Effects of Seed Quality, Seed Treatment, Soil Source, and Initial Soil Moisture on Soybean Seedling Performance

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ABSTRACT

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Greenhouse experiments were conducted in which untreated and carboxin-thiram treated seeds from relatively high or low quality seed lots were incubated for 3 days in five soils adjusted to specific matric potentials. Soils were then adjusted to matric potentials near-optimum for germination. Greenhouse results were compared with results of concurrent field experiments. In general, seed lot quality and seed treatment had more effect on establishment (proportion of seeds producing seedlings with opened true leaves) than did soil source or nonsaturated soil moisture treatments. Seed treatment increased establishment more for lower than for higher quality seeds. Low soil moisture levels decreased establishment more from lower than from higher quality seeds. Establishment was greatly reduced after incubation in saturated soil compared with all nonsaturated soil moisture levels. Establishment after incubation in saturated soil was increased greatly by seed treatment, but was affected little by seed lot. Symptoms of root and hypocotyl disease were most severe after incubation at nonsaturated soil water potentials greater than approximately −1.0 bar, were reduced by seed treatment, but were not affected by seed lot. There was no discernible difference in the performance of seeds that had been treated before or after 5-mo storage. In general, field and greenhouse results were similar; however, soil pasteurization by methyl bromide-chloropicrin fumigation resulted in a greater increase in establishment in the field than in the greenhouse. These findings indicate that caution should be used in comparing the results of seed quality tests of fungicide treated seed lots with those for untreated seed lots, that seed treatment can improve the performance of both high and low quality seed lots, and that it may be advantageous to treat all soybean seeds at the time of cleaning.

Additional key words: damping-off, *Glycine max*, *Phomopsis longicolla*, *Phomopsis* sp., pod and stem blight, seed vigor.

A number of studies have investigated relationships of soybean (*Glycine max* (L.) Merr.) seed quality and/or treatment with field performance (2,4,11,12,17,18,22). In general, seed lots of low quality and/or with high levels of infection by pod and stem blight fungi have been found to be more likely to perform poorly in the field and to respond to seed treatment. However, these results have been somewhat variable, and variations in soil moisture, soil temperature, and the activities of soilborne pathogens have been postulated as causes of this variable field performance. Soil moisture, soil temperature, and aeration have been shown to influence the effects of soilborne pathogens (primarily *Pythium ultimum*) on soybean emergence (3,14,19,20); however, there has been little work to evaluate the interactions of these factors with seed quality. The purpose of the research described herein was to evaluate the relative importance and interacting effects of soil source, soil moisture, seed quality, and seed treatment on soybean performance in the greenhouse and to compare these greenhouse results with those of concurrent field experiments. A preliminary report has been published (7).

MATERIALS AND METHODS

The investigation consisted of two pairs of experiments. Each pair consisted of a greenhouse experiment and a field experiment. The first pair of experiments was conducted in late May of 1982, and the second pair was conducted in late May and early June of 1984. Results and observations from the first pair of experiments were considered in the design of the second pair.

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Soil collection. In each greenhouse experiment, topsoil was collected from four fields in Kentucky in late April and early May, 2–3 wk before use. Soil from each field was collected to a depth of approximately 20 cm, transported in plastic bags, shredded, and stored in plastic bags at room temperature (20–25 C) until use. In the 1982 experiment, soils 1, 2, and 3 were collected from commercial soybean fields in Carlisle, Simpson, and Webster counties, respectively. Soil 4 was collected from the location of the field experiment at Spindletop Research Farm of the University of Kentucky near Lexington. All fields had been planted with soybeans the previous growing season, and soybean samples from them had been diagnosed as having early season root rot of undetermined etiology. To obtain soil 5, soil 4 was pasteurized by microwave oven treatment of 4-kg bags of soil for 425 sec (6). In the 1984 experiment, soils 1 and 2 were from experimental plots at the Princeton Experiment Station of the University of Kentucky on which soybeans had been grown the previous year. Soil 3 was from a commercial field in McLean County that was heavily infested with soybean cyst nematode. Soil 4 was collected from the location of the field experiment on the South Farm experiment facility of the University of Kentucky, near Lexington. The field was well drained, had been previously cropped with squash, had never been cropped with soybeans and had been cultivated 3 wk before collection. Soil 5 was collected from areas of the same field from which soil 4 was collected that had been fumigated with methyl bromide-chloropicrin (98-2) at the rate of 68 g of product per square meter 2 wk before collection.

Soil assays. Each soil was assayed for populations of soil microorganisms within 3 wk of the beginning of each experiment. A 25-g (wet weight) soil sample was comminuted with 100 ml of autoclaved water in a Waring Blendor, and a dilution series was prepared. Appropriate dilutions were plated on media selective for *Pythium*, *Fusarium*, *Rhizoctonia*, and total fungi as described

previously (9). Assays of *Rhizoctonia* were of *R. solani* AG-4 in 1982 and total *Rhizoctonia* spp. in 1984. Three and four replicate samples were assayed per soil in the 1982 and 1984 experiments, respectively. In 1984, populations of *P. ultimum* were enumerated by plating drops of soil suspension on water agar and examining for hyphae of *P. ultimum* emerging from drops after 24 hr incubation at 25 C (16). Populations of soybean cyst nematode (*Heterodera glycines*) were counted after sieving 200-g soil samples through a 250-μm (60 mesh) sieve. In the 1984 experiment, soils were analyzed for chemical and physical characteristics by the University of Kentucky Soil Testing Laboratory.

Soil moisture characteristics. Relationships between soil water content and soil water potential were determined separately for each soil. In the 1982 experiment, soil water contents (weight water per dry weight soil) corresponding to between 0 and −0.1 bar matric potential were estimated by placing a 100-g wet-weight sample on a tension plate apparatus, saturating the soil, then determining water content at progressively greater tensions up to 100 cm (21). Water contents at matric potentials less than −0.1 bar were estimated from water contents of Whatman No. 42 filter paper at equilibrium with soils at a range of water contents (5). In the 1984 experiment, soil water contents corresponding to 0 to −0.1 bar matric potential on the wetting curve were estimated by equilibrating an 80-g wet-weight sample on a tension plate adjusted to 100-cm tension, and then progressively lowering the tension to 0 cm. This wetting curve procedure was used because soil moisture treatments in this range were obtained by addition of water in the experiments. Values between −0.33 and −15.0 bars matric potential were determined using a pressure plate apparatus (13), and values for less than −15.0 bars were estimated using filter paper. In all experiments, soil water contents were determined from sample weights before and after drying at 105 C for 24 hr. For each method and soil, linear and quadratic equations were fitted to water content and water potential data after logarithmic transformation. The fitted linear or quadratic equation was used to estimate water potential for treatments having water contents within the range of water contents used in the derivation of the equation (Table 1). The fitted linear equation was used to estimate water potentials outside the range.

Seed lots and seed treatment. Two soybean seed lots of cultivar Williams were used in each experiment. Both seed lots used in the 1982 experiment were produced in 1981. Seeds were treated with 17% thiram-17% carboxin (Vitavax 200 F, Gustafson, Inc., Dallas, TX) at a rate of 0.46 g total a.i. per kilogram (2 fl oz of product per cwt) in January 1982 and December 1981 for seed lots 1 and 2, respectively, or in May, 1982 for both seed lots. Seeds were stored under conditions similar to those used for certified seeds (10–18 C in multiwalled paper bags). Incidence of seedborne fungi was assayed by plating surface disinfested seeds on acidified Difco potato-dextrose agar (PDA). Standard germination was assayed according to standardized procedures adopted by the Association of Official Seed Analysts (1). The accelerated aging test consisted of placing 200 seeds in cloth bags, putting the bags into germination trays, subjecting the seeds to approximately 100% relative humidity at 42 C for 72 hr and testing for standard germination.

In the 1984 experiment, seed lot 1 was produced in 1982 and was considered to be of moderately high quality on the basis of preliminary tests. Seed lot 2 was produced in 1983 in a field with high pod and stem blight disease pressure, was harvested 2 wk after harvest maturity, and was considered to be of medium quality. Before testing or use in experiments, the water contents of the seed lots were raised to 11–12% (weight water per wet weight seed) by incubation at 100% relative humidity at 25 C. Incidence of seedborne fungi was assayed by plating surface disinfested seeds on PDA amended with 100 mg/L of streptomycin sulfate, 50 mg/L of chloramphemicol, and 1 ml/L of nonionic surfactant (Tergitol NP10, Sigma, St. Louis, MO). Each seed lot was tested using standardized procedures for standard germination, accelerated aging, conductivity, and cold test performance by the laboratory of D. M. TeKrony in the University of Kentucky Department of Agronomy (1). Seeds from each seed lot were treated with 17%

TABLE 1. Initial soil moisture treatments used in experiments

Location	Year	Treatment	Time[b]	Water content (%)[a]					Method[c]	Estimated water potential (−bars)				
				Soil 1	Soil 2	Soil 3	Soil 4	Soil 5		Soil 1	Soil 2	Soil 3	Soil 4	Soil 5
Greenhouse	1982	Saturated	Start	46.0	37.0	42.0	57.0	57.0	T	0	0	0	0	0
			End	42.7	36.7	38.8	51.0	49.6	T	0.004	0	0.006	0.005	0.006
		Wet	Start	31.0	23.0	31.0	30.0	30.0	T	0.090	0.100	0.090	0.096	0.096
			End	30.3	21.7	26.1	30.1	29.5	T	0.100	0.128*[d]	0.0170*	0.094	0.110*
		Dry	Start	19.0	17.0	21.0	19.0	19.0	F	0.60	0.36	0.32	1.9	1.9
			End	8.4	8.9	8.3	15.4	13.3	F	32.0	3.5*	5.4*	8.0	11.7*
Greenhouse	1984	Saturated	Start	85.4	64.8	53.5	57.9	57.9	T	0	0	0	0	0
			End	...	...	...	...	...		...	...	...	...	...
		Wet	Start	30.8	31.1	26.7	30.8	30.8	T	0.010	0.010	0.010	0.010	0.010
			End	29.7	30.2	24.9	30.3	28.7	T	0.011	0.010	0.016	0.011	0.015
		Field[e]	Start	25.9	21.8	21.6	21.3	21.3	P	0.29	0.46	0.40	0.62	0.62
			End	25.6	21.2	21.4	20.2	20.3	P	0.30	0.49	0.40	0.76	0.75
		Dry	Start	11.5	9.5	6.0	11.6	11.1	P	10.7	7.0	7.9	10.3	12.4
			End	12.7	9.7	6.0	12.3	11.7	P	6.0	6.5	7.9	8.0	9.9
		Very dry	Start	8.8	6.5	3.8	7.4	6.8	F	43.1	23.2	41.2	48.7*	63.1*
			End	9.6	6.7	4.1	8.2	7.1	F	27.5	21.6	31.6	35.5	55.3*
Field	1984	Wet	Start	...	...	...	34.8	36.3	T	...	...	...	0.004	0.003
			End	...	...	...	36.4	36.6	T	...	...	...	0.003	0.003
		Medium	Start	...	...	...	27.4	28.3	P	...	...	...	0.28	0.25
			End	...	...	...	25.9	24.9	P	...	...	...	0.32	0.36
		Dry	Start	...	...	...	4.8	6.1	F	...	...	...	184*	88*
			End	...	...	...	6.6	7.8	F	...	...	...	69*	41
		Very dry	Start	...	...	...	3.7	4.3	F	...	...	...	408*	257*
			End	...	...	...	5.1	4.9	F	...	...	...	152*	172*

[a] Soil water content (weight water per dry weight soil) estimated at start of the initial 3-day incubation period from water content before set up and water added at set up, and at end of the initial 3-day incubation period from water content of soil samples. Water contents of all treatments were maintained at near optimum for germination following the initial incubation period.

[b] Time during the initial 3-day incubation period.

[c] Method used to determine soil water content-water potential relationship for estimation of water potential. T = tension plate, P = pressure plate, F = filter paper.

[d] * indicates that the water potential value was obtained by extrapolation beyond the water content range over which the water content-water potential relationship was derived.

[e] For the "field" treatment, soils used at the water content attained after shredding and storage.

thiram-17% carboxin (Vitavax 200 F) at a rate of 0.92 g total a.i. per kilogram of seed (4 fl oz of product per cwt) applied as 5 ml of suspension per kilogram of seed. Untreated seeds received an equivalent amount of deionized water.

Greenhouse experiments. Treated and untreated seeds of each seed lot were planted in each soil adjusted to three (1982) or five (1984) soil water contents (Table 1), incubated for 3 days, and then soils were adjusted to water contents near optimum for germination. In both experiments, seeds were planted in 52- × 25.5- × 6.5-cm plastic flats that had two 1.2-cm-diameter drainage holes in each end. A weighed amount of soil was placed in the flat, three rows of 20 seeds each were placed on the soil surface and lightly tamped, and the seeds were covered with another weighed amount of soil. In the 1982 experiment, the initial wet weights of the bottom and top layers of soil were 2 and 3 kg, respectively. In the 1984 experiment, the wet weights of the bottom and top layers corresponded to 1.6 and 2.4 kg dry weight, respectively. These configurations were equivalent to a planting rate of 39 seeds per meter (12 seeds per foot) of row at a depth of approximately 2.5 cm. Two flats were set up for each soil-moisture-seed lot-seed treatment combination. Flats were arranged in a split-split plot randomized block design in a greenhouse equipped with steam heat and evaporative cooling.

Soil water contents in the saturated treatments were established by plugging the drainage holes, adding water, and covering the flat with aluminum foil to restrict evaporation. In the 1982 experiment, volumes of water calculated to raise soil water potential to 0 bars on the basis of tension plate results were added to flats of the saturated treatments. In the 1984 experiment, water was added to saturated treatment flats until free water was visible on the soil surface. Similarly, specific amounts of water were added to flats of the wet treatment before covering. One week before the 1984 experiment, soil lots for the dry and very dry treatments were air-dried in the greenhouse at 22–30 C until samples of known initial weight and water content reached target weights. In the 1982 experiment, flats of the dry treatment were set up at field moisture (the water content attained after shredding and storage), left uncovered for one day after placement, then covered with aluminum foil. All other flats were covered with aluminum foil immediately after placement on the greenhouse bench.

In both experiments, the aluminum foil was removed from the flats 3 days after planting, at which time soil water contents were equalized by removing plugs from holes in flats of the saturated and wet treatments and adding amounts of water to other treatments equivalent to that needed to raise soil moisture to approximately −0.01 bar matric potential. After uncovering, flats were watered once or twice per day according to soil surface appearance to maintain water potential between approximately −0.01 and −1.0 bar.

In the 1982 experiment, establishment (the proportion of planted seeds that produced seedlings with true leaves unfolded) in each row of each flat was counted 2 wk after planting for the wet and dry treatments and 3 wk after planting for the saturated treatment. In the 1984 experiment, emergence (the proportion of planted seeds with any part of the seedling visible above the soil surface) and establishment were counted every 1 or 2 days from uncovering until 3 wk after planting. Postemergence failure was calculated as the difference between final emergence and establishment, and thus included failure due to both biotic and abiotic causes. Times until 50% emergence (emergence t_{50}) and establishment (establishment t_{50}) were calculated for each row of seeds by interpolation between the time of the last count below and the time of the first count above 50% of the 20 seeds planted. In the 1984 experiment, emerged plants in the center row of each flat were removed from the soil 3 wk after planting and were assigned a disease index rating on the basis of the incidence and severity of root and hypocotyl discoloration on a 0–5 scale (0 = no symptoms on any plants, 5 = severe root and hypocotyl rot on all plants).

Field experiments. Seeds were planted in the field 2 days before and 10 days after those in the corresponding greenhouse experiment in 1982 and 1984, respectively. Seed lots and seed treatments were identical to those used in the corresponding greenhouse experiments. In the 1982 experiment, seeds of each lot-treatment combination were planted at 32 seeds per meter of row in four row plots, 6.1 m long with 0.76 m between rows, in a randomized block design with four replications. Alachlor was applied preplant incorporated for weed control. Counts of emergence were made 8 and 23 days after planting. In the 1984 experiment, seeds were planted in fumigated or nonfumigated plots under four soil moisture regimes. One month before planting, eight 2.4- × 5.5-m plots were prepared in each of two blocks. Four plots in each block were covered with 4 ml black polyethylene film and were fumigated with methyl bromide-chloropicrin (98-2) at the rate of 68 g of product per square meter. Plots were uncovered 10 days after release of the fumigant. Beginning 1 wk before planting, dry and very dry moisture treatment plots were rototilled every day and were covered with black polyethylene when rain was expected and after soil water contents reached target values. In each plot, four 2.2-m rows of 50 seeds of each seed lot-treatment combination were planted approximately 2.5 cm deep. Dry and very dry moisture treatment plots were covered with black polyethylene immediately after planting. Medium moisture treatments plots were irrigated using a perforated ("soaker") hose for 2 hr immediately after planting and were then covered with black polyethylene. Wet moisture treatment plots were irrigated continuously by using perforated hose starting immediately after planting and were not covered. Three days after planting, irrigation was discontinued in the wet moisture treatment plots, the other plots were uncovered, and dry and very dry moisture treatment plots were irrigated using a hand-held hose. All plots were then irrigated

TABLE 2. Microbial populations in soils used in experiments

Year of experiment	Soil	Population (propagules/g dry soil) *Pythium*	*P. ultimum*	*Rhizoctonia*	*Fusarium* (× 10^3)	Total fungi (× 10^5)
1982	1	439 a[z]	···	0.18 a	7.2 a	1.8 b
	2	362 a	···	0.03 b	6.5 a	6.3 a
	3	310 a	···	0.04 b	5.9 a	5.8 a
	4	527 a	···	0.05 b	1.1 b	2.6 c
	5	0 b	···	0 b	0 c	0.001 c
1984	1	1,038 a	41 b	4.8 a	33.6 b	6.8 a
	2	734 ab	120 a	1.2 b	15.9 c	1.9 b
	3	405 bc	88 ab	1.8 b	4.2 d	1.1 c
	4	318 c	101 ab	0.25 c	68.9 a	2.0 b
	5	5 d	0 c	0 c	5.2 d	2.0 b

[z] For each organism and year, means followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

TABLE 3. Physical and chemical characteristics of soils used in the 1984 greenhouse experiment

Soil	Source[a]	Particle size distribution (%) Sand	Silt	Clay	pH	Organic matter (%)	Nutrient concentration (μg/g dry soil) Total N	P	K	Ca	Mg	Zn	Mn
1	Princeton Experiment Station	12.6	73.1	14.3	6.7	3.5	1,852	51	184	1,770	118	3.0	4.8
2	Princeton Experiment Station	1.8	86.5	11.7	6.9	2.1	1,443	42	150	1,555	66	2.8	1.0
3	McLean County	2.8	92.4	4.8	7.8	1.3	981	45	45	1,553	69	2.3	0.4
4	South Farm, Lexington	7.0	78.2	14.8	6.0	2.2	1,660	119	236	1,186	81	2.3	3.0

[a] All locations are in Kentucky.

TABLE 4. Quality characteristics of seed lots used

Experiment	Seedlot	Seed treatment[r] Material	Date	Towel germination (%)[s] Standard	Abnormal	Dead	Cold test (%)[u]	Artificial aging (%)[v]	Conductivity[w] (μmho/cm-g)	Seed infection (%)[t] Pod & stem[x]	Other fungi
1982	1	None	···	98 a[y]	···	···	···	95 a	···	1 cd	14 b
		Thiram-carboxin	Jan.	98 a	···	···	···	94 a	···	0 d	6 c
		Thiram-carboxin	May	95 ab	···	···	···	95 a	···	0 d	3 c
	2	None	···	61 d	···	···	···	50 c	···	47 a	24 a
		Thiram-carboxin	Dec.	87 c	···	···	···	75 b	···	19 b	31 a
		Thiram-carboxin	May	92 bc	···	···	···	77 b	···	3 c	30 a
1984	1	None	···	94 *[z]	5 *	1	50	69 *	51	0 *	35 *
	2	None	···	71	26	3	37	50	55	21	22

[r] In 1982 experiment, seeds were treated with thiram-carboxin in either December 1981, or January 1982, and in May 1982. Seeds were stored at 10–18 C in multiwall paper bags until testing in May 1982.
[s] Germination after 7 days incubation in rolled germination towels at alternating 20 and 30 C.
[t] Percentage of seeds yielding fungi after 10 days incubation on acidified PDA (1982) or PDA amended with antibiotics and nonionic surfactant (1984).
[u] Germination after 7 days incubation at 10 C on cellulose germination medium covered with untreated soil followed by 4 days incubation at 25 C.
[v] Standard germination after 72 hr at 41 C and 100% relative humidity followed by 7 days incubation on rolled germination towels at alternating 20 and 30 C.
[w] Conductivity of 75 ml of deionized water in which 50 seeds had been incubated for 24 hr at 25 C.
[x] Includes *Diaporthe phaseolorum* var. *sojae, D. phaseolorum* var. *caulivora,* and *Phomopsis longicolla. Phomopsis longicolla* was the most prevalent.
[y] For the 1982 experiment, means followed by the same letter are not significantly different ($P = 0.05$) according to a Z-test.
[z] For the 1984 experiment, * indicates that the mean for lot 1 is significantly different ($P = 0.05$) from that for lot 2 according to a Z-test.

TABLE 5. Mean squares from analysis of variance for establishment in the 1982 greenhouse experiment

	df	Moisture Saturated	Wet	Dry
Soil source	4	0.0685**[a]	0.0006	0.0045
Error(a)	4	0.0029	0.0014	0.0009
Block	1	0.0214	0.0004	0.0010
Lot	1	1.2327***	0.0678***	0.2100***
Treat-1[b]	1	0.7407***	<0.0001	<0.0001
Treat-2	1	1.0845***	0.0581***	0.1927***
Date-1	1	0.0014	0.0002	<0.0001
Date-2	1	0.0109	0.0005	<0.0001
Soil × Lot	4	0.0105	0.0051	0.0019
Soil × Treat-1	4	0.0478**[c]	<0.0001	0.0003
Soil × Treat-2	4	0.0072	0.0004	0.0074**[d]
Soil × Date-1	4	0.0094	0.0008	<0.0001
Soil × Date-2	4	0.0049	0.0010	0.0007
Error(b)	25	0.0113	0.0005	0.0010

[a] ** and *** indicate significance at $0.01 \geq P > 0.0001$ and $P \leq 0.0001$, respectively.
[b] Treat-1 and treat-2 test the effect of seed treatment for lots 1 and 2, respectively; date-1 and date-2 test the effect of treatment date for lots 1 and 2, respectively.
[c] Further analysis by soil indicated a significant treat-1 effect ($P < 0.01$) for soils 2, 3, and 4, but not soils 1 or 5.
[d] Further analysis by soil indicated a significant treat-2 effect ($P < 0.05$) for all soils.

daily with a hand-held hose to maintain soil water contents between 15 and 35% at a depth of 0–5 cm. Emerged and established plants in each row were counted 19 days after planting.

Statistical analyses. Data from each experiment were analyzed by appropriate analysis of variance (ANOVA) according to the design (randomized block, split plot randomized block, or split-split plot randomized block). In order to more clearly evaluate the influence of seed treatment for each seed lot, effects that tested the effect of seed treatment for each lot were substituted for the more traditional tests of effects of seed treatment and seed lot × seed treatment (8). Because of the presence of interactions between factors, incomplete data and/or heterogeneity of error variances, analyses were performed for all data from each experiment and for data partitioned by moisture or soil treatments. Relationships between dependent variables within seed lot-treatment combinations in the 1984 greenhouse experiment were examined using Spearman's coefficient of rank correlation. Results for establishment in the 1984 field and greenhouse experiments were compared with ANOVA using data from the field and very dry treatments for soils 4 and 5 in the greenhouse and data from the medium and dry treatments in the field.

RESULTS

Soil assays. In both the 1982 and 1984 experiments, populations of soil fungi varied considerably among soils, and the soil pasteurization treatment eliminated or greatly reduced populations of *Pythium*, *Rhizoctonia*, and *Fusarium* (Table 2). A population of *Heterodera glycines* of 0.21 viable cysts per gram of dry soil was enumerated in soil 3 in the 1984 greenhouse experiment. No *H. glycines* was recovered from any other soil. Other soil characteristics in the 1984 experiment also varied, with soil 3 being notable for its relatively high pH, low organic matter, and low K (Table 3).

Seed lot evaluations. The untreated seed lots used in the 1982 experiment differed greatly in standard germination, artificial aging, and infection by pod and stem blight fungi (Table 4). Seed treatment significantly increased standard and artificial aging germination and reduced pod and stem blight for lot 2, but not lot 1. The lots used in the 1984 experiments also differed substantially, but were more similar in quality than were the lots used in 1982. Results of the standard germination, artificial aging, and plate bioassay tests were used to rank the four seed lots: highest quality = lot 1, 1982 > lot 1, 1984 > lot 2, 1984 > lot 2, 1982.

Moisture and temperature conditions. Among the soil moisture regimes used, water potential was much more similar for different soils with the same moisture treatment than for the same soil with different moisture treatments (Table 1). However, water potentials for the same moisture treatment varied somewhat, particularly in the field, dry and very dry treatments. The dry and very dry treatments in the 1984 field experiment were considerably drier than had been intended, and increases in surface soil water content occurred during the initial incubation period–possibly because of a solar still effect under the polyethylene mulch. Soil moisture data were not taken during the 1982 field experiment; however, greater than 2 mm of rain per day was not recorded at a weather station approximately 1 km from the plot site 5 days before and 10 days after planting, and 8-day emergence was 50–75% of final emergence, indicating that soil moisture was probably below optimum for germination. Soil temperatures ranged from approximately 20–28 C in the greenhouse experiments and from approximately 20–38 C in the 1984 field experiment.

Establishment. In the 1982 greenhouse experiment, overall ANOVA for establishment indicated significant interactions of seed lot and treatment effects with soil moisture. Further analyses by moisture indicated that establishment was greater from lot 1 than lot 2 under all conditions, and that seed treatment increased establishment from lot 2 under all conditions, but only in saturated soil for lot 1 (Table 5). Compared with wet, the dry soil moisture treatment had no significant effect on establishment of treated or untreated seeds of lot 1 or treated seeds of lot 2 (contrasts,

$P < 0.05$), but significantly decreased establishment for untreated lot 2 seeds in soils 1, 4, and 5 (Fig. 1). Time of seed treatment had no significant effect on establishment for any soil source-moisture-seed lot combination. In the 1982 field experiment, seed treatment increased establishment for lot 2, but not lot 1. Compared with results for the same soil in the greenhouse experiment (soil 4-dry), establishment in the field was significantly lower for both treated and untreated lot 2 seeds, but not lot 1 seeds (Student's t tests, $P < 0.05$). In the 1984 greenhouse experiment, overall ANOVA for establishment indicated significant interactions of lot and treatment effects with soil and/or moisture. Separate analyses of data from saturated and nonsaturated treatments eliminated some, but not all, of these interactions (Table 6). In general, the effect of seed lot was greatest for the dry and very dry moisture treatments due to decreased establishment from lot 2, but not lot 1; seed treatment had more of an effect on lot 2 than lot 1; the effect of treatment varied with soil for lot 2, but not lot 1; and the effect of soil source was greatest for very dry and least for wet and field moisture treatments (Fig. 2). Under saturated conditions, the effect of lot on establishment was much less than was the effect of seed treatment of either lot. In the 1984 field experiment, overall ANOVA for establishment indicated significant effects of soil, moisture, lot, and seed treatment of either lot and significant interactions of lot and seed treatment of either lot with soil. Analyses by soil indicated that establishment was greater from lot 1 than lot 2 in both fumigated and untreated soil, but that seed treatment of either lot increased establishment only in untreated soil (Fig. 3). ANOVA comparing field and greenhouse establishment indicated significant effects of seed lot and treatment of either lot. No significant effect of moisture was detected. A significant site × soil × lot interaction was indicated, but further analyses indicated that this was due to differences in the magnitude, but not the direction, of the lot effect in different site × soil combinations.

Other variables. Overall ANOVA for postemergence failure indicated significant effects of moisture and lot × moisture. Further analyses indicated significant effects of lot and treatment of lot 1 for nonsaturated treatments and no significant effects of any treatments under saturated conditions (Table 6). For nonsaturated treatments, both emergence t_{50} and establishment t_{50} decreased with increasing moisture; however, emergence t_{50} was more strongly affected by moisture, with a range of 4.2 days between the mean for the wet and very dry moisture treatments versus a range of 1.3 days for establishment t_{50}. Values of both variables were significantly greater for lot 2 than for lot 1, and for treated versus untreated seeds of lot 2. Although quite variable, the root and hypocotyl disease index was greatly affected by moisture,

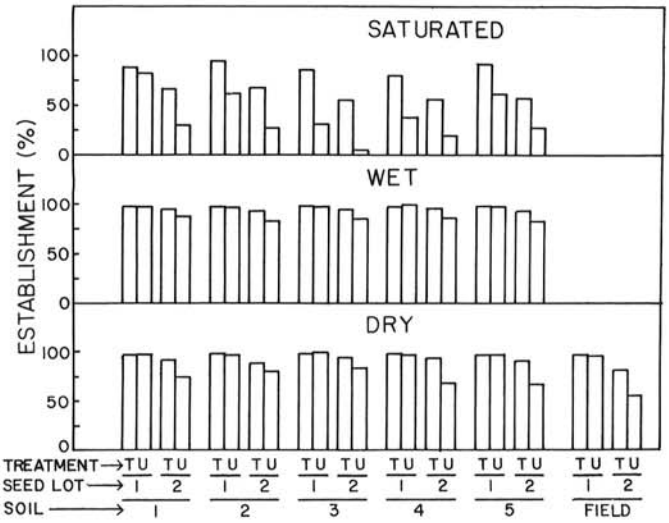

Fig. 1. Effects of soil moisture, soil source, seed lot, and seed treatment on soybean establishment in the 1982 greenhouse and field experiments. Seeds were treated with thiram-carboxin (T) or were untreated (U). Soils 1 through 4 were untreated and soil 5 had been pasteurized by microwave oven treatment. Soil 4 was collected from the field used in the field experiment.

TABLE 6. Mean squares from analysis of variance for dependent variables in the 1984 greenhouse experiment

	Without saturated data							Saturated data only			
Source of variation	df	Establishment	Postemergence failure	Emergence t_{50}	Establishment t_{50}	Disease index	Plant weight	df	Establishment	Postemergence failure	Plant weight
Soil source	4	0.0123	0.00083	3.569	3.156	3.809	2.195	4	0.0372	0.00785	1.422
Error(a)	4	0.0022	0.00023	0.638	1.152	1.830	2.253	4	0.0134	0.00197	0.313
Moisture	3	0.0228***[a]	0.00146**	171.818***	16.508***	23.234***	0.990*	...	...	...	...
Soil × moisture	12	0.0029*	0.00040	1.925*	1.275	1.795	0.544*	...	...	...	...
Error(b)	15	0.0010	0.00026	0.543	1.079	0.833	0.206	...	...	...	...
Block	1	0.0020	0.00117	1.849	6.506	0.028	0.049	1	0.0456	0.01534*	0.002
Lot	1	0.7608***	0.00306**	7.091***	15.352***	0.043	10.342***	1	0.1542**	0.00667	4.440**
Treat-1[b]	1	0.0125**	0.00200*	0.008	0.030	6.830**	1.248**	1	1.2667***	0.00006	0.017
Treat-2	1	0.2033***	0.00089	1.365**	1.938**	6.613**	0.271	1	1.0200***	0.00168	0.385
Soil × lot	4	0.0011	0.00058	0.231	0.118	0.881	0.149	4	0.0052	0.00164	0.170
Soil × treat-1	4	0.0039	0.00064	0.090	0.211	2.155*	0.051	4	0.0103	0.00318	0.201
Soil × treat-2	4	0.0046*	0.00043	0.300	0.338	0.855	0.124	4	0.0095	0.00074	0.545
Moisture × lot	3	0.0133**[c]	0.00026	0.187	1.070**[d]	0.194	0.056	...	...	...	...
Moisture × treat-1	3	0.0007	0.00023	0.036	0.033	1.776	0.472*	...	...	...	...
Moisture × treat-2	3	0.0008	0.00064	0.059	0.050	1.298	0.042	...	...	...	...
Soil × moisture × lot	12	0.0030	0.00015	0.199	0.131	1.236	0.109	...	...	...	...
Soil × moisture × treat-1	12	0.0010	0.00030	0.051	0.082	0.325	0.075	...	...	...	...
Soil × moisture × treat-2	12	0.0033*	0.00040	0.078	0.071	0.217	0.135	...	...	...	...
Error(c)	60	0.0016	0.00030	0.131	0.151	0.736	0.130	15	0.0063	0.00279	0.401

[a] *, **, and *** indicate significance at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.0001$, and $P \leq 0.0001$, respectively.

[b] Treat-1 = effect of seed treatment of lot 1.

[c] Further analyses by moisture indicated significantly ($P < 0.0001$) higher establishment for lot 1 than lot 2 at all moistures, with magnitude being least under wet and field, greater under dry and greatest under very dry moistures.

[d] Further analyses by moisture indicated significantly ($P < 0.01$) longer establishment t_{50} for lot 2 than lot 1 at all moistures, with the magnitude being least under wet and field, greater under dry and greatest under very dry moistures.

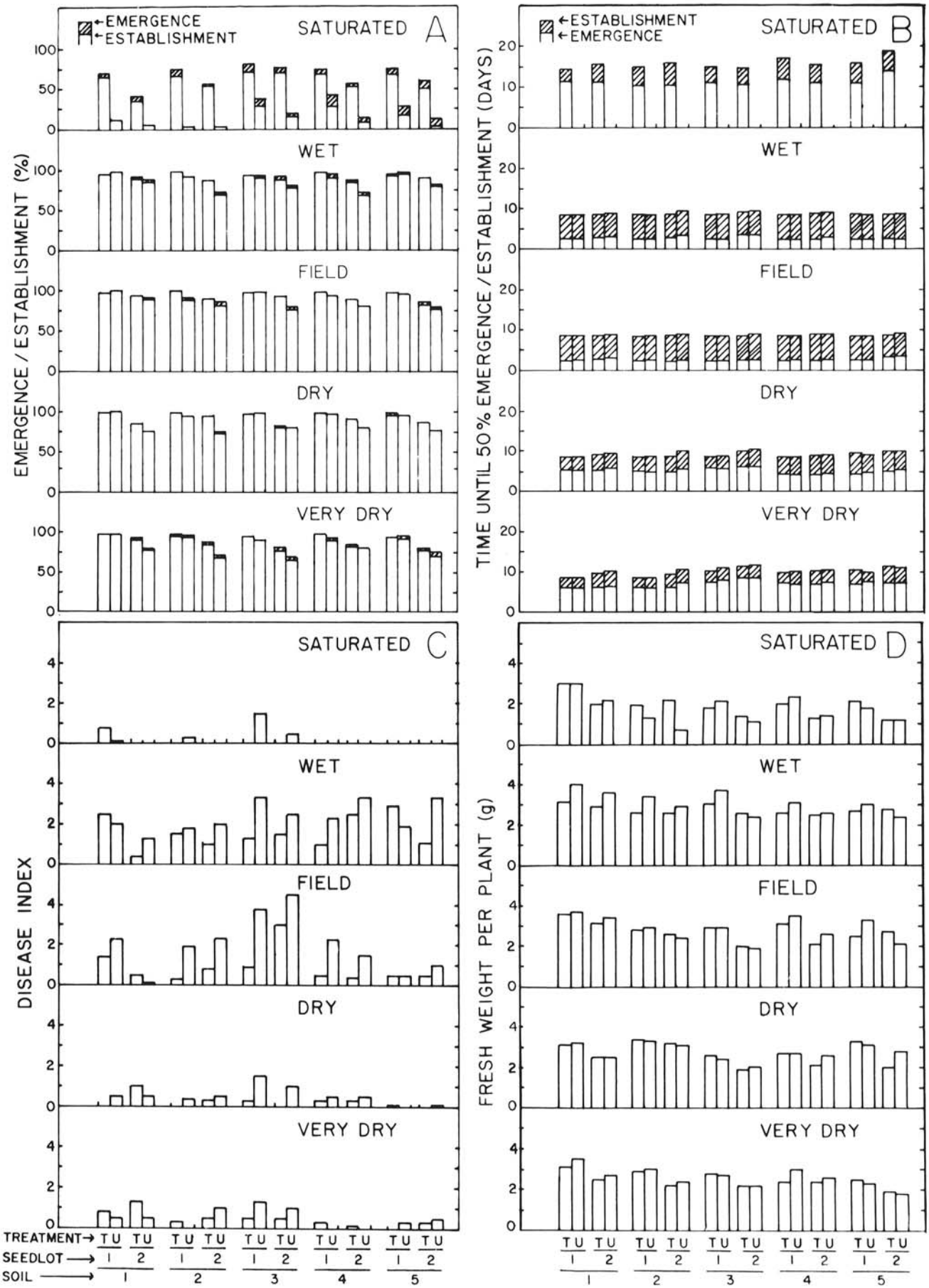

Fig. 2. Effects of soil moisture, soil source, seed lot, and seed treatment on dependent variables in the 1984 greenhouse experiment. Seeds were treated with thiram-carboxin (T) or were untreated (U). Soils 1 through 4 were untreated and soil 5 had been pasteurized by methyl bromide-chloropicrin fumigation. **A,** Establishment and emergence. **B,** Time to 50 % emergence and establishment. **C,** Disease index. **D,** Fresh weight of emerged plants.

being relatively high only for the wet and field moisture treatments. The disease index was not affected by seed lot, and values were reduced with treatment for both lots. At nonsaturated moistures, plant weight was affected slightly by moisture and greatly by lot and treatment of lot 1. Soil had a slight influence on the effects of both moisture and treatment of lot 1. The only nonparametric correlations between the dependent variables that were significant for all lot-treatment combinations were those for establishment versus establishment t_{50} and emergence, and emergence t_{50} versus establishment t_{50} and disease index. Other pairs of variables were significantly correlated for one to three lot-treatment combinations (Table 7).

DISCUSSION

In general, similar effects on seedling establishment of seed lot, seed treatment, soil and soil moisture were observed in both the greenhouse and field experiments; however, the differences that were observed indicate that the greenhouse results should be extrapolated to the field with caution. In all of the experiments, seed lot and seed treatment had a much greater effect on establishment than did nonsaturated soil moisture treatments. Soil source/pasteurization had a definite effect only in the 1984 field experiment. The lack of a soil source effect in the greenhouse experiments is surprising in view of the great variation in populations of soilborne pathogens among the soils. Taken alone, the greenhouse results could be interpreted as indicating that damping-off is primarily a function of seed quality characteristics such as nutrient leakage and tolerance of infections. However, the marked response to soil fumigation in the 1984 field experiment suggests that soilborne pathogens also can be important. The field-greenhouse inconsistency could have been because of a decrease in soilborne pathogen activity associated with the collection, shredding and storage of the soil for the greenhouse experiment or a favoring of soilborne pathogens in the field by wider fluctuations of soil temperature and moisture.

Establishment was consistently greater from higher than lower quality seeds and from treated than untreated lower quality seeds. However, under nonsaturated moisture regimes, both of these effects interacted with soil moisture. In the 1982 greenhouse experiment, establishment from untreated lower quality seeds was greater in wet than in dry treatments for the three soils with the lowest estimated dry treatment water potentials (soils 1, 4, and 5), whereas establishment was high at both moistures from treated lower quality seeds and treated or untreated higher quality seeds. Although a similar interaction of seed treatment with moisture was not observed in the 1984 experiments, differences between lots in the greenhouse were greater at lower soil moistures, and the effect of treatment of the lower quality lot in the field increased with decreasing moisture in fumigated soil. Overall, these results indicate that lower quality seeds are more susceptible to damping-off under relatively dry conditions. Although *Fusarium* spp. have been reported to be more damaging to soybean seeds in relatively dry soil (15), the facts that the soil moisture effect that we observed took place in both pasteurized and untreated soil and was greater for the seed lots with a higher incidence of seedborne fungi indicate that a seedborne pathogen(s) was at least partially responsible. Further work initiated on the basis of these observations has shown that *Phomopsis longicolla* is more damaging when seeds are planted in low-moisture soils (9).

In saturated soils in both greenhouse experiments, establishment was much less than for any of the nonsaturated moisture treatment, and the effect of seed treatment was much greater. This difference in behavior suggests that different processes take place in saturated compared with nonsaturated soils and should be expected in a comparison of hypoxic with aerobic conditions. An effect of soil source under saturated conditions was observed in the 1982 experiment; however, this effect was not observed in 1984, and may have been due to failure to achieve complete saturation in some soils.

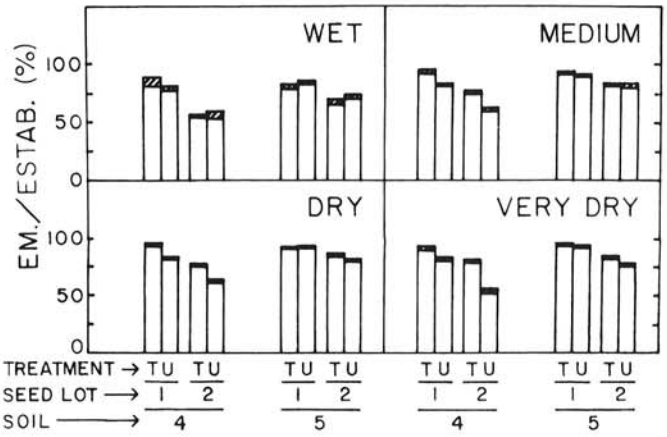

Fig. 3. Effects of soil moisture, soil fumigation, seed lot, and seed treatment on soybean emergence (full bars) and establishment (unshaded bars) in the 1984 field experiment. Seeds were treated with thiram-carboxin (T) or were untreated (U). Soil 5 was fumigated with methyl bromide-chloropicrin and soil 4 was not fumigated. Soils 4 and 5 in the 1984 greenhouse experiment were collected from corresponding sites in the field experiment.

TABLE 7. Nonparametric correlations (Spearman's *r*) between dependent variables for nonsaturated treatments in the 1984 greenhouse experiment

	Untreated seeds						Treated seeds					
Variable	Emergence	Establishment t_{50}	Emergence t_{50}	Postemergence failure	Disease index	Plant weight	Emergence	Establishment t_{50}	Emergence t_{50}	Postemergence failure	Disease index	Plant weight
Lot 1												
Establishment	0.94***[a]	−0.60***	−0.05	−0.62***	−0.01	−0.06	0.81***	−0.69***	−0.37*	−0.64***	−0.20	0.12
Emergence		−0.53**	0.01	−0.34*	−0.11	0.04		−0.61***	−0.36*	−0.15	−0.28	0.07
Establishment t_{50}			0.57***	0.38*	−0.24	−0.39*			0.60***	0.43**	−0.07	−0.06
Emergence t_{50}				0.16	−0.65***	−0.35*				0.21	−0.47**	−0.10
Postemergence failure					−0.22	−0.11					0.01	−0.26
Disease index						0.23						−0.03
Lot 2												
Establishment	0.94***	−0.76***	−0.51**	−0.26	−0.07	0.19	0.94***	−0.81***	−0.61***	−0.51**	0.31	0.35*
Emergence		−0.76***	−0.55**	0.02	0.01	0.16		−0.69***	−0.51**	−0.22	0.31	0.39*
Establishment t_{50}			0.85***	0.05	−0.27	−0.20			0.79***	0.60***	−0.29	−0.47**
Emergence t_{50}				−0.11	−0.50**	−0.12				0.49**	−0.49**	−0.31
Postemergence failure					0.23	0.06					−0.11	−0.14
Disease index						−0.44**						0.19

[a] *, **, *** = significant at $0.05 \geq P > 0.01$, $0.01 \geq P > 0.0001$, and $P \leq 0.0001$, respectively.

The results of the 1984 greenhouse experiment indicate that seed lot, seed treatment, soil source, and initial soil moisture have different effects on different aspects of seedling behavior. Postemergence failure was sporadic at nonsaturated initial soil moistures; however, it was significantly greater for lower quality seeds and at the wet and very dry moisture treatments. This similarity of behavior at extremes of aerobic moisture may indicate that postemergence failure is favored by a large, rapid increase in water potential (such as occurred in the wet treatment at day 0 and in the very dry treatment at day 3). Under saturated conditions, the high incidence of postemergence failure may have been due to the same process(es) that resulted in low establishment. The root and hypocotyl disease index was strongly affected by moisture and seed treatment of either lot, minimally affected by soil (through interaction with treatment of the higher quality lot) and not affected by seed lot. The low ratings observed for the saturated, dry, and very dry moisture treatments may indicate that potential inoculum activity declined during the initial incubation period at these moistures or that a relatively high moisture level is necessary immediately after planting in order for the organisms responsible to be active. The lack of a seed lot effect and the very strong moisture effect on the disease index contrast sharply with the effects of these variables on establishment. Apparently, different factors affect the processes responsible for preemergence damping-off and root and hypocotyl symptoms. Consequently, attempts to determine the organisms responsible for preemergence damping-off by isolations from emerged seedlings may give spurious results.

In nonsaturated soils, times until 50% emergence and 50% establishment were longer at drier initial moistures, were little affected by soil, were shorter for the higher quality lot, and were increased slightly by treatment of the lower quality lot. Differences between lots in establishment t_{50}, but not emergence t_{50}, were least at wet and field, greater at dry and greatest at very dry moisture treatments. This lot-moisture interaction was similar to that observed for establishment, and was also reflected in the high negative correlations of establishment with establishment t_{50} for all lot-treatment combinations. The similarity may indicate that the poorer establishment of the lower quality lot in drier soil is partially due to a longer period during which the apical meristem is protected from desiccation and thus a longer period during which associated microorganisms can be active. The much smaller magnitude of the increase in establishment t_{50} than emergence t_{50} in drier soil may indicate that development of the plumule took place during the 3-day incubation period at these low water potentials, even though radicle growth and hypocotyl elongation were inhibited. Such a process would be analogous to that which occurs when the vigor of unplanted seeds is increased by incubation at water potentials high enough to allow metabolic activity, but too low to allow radicle emergence (10).

Mean fresh weight per emerged plant was consistently greater for plants from the higher quality seed lot. This could be a reflection of the same faster growth of the higher quality lot that resulted in lower t_{50} values. However, while the t_{50} values were influenced by initial moisture and treatment of the lower quality lot, these factors had little or no effect on plant weight, and treatment of the higher quality lot reduced plant weight in the wet soil moisture treatment but did not affect t_{50} values. Plant weight is apparently a function of interactions between growth rates (reflected in t_{50} values), factors that might affect transport between root and shoot (such as hypocotyl disease), and factors that might affect the photosynthetic activity of emerged plants (such as seed lot and treatment).

In all experiments, untreated higher quality seeds had consistently greater establishment than untreated lower quality seeds and had higher values in the seed quality tests. However, standard germination values for untreated lower quality seeds tended to underestimate establishment in the greenhouse and overestimate it in the field. This consistency in ranking of seed lot-treatment combinations, but variability in actual percentage values, is similar to that found in other investigations (17). Hypothetically, because the conditions of the physical and biological environment vary among different seed quality tests and among different planting situations, it is to be expected that no quality test can accurately predict establishment in all soil situations. Conversely, if seed quality is considered to be one-dimensional, then any seed quality test would be expected to accurately rank the performance of seed lots in all soil situations (within the bounds of statistical uncertainty). However, our observation of an apparent interaction of seed quality with soil moisture makes this latter hypothesis questionable; and furthermore, the 1982 results for saturated soil can be interpreted as indicating that seed treatment introduces a new dimension into seed quality that is not accounted for in seed quality tests. The ranking observed for the seed quality tests and for establishment in nonsaturated soil was higher quality-treated = higher quality-untreated > lower quality-treated > lower quality untreated. However, in three out of five saturated soils the observed ranking of establishment was higher quality-treated > lower quality treated > higher quality-untreated > lower quality-untreated. It can be argued from these results that, although the performances of treated seeds of different seed lots can be expected to rank similarly in seed quality tests and in soil, seed quality tests cannot be used to compare treated seed lots with other, untreated, seed lots.

Although the experiments did not address some factors that would be expected to affect seedling disease (such as temperature and changes in moisture and temperature with time), it is possible to draw some tentative conclusions that relate to soybean production practices. Our results support the recommendations that high quality seeds should be used in soybean production, and that lower quality seeds should be treated before planting. However, the results for saturated soils indicate that even very high quality seed lots can respond to seed treatment if flooding occurs, and the disease index data also indicate that treatment of high quality seeds can be advantageous. These observations, along with the lack of any effect of time of seed treatment, indicate that it might be a reasonable practice for seed conditioners to treat all soybean seed lots at the time of cleaning, such as is currently done with corn. If a single seed treatment material were used, such a practice would overcome the problems associated with comparing the quality characteristics of treated with untreated seed lots. However, widespread use of a single seed treatment material would bring up the problem of determining the identity of the optimum material to use, and might inhibit innovation in the development of seed treatment materials.

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