (8), could reduce incidence of some of the pest damage present in tropical field conditions. Damage caused by three of the most common soybean fungal diseases in tropical locations (4), pod blight, purple seed stain, and anthracnose, and the damage by insects was reduced by harvesting immature seeds. This harvest procedure seems to diminish diseases in soybean seeds grown under tropical conditions, particularly hot rainy periods that favor pest activity.

RESUMEN

Daño de hongos e insectos a semillas inmaduras de soja en el trópico

El propósito del trabajo que informamos fue estudiar si la incidencia de plagas y sus daños cambian al cosechar semilla de soja en diferentes etapas de desarrollo en condiciones tropicales. Las cultivares 'BSR 201', del Grupo de Madurez II, y 'Cumberland', del Grupo de Madurez III, se sembraron en tres ambientes en Puerto Rico en 1981 y 1982. Para determinar la edad de las semillas en desarrollo, las flores se identificaron el día que abrieron con una etiqueta colocada inmediatamente debajo del nudo donde estaban las flores. La etiqueta decía la fecha de floración y el número de flores por nudo. Las semillas se comenzaron a cosechar a los 24 días después de la floración (DAF) y continuó semanalmente hasta que las plantas alcanzaron su madurez fisiológica a los 59 DAF. Los síntomas de cada plaga se identificaron y se contó su incidencia en una muestra de 50 semillas de cada

cultivar, cada fecha de cosecha y cada ambiente. Las semillas inmaduras cosechadas a los 24 DAF presentaron síntomas de mancha púrpura [*Cercospora kikuchii* (T. Matsu. & Tomoyasu) Gardner], añublo pulverulento [*Peronospora manshurica* (Naum.) Syd. ex Gaum.], antracnosis [*Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncatum* (Schw.) Arx.], tizón de la vaina (*Phomopsis* spp.) y daños de las larvas del barrenador de la vaina [*Etiella zinckenella* (Treitschke)] y de la chinche apestosa [*Nezara viridula* (L.)]. Los daños máximos causados por estas plagas se observaron en las semillas cosechadas 38 DAF o más edad dependiendo de la plaga y del ambiente. Nuestros resultados indican que la cosecha de semillas de soja inmaduras a los 31 DAF disminuye la incidencia del daño de las plagas indentificadas en este estudio.

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