Field Crops Research, 4 (1981) 113—121
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

SOYBEAN SEED DETERIORATION IN THE TROPICS. I. THE ROLE OF PHYSIOLOGICAL FACTORS AND FUNGAL PATHOGENS

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(Accepted 17 December 1980)

ABSTRACT

Ndimande, B.N., Wien, H.C. and Kueneman, E.A., 1981. Soybean seed deterioration in the tropics. I. The role of physiological factors and fungal pathogens. *Field Crops Res.*, 4: 113—121.

Two field experiments were conducted to determine whether the rapid loss of soybean seed viability in the lowland humid tropics is primarily due to physiological factors or to seed-borne microorganisms, or to both. In both experiments benomyl fungicide was applied (1 g/l) to four cultivars of contrasting storability as a foliar spray weekly from mid-flowering to maturity. The seeds were harvested at maturity or after a delay of two weeks.

Plants in Experiment 1 matured under dry conditions and low fungi incidence. Harvest delay and benomyl treatment had little effect on seed pathogen incidence or germination at harvest. In Experiment 2, which matured in moist conditions, delayed harvest reduced viability by 25%, with significant cultivar × harvest time interactions. Seeds from plants treated with benomyl had higher viability than seed from untreated plants, and significantly lower incidence of *Phomopsis* species, *Macrophomina phaseoli, Fusarium* species and *Colletotrichum truncatum*. Pathogens seem to be important in seed deterioration prior to harvest.

To determine the effect of benomyl treatment on rate of decline in seed viability in storage, seeds from Experiment 1 and 2 were stored at 80% relative humidity and 28 or 35°C and sampled periodically. Seeds from benomyl-treated and untreated plants declined in viability at equal rates.

Dusting of benomyl on seed of Bossier soybean equilibrated to 10, 13 or 16% moisture content before the start of storage at 28°C completely inhibited surface fungal growth, but did not influence rate of viability decline. Rate of viability decline varied directly with seed moisture content. Extracted embryos from freshly harvested Bossier seed grew on agar and were free from fungal pathogens, while embryos from seed stored for 9 months failed to grow even though little infection (0—5%) was observed. These results strongly suggest that the role of pathogens in seed deterioration during storage may frequently be secondary to that played by physiological factors.

INTRODUCTION

Production of good quality soybean [*Glycine max* (L.) Merr.] seed and maintenance of viability in storage is difficult in the humid tropics. Previous research suggests that loss of seed viability might result from pathogens or from physiological factors, and from biochemical changes which are accelerated by high temperature and high relative humidity (RH). Fungal pathogens have been implicated in seed deterioration before harvest (Wilcox et al., 1974; Ellis et al., 1974; Roy and Abney, 1977; Kmetz et al., 1978; Paschal and Ellis, 1978). Physical disruption of seed tissue resulting from alternate wetting and drying may also be responsible for field weathering of seed (Moore, 1965; Moore, 1971; Mondragon and Potts, 1974). While numerous reports suggest that pathogens cause seed deterioration during storage (Nicholson et al., 1972; Dhingra et al., 1973; Ellis et al., 1974; Tenne et al., 1974), there is also substantial evidence indicating non-pathological causes (Edje and Burris, 1970; Abdul and Burris, 1971; Roberts, 1973a, b; Chapman and Robertson, 1977).

The objective of the present work was to determine whether the pre- and post-harvest loss of soybean seed viability in the lowland humid tropics is primarily due to physiological factors or to seed-borne microorganisms, or to both. The conclusions are prerequisites for determining an efficient methodology for breeding varieties for the tropics with resistance to field weathering of seed, and with improved seed storability. The work was divided into two areas of study: the role of fungi in seed deterioration before harvest and during storage.

MATERIALS AND METHODS

Two experiments were conducted at IITA (Ibadan, Nigeria) to determine the role of fungal pathogens in the loss of soybean seed viability before harvest. Experiment 1, from September to December 1977, was harvested under dry conditions; Experiment 2, from April to July 1978, had humid conditions at harvest time.

Both experiments were in split-split designs with benomyl application (with and without) as whole plots, cultivars (TGm 80, TGm 294-4, TGm 686, and TGm 685) as sub-plots and harvest time (prompt or 2 weeks delay) as sub-sub plots; there were four replications. TGm 80 (varietal name, Bossier) and TGm 294-4 are cultivars of US origin and known to have high yield potential but poor storability, while TGm 685 and TGm 686 are introductions of Indonesian origin with good storability. Benlate with 50% a.i. of benomyl (methyl-1 (butylcarbamyl)-2 benzimidazole carbamate) was applied weekly at a rate of 1 g/l as a foliar spray from mid-flowering to maturity. At each harvest, seeds were tested for germination and analyzed for incidence of seed-borne fungi by the "blotter test method" (P. Neergaard, personal communication, 1977), a technique involving surface pre-treatment with 1% sodium hypochlorite for 3 min. Seeds were plated in petri dishes on three layers of moistened blotter paper 2 cm apart, and incubated at 26° C ± 2° C. A near ultraviolet light/darkness cycle of 12/12 h adopted as a standard procedure by International Seed Testing Association (ISTA, 1966) was used. Recording for germination and fungi was made after 8 days incubation. Seeds were considered germinated when the radicle grew twice the length of the seed. Fungi analyses were made with a stereomicroscope. Arcsine transformations were made on all percentage data before statistical analysis.

To determine the effect of a foliar benomyl treatment on the death of seed in storage, seeds from Experiments 1 and 2 were stored at 80% RH and 28 to 35° C and were sampled periodically to monitor incidence of pathogens and germinability.

Experiment 3 was designed to determine whether seed pathogens or physical factors are primary causal agents in the deterioration of soybeans during storage. Seeds of Bossier, a line known to have poor storability, were equilibrated to 10, 13 or 16% moisture content by suspending them over water at 5° C before fungicide treatment. Before storage, seeds were kept for 3 h at 5° C, resulting in the condensation of water on the seed surface, when they were removed to ambient temperature, which facilitated adherence of fungicide particles to the seeds. Benomyl was applied as dust at the rate of 30 mg/ 100 seeds. Untreated seeds served as controls. Seeds were sealed in air-tight containers to maintain moisture content at the initial level and stored at ambient temperature (28° C). At 2 month intervals a sample of 100 seeds from each of four replications was examined for germination and fungal incidence.

To determine if loss of viability was due to embryo infection, four sets of 100 Bossier seeds were equilibrated to 10, 13 and 16% moisture content and stored for 9 months; freshly harvested Bossier seeds were included in subsequent tests for comparison. Seeds were surface sterilized by dipping them into 95% ethanol and flaming them over a bunsen burner. Embryos were excised aseptically and planted on potato dextrose agar. After 7 days incubation at 28° C the embryos were examined for viability and infection by fungi and bacteria.

RESULTS

The effects of delayed harvest and benomyl on seed deterioration prior to harvest

Delayed harvest significantly decreased germination by 21% in Experiment 1, when weather conditions at harvest time were relatively dry, and by 22.5% in Experiment 2 when conditions at harvest were wet. Bossier was more adversely affected by the delayed harvest than other varieties in Experiment 2, giving rise to a highly significant variety × harvest date interaction (Table I).

Delayed harvest also caused significant increases in seed-borne fungi, namely *Macrophomina phaseoli* (Maubl.) Ashby, *Aspergillus* species and *Cercospora kikuchii* (Matsatomoy) M.W. Gardner in Experiment 1 and *M. phaseoli*, *Fusarium* species, and *C. kikuchii* in Experiment 2 (Table II). It is of interest to note that seeds of the two varieties of Indonesian origin (TGm 686, and TGm 685) were significantly less infected by *Phomopsis* species than were seeds of

TABLE I

Effect of delayed harvest and pre-harvest benlate treatment on germination, prior to storage, of four soybeans varieties for two seasons

Variety	% Germination — harvest effects (averaged over benlate treatments)			% Germination — benlate effects (averaged over harvest times)		
	Prompt harvest	Delayed harvest	Δ	With benlate	Without benlate	Δ
Experiment 1 (dry)						
Bossier	96.7	90.1	6.6	97.3	90.2	7.1
TGm 294	95.5	97.0	−1.5	96.7	95.7	1.2
TGm 686	99.0	97.0	2.0	97.2	98.7	−1.5
TGm 685	98.0	96.1	1.9	96.3	97.7	−1.4
Mean	97.3	95.2	2.1*	96.9	95.6	V× T**
Experiment 2 (wet)						
Bossier	87.6	54.8	32.8	74.7	67.7	7.0
TGm 294	86.1	63.1	23.0	75.8	73.3	2.5
TGm 686	89.8	75.7	14.1	84.5	81.1	3.4
TGm 685	93.2	73.1	20.1	84.6	81.7	2.9
Mean	89.2	66.7	22.5** V× H**	79.9	76.0	3.9*

V = variety; H = harvest time; Δ = difference.
*, **Significantly different (arcsine transformation) at $P = 0.05$ and $P = 0.01$ respectively.
V× H = variety × harvest time interaction. V× T = variety × benlate treatment interaction.

the two varieties (Bossier and TGm 294) of US origin. TGm 686 was significantly more susceptible to *Colletotrichum truncatum* (Schw.) Andr. & Moore than other varieties in both experiments, and TGm 685 was significantly more resistant than other varieties to *C. kikuchii* (Table II).

Foliar application of benomyl before harvest significantly improved subsequent germination by 5% in Experiment 2 (Table I). In Experiment 1 the effect of benomyl on germination across varieties was not significant but the variety × benomyl interaction was highly significant; benomyl improved the germination of Bossier seed while the germination of other varieties was very high both with and without benomyl. Seeds from benomyl treated plants had significantly lower incidence of *Phomopsis* species, *M. phaseoli*, *Fusarium* species and *C. truncatum* in both experiments; only the incidence of *C. kikuchii* was significantly reduced by benomyl in Experiment 1.

Role of fungal pathogens in seed deterioration during storage

Foliar application of benomyl did not prevent the subsequent rapid deterioration of seed in storage (Table III). For example, before storage the germina-

TABLE II

Effects of delayed harvest on incidence of seed-borne fungal pathogens prior to storage for four soybean varieties averaged across fungicide treatments for two seasons

Variety	% Seeds infected																	
	Phomopsis sp.			*Macrophomina phaseoli*			*Fusarium* sp.			*Colletotrichum truncatum*			*Aspergillus* sp.			*Cercospora kikuchii*		
	P	D	Δ	P	D	Δ	P	D	Δ	P	D	Δ	P	D	Δ	P	D	Δ
Experiment 1 (dry)																		
Bossier	12.9	13.5	0.6	1.0	3.0	0.2	6.5	6.5	0	0.0	1.2	1.2	1.0	5.2	4.2	9.5	14.9	5
TGm 294	6.6	9.8	3.2	0.0	1.5	1.5	5.5	11.0	5.5	0.0	1.5	1.5	1.2	2.9	1.7	3.1	8.0	4
TGm 686	1.8	7.5	5.7	2.0	6.4	4.4	7.6	8.0	0.4	12.0	9.9	—2.1	0.0	1.8	1.8	8.9	14.6	5
TGm 685	4.5	0.6	3.9	2.5	4.9	2.4	5.6	8.3	2.7	1.6	0.0	—1.6	1.5	0.5	—1.0	0.0	3.5	3
$\bar{x}$	6.5	7.8	1.3	1.5	3.9	2.4**	6.3	8.5	2.2	3.4	3.2	—0.2	0.9	2.6	1.7**	5.4	10.2	4
		V×H**			V×H*						V×H**							
Experiment 2 (wet)																		
Bossier	10.9	14.5	3.6	4.0	5.0	1.0	14.0	19.4	5.4	2.6	3.6	1.0	5.5	5.9	0.4	23.6	30.1	6
TGm 294	9.6	10.2	0.6	3.9	7.9	3.9	9.4	13.2	3.8	2.9	2.1	—0.8	2.0	4.2	2.2	16.5	20.1	3
TGm 686	2.4	3.6	1.2	1.1	5.1	4.0	11.4	15.2	3.8	15.1	11.1	—4.0	1.5	1.5	0.0	17.1	17.1	0
TGm 685	1.5	1.1	—0.4	4.6	4.6	0.0	10.0	13.0	3.0	4.5	5.1	0.6	2.8	4.2	1.4	6.2	8.0	1
$\bar{x}$	6.1	7.5	1.4*	3.4	5.6	2.2*	11.2	15.2	4.0**	6.3	5.5	—0.8	3.0	4.0	1.0	15.9	18.8	2

P = prompt harvest; D = delayed harvest; Δ = difference.
*, **Significantly different (arcsine transformation) at P = 0.05 and P = 0.01, respectively.
V×H = Variety × harvest time interaction.

TABLE III

Effects of 10 weeks of storage, harvest time, and pre-harvest fungicide application on the germination of four varieties of soybean, Experiment 2

	Before storage				After 10 weeks storage			
	Benomyl treated plants		Not treated		Benomyl treated plants		Not treated	
	P[1]	D[2]	P	D	P	D	P	D
TGm 80	90	59	86	50	18	11	12	6
TGm 294	88	64	85	62	27	15	6	6
TGm 686	90	79	89	73	92	69	87	60
TGm 685	94	76	93	71	88	72	87	55
S.E. ($\bar{x}$) = 2.39								

[1] Prompt harvest; [2] Delayed harvest (2 weeks).

tion of promptly harvested TGm 80 was 90% and 86% from benomyl treated and untreated plants, respectively, and 18% and 12%, respectively, after 10 weeks of storage.

The two varieties of Indonesian origin, TGm 685 and TGm 686, maintained higher germinability in storage than did the varieties of US origin, TGm 80 and TGm 294-4, in both experiments; data from Experiment 2 are shown on Table III.

Dusting of benomyl on seed of Bossier equilibrated to 10, 13 or 16% moisture content before the start of storage at 28° C appeared to control growth of fungi completely, but did not influence rate of loss in viability (Fig. 1). Rate of viability decline varied directly with seed moisture content.

While the incidence of embryo infection of aged seed remained very low (only *Aspergillus* sp. was isolated, and from only a few embryos), embryo viability was greatly reduced by storing seed at high moisture content (Table IV).

DISCUSSION

Our data confirm previous reports that delayed harvest results in the increase of seed-borne fungi and reduction in seed germination (Ellis and Sinclair, 1976; Ellis et al., 1976; Anon, 1977). Seeds harvested from the drier conditions of Experiment 1 had better storability than seed from the wetter conditions of Experiment 2.

These data agree with the previous report that foliar application of benomyl decreases seed-borne microorganisms, resulting in improved germination (Ellis and Sinclair, 1976). Since benomyl reduced the incidence of seed-borne pathogens and increased seed viability, it is probable that pathogens play an important role in the loss of seed viability before harvest.

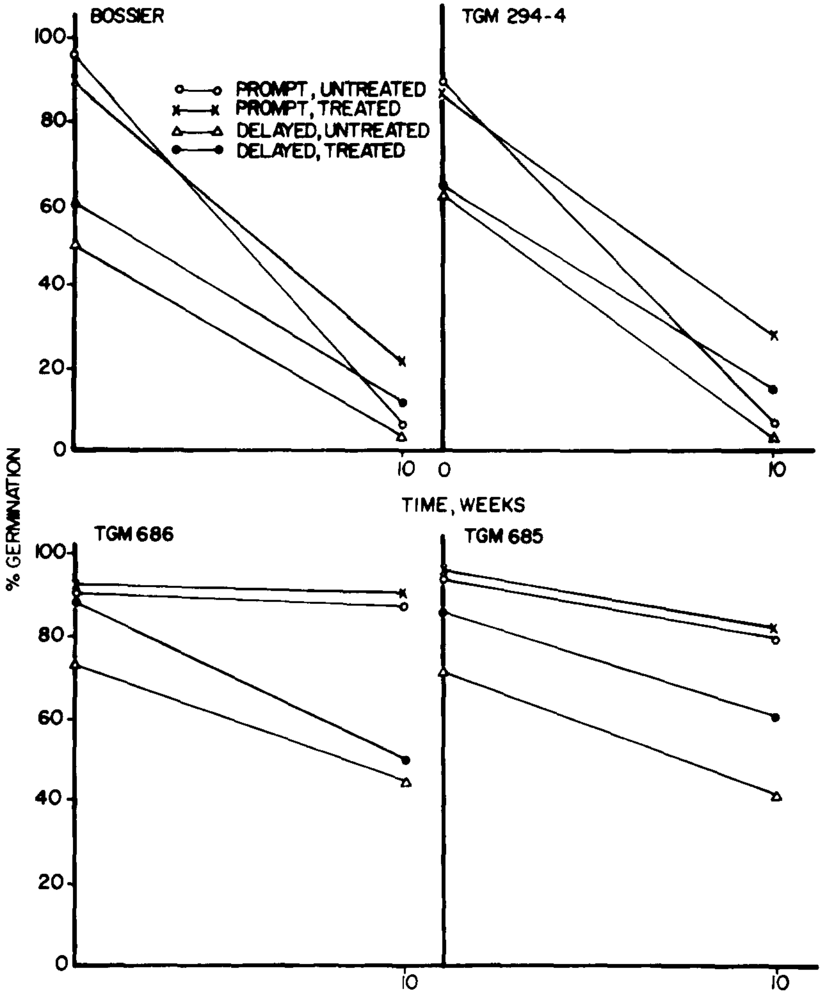

Fig. 1. Effect of seed moisture content and of dusting seed with benlate on germination of Bossier seed after 2 to 8 months in storage.

TABLE IV

The percentage of viable embryos and embryos infected by fungal pathogens in seeds of different initial moisture contents stored for 9 months or freshly harvested

Seed treatment		Percentage viable embryos	Percentage infected embryos
Storage time (months)	Moisture content (%)		
0	13	95	0
9	10	45	1
9	13	0	3
9	16	0	5

Since the fungicide treatment was not entirely effective, it was not possible to determine the level of importance of physiological factors in field weathering nor the nature of the interaction which probably exists between pathogens and physiological factors. Ellis and Sinclair (1976) reported that benomyl sprays on soybean could be used to assure high quality seeds; under our wet-season conditions, when many seeds die before harvest, benomyl sprays are not entirely effective.

Physiological factors may be the major cause in most cases of viability loss during storage. This premise is based on the following evidence:

(1) Reducing the pathogens responsible for field deterioration of seed quality by foliar application of benomyl did not modify the rate of seed decay in storage.

(2) There was a loss of viability in storage at 35° C in spite of low incidence of seed-borne fungi.

(3) Control of fungi by dusting seed with benomyl did not improve storability.

(4) High moisture content of seed stored at 28° C greatly decreased storability, but did not result in a proportional increase in fungal pathogens.

(5) Embryos asceptically excised from stored seed were dead but virtually free of fungal pathogens.

CONCLUSION

The results suggest that seed-borne fungi may play an important role before harvest, but a minor role during storage. Physiological factors favored by high temperature and moisture content are most probably the principal agent of seed deterioration in storage. Consequently screening methods used in breeding for resistance to field weathering may logically include identification of resistance to specific pathogens. However, the efficiency of screening lines for resistance to deterioration in storage probably will not be improved by selection for resistance to pathogens.

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