Influence of Soil Water Potential on Performance of Soybean Seeds Infected by *Phomopsis* sp.

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ABSTRACT

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Effects of soil water potential on emergence and establishment of seedlings from soybean seeds infected by *Phomopsis* sp. were examined in growth chamber and greenhouse experiments. Seeds were incubated for 3 days in untreated or pasteurized soil at soil matric potentials of approximately 0, −0.008, −0.01, −0.1, −4, −17, or −51 bars, then for 16–18 days at −0.008 to −2.2 bars. Seed lots with high proportions of seeds infected by *Phomopsis* sp. (either naturally or by inoculation) had lower percent emergence and establishment than lightly infected lots, particularly when the initial incubation period was in soil at $\psi_m \leq -17$ bars. Growth of the fungus from infected seed coats to cotyledons (as indicated by increases in percent incidence in cotyledons after incubation in pasteurized soil) was much greater in soil at $\psi_m \leq -0.1$ bar than at $\psi_m \geq -0.01$ bar. *Phomopsis* sp. was the only seedborne fungus whose incidence in seeds of five seed lots was significantly negatively correlated with emergence in unsaturated soil. The results suggest that seedborne *Phomopsis* sp. reduces emergence and establishment most when seeds are incubated in dry soil. Evidence that low soil water potential inhibits seedling growth more than growth of *Phomopsis* sp. may help explain this phenomenon.

Additional key words: Diaporthe phaseolorum, Glycine max, pod and stem blight, seed vigor, seedborne pathogens.

Pod and stem blight is a major cause of poor quality soybean (*Glycine max* (L.) Merr.) seed in the United States (30). The fungi that cause pod and stem blight, *Phomopsis* sp. (sensu Kmetz et al [15]) and *Diaporthe phaseolorum* (Cke. & Ell.) Sacc., grow through the pod walls and infect seeds during maturation (13,31). *Phomopsis* sp. is frequently more abundant than *D. phaseolorum* in infected seed lots and appears to be more pathogenic (14,15,28).

The major damage done by pod and stem blight is reduction of germination and emergence (14,28). Seed lots with a high incidence of pod and stem blight infection often receive low ratings in laboratory vigor tests and/or emerge poorly in the field (4,14,16,21). Reducing the incidence of *Phomopsis* sp. and *D. phaseolorum* on seeds by fungicide treatment (24,35) or during storage (34) can raise seed lot performance.

The impact of pod and stem blight on seed lot performance in the field is highly variable. Neither percent pod and stem blight incidence nor laboratory vigor test ratings of seed lots have been consistently correlated with field emergence (16,33). A better understanding of interactions between diseased seeds and the soil environment is needed to improve vigor testing procedures and fungicide treatment recommendations.

Most research on soil moisture–plant disease relationships has focused on effects in moist-to-wet soil (3). Relatively little is known about influences of dry soil on preemergence mortality. Hunter and Erickson (12) observed that seeds placed in dry soil sometimes failed to germinate even after moisture became optimal and suggested that seedborne or soilborne fungi can contribute to seed mortality in such situations. This idea also is supported by data of Schlub et al (27) showing that emergence from soybean seeds in soil infested with *Fusarium* spp. was much lower when seeds were initially incubated at matric potentials (ψ_m) = −15 bars than at ψ_m = −2, −4, or −8 bars.

Few reports have focused on relationships between soil moisture and seedborne pathogens. Wallace (36) noted that a high proportion of cereal seeds invaded after planting by xerophilic fungi, such as *Aspergillus* spp. and *Penicillium* spp., had cracked seed coats or fungal infections before planting. Seedlings from wheat seeds infected by *Fusarium nivale* (Fr.) Cesati and planted in dry soil had more symptoms and weighed less than seedlings from seeds planted in moist soil (22). Wallen and Seaman (37) speculated that soybean seed lots with a high incidence of pod and stem blight infection might be particularly vulnerable to attack by soilborne pathogens under suboptimal moisture conditions. Although Kulik and Schoen (16) found no relationship between soil moisture and field emergence of soybean seed lots heavily infected with pod and stem blight fungi, they tested only two moisture levels and did not quantify these. Ferriss et al (8) noted that a seed lot with a high incidence of *Phomopsis* sp. performed much more poorly in certain dry (−3 and −12 bars) and saturated soils than either the same lot in moderately moist soils (−0.2 to −0.4 bar) or a healthy seed lot. The lot with a high incidence of *Phomopsis* sp. performed poorly in both pasteurized and untreated soils, suggesting that the seedborne microflora contributed to seed mortality.

The present work investigated the possible influence of soil moisture on the behavior of soybean seed lots infected by *Phomopsis* sp. and the role of *Phomopsis* sp. in this relationship. Two preliminary reports have been published (10,11).

MATERIALS AND METHODS

Soil moisture characteristics. Silty clay loam soil (12% sand, 58% silt, 30% clay) at 20–25% water content (weight water per dry weight soil) was collected from a field that had been in continuous soybeans for at least 5 yr. The soil was mechanically shredded before determination of moisture characteristics and use in experiments. Soil was stored in plastic bags at room temperature. Moisture characteristic curves were determined separately for three ranges of soil water potential. For the range −0.1 bar to saturation, a curve was derived by adding water to a soil column on a tension plate apparatus (Fig. 1A). This procedure was used to imitate the pore filling and soil settling that occurred when water was added to soil during setup of treatments in this moisture range. A pressure plate was used to derive a drying curve for the range −0.33 to −15 bars, and water potentials <−15 bars were estimated by a filter

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paper method (5) after air-drying of soil samples to specific water contents (Fig. 1B).

Seed quality tests. Seed vigor tests (3-day germination, accelerated aging, conductivity, and cold test) and standard germination were conducted according to standardized procedures (1). Immediately before use, soybean seeds (seven lots of cv. Williams, one lot of cv. Cumberland) were assayed for percent incidence of pod and stem blight fungi and other microorganisms. Seeds were surface-sterilized for 30 sec in 0.5% NaOCl, rinsed three times in deionized water, then soaked for 2–4 hr in deionized water. Seed coats and cotyledons were separated aseptically and placed on Difco potato-dextrose agar (PDA) amended with 100 mg of streptomycin sulfate, 5 mg of chlortetracycline HCl, and 0.5 ml of Tergitol NP-10 (Union Carbide Corp., New York, NY) per liter. Fungal colonization was determined after incubation at 21–24 C for 8–12 days and illumination at 15–20 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 9–12 hr per day.

Artificial inoculation of seeds. Seeds used in two experiments were artificially inoculated with *Phomopsis* sp. before planting. Subsamples from two seed lots having less than 2% seeds infected by *Phomopsis* sp. were surface-sterilized for 30 sec in 0.5% NaOCl, rinsed three times with autoclaved deionized water, then raised to 37% water content (weight water per wet weight seed) over a 12-hr period by contact with germination paper wetted with autoclaved deionized water. The lots were stored for 1–3 days at 5 C to allow moisture equilibration among seeds. Inoculum was produced by culturing an isolate of *Phomopsis* sp. originally obtained from a soybean seed in Difco potato-dextrose broth for 4 days at 26 C. The resulting mycelial mats were comminuted in a Waring Blendor for 90 sec and centrifuged for 5 min at $1,820 \times g$. After the supernatant was poured off and replaced with autoclaved deionized water, the pellet was resuspended using a vortex mixer. Seeds were sprayed with this suspension (4.8×10^4 viable mycelial fragments per gram dry weight seed) or the same volume of autoclaved deionized water as a control. The spray raised seed moisture to $40 \pm 1\%$. Inoculated seeds were incubated in a closed container for 18 hr at 25 C, then dried to 12% water content at 21–24 C.

Assays of soil microorganisms and soil treatments. A 50-g (wet weight) soil sample was comminuted with 100 ml of autoclaved deionized water in a Waring Blendor, a dilution series was prepared, and appropriate dilutions were plated on the following media: *Pythium* spp. were enumerated on a medium containing 17 g of Difco cornmeal agar, 25 mg of ampicillin, 100 mg of pentachloronitrobenzene (PCNB), 10 mg of rifampicin, 10 mg of rose bengal, and 5 mg of pimaricin in 1 L of water (23). *Fusarium* spp. were enumerated on modified PCNB medium (25). Total fungi were enumerated on a medium containing 39 g of Difco PDA, 100 mg of streptomycin sulfate, 20 mg of chlortetracycline HCl, and 1 ml of Tergitol NP-10 in 1 L of water (32). Total bacteria and actinomycetes were enumerated on 0.3% tryptic soy agar (20). For assay of *Rhizoctonia* spp., the initial soil suspension was screened on a sieve with 125-μm openings, the residue was suspended in 15 ml of 3.0% agar at 50 C, and the agar-soil mixture was cut into 100 blocks after solidification. Blocks were plated on a medium containing 0.18 g of enzymatic digest of casein, 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$, 0.12 g of tannic acid, 0.7 g of neomycin sulfate, 95 mg of pyroxychlor, 0.5 mg of benomyl, and 15.0 g of agar in 1 L of water (7,9).

Soil used in experiments was either untreated or pasteurized (microwave oven treatment of 4-kg bags of soil for 425 sec [6]). Populations of soilborne fungi were analyzed 3 wk after microwave oven treatment.

Experimental design. A series of growth chamber and greenhouse experiments were carried out to determine emergence (elevation of a seedling above the plane of the soil surface) and establishment (full opening of a true leaf) of selected seed lots in response to a range of soil treatments. Several days before the experiments were conducted, seeds were adjusted to 12% water content in a 100% relative humidity container at room temperature. In some experiments, seeds were treated with carboxin-thiram (Vitavax 200) at the rate of 0.92 g a.i. of each material (5 ml of flowable product) per kilogram of seed wet weight (2 fl oz/cwt); control seeds received an equivalent volume of autoclaved deionized water. Seeds were planted in soil at a depth of 2.5 cm in $6 \times 11 \times 15$ cm plastic trays (for growth chamber experiments) or $6 \times 24 \times 50$ cm plastic flats (for greenhouse experiments). Dry weight of soil per container was equal for all treatments in each experiment. There were five replicate trays (15 seeds each) per treatment in each growth chamber experiment and six replicates (20 seeds in each of three rows in two flats) per treatment in each greenhouse experiment. Following planting, seeds were incubated in soil for 3 days at 0 (59% soil water content), −0.008 (37%), −0.01 (30%), −0.1 (23%), −4 (18%), −17 (13%), and −51 bars (9%) ψ_m. Soil water potentials ≤ -4 bars were obtained by air-drying soil to specific water contents before planting. Soil water potentials ≥ -0.1 bar were obtained by adding specific amounts of water immediately after planting. Containers were enclosed in polyethylene bags or covered with aluminum foil to retard moisture loss during the 3-day incubation period. After 3 days, soil moisture was brought to −0.008 bar in all treatments by adding appropriate volumes of water. Soil moisture was maintained within the range of −0.008 to −2.2 bars for the next 18 days by daily watering. An exception to this regime was the saturated-soil treatment, which required about 2 days (days 3–5) to drain from 0 to −0.008 bar ψ_m. A 12-hr photoperiod was maintained after day 3 in the growth chamber (40-W fluorescent lamps, 120 $\mu E \cdot m^{-2} \cdot s^{-1}$) and greenhouse (natural light supplemented with 40-W fluorescent lamps). Air temperatures ranged from 22 to 25 C in growth chamber

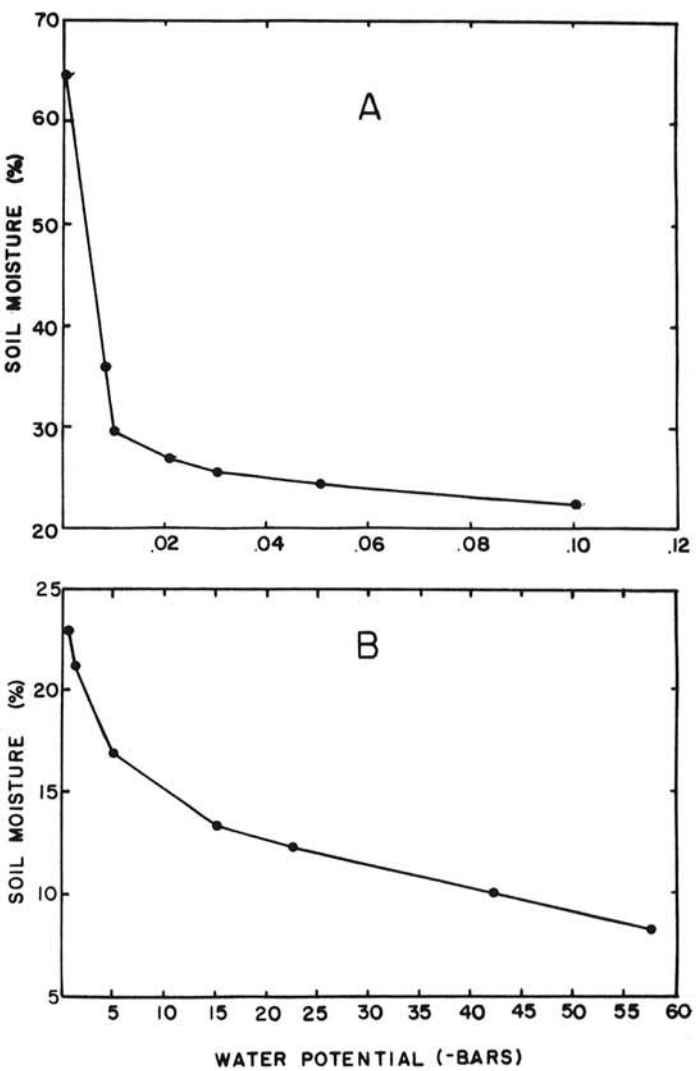

Fig. 1. Moisture characteristic curves of silty clay loam soil used in experiments. **A,** Curve derived from tension plate determinations by wetting soil. **B,** Curve derived from pressure plate ($\psi_m \geq -15$ bars) and filter paper ($\psi_m < -15$ bars) methods.

experiments and from 21 to 27 C in the greenhouse. Emergence and establishment were counted 3, 5, 7, 9, 11, 16, and 21 days after planting. The elapsed time between planting and emergence of 20% of planted seeds (T_{20}) was determined by linear interpolation between the data points closest to 20% emergence, assuming that emergence 1 day after planting was zero.

In some growth chamber experiments, half the seeds were removed from soil after 3 days, and microbial colonization of seed coats and cotyledons was assayed in the same manner as for unplanted seeds. The remaining seeds were treated as in the above experiments from day 3 to day 20 or 21.

RESULTS

Seed quality tests. Table 1 lists germination, vigor, and fungal colonization data for eight seed lots before use.

Microwave treatment of soil. On the basis of three replicate samples, microwave treatment of soil reduced *Fusarium* spp. to 1.5%, *Pythium* spp. to 0.05%, *Rhizoctonia* spp. to 11.8%, total fungi to 10.1%, and total bacteria to 36.3% of populations in untreated soil. Total actinomycete populations rose to 190% of levels in untreated soil.

Establishment from naturally infected seeds. Effects of soil moisture treatments on seedling establishment from pairs of seed lots heavily or lightly infected with *Phomopsis* sp. were evaluated in three greenhouse and four growth chamber experiments. Overall results were similar in each experiment. In a representative experiment, establishment of untreated seeds from a lot heavily infected with *Phomopsis* sp. (lot 5, Table 1) in pasteurized soil was highest at −0.008 to −0.1 bar, declined progressively between −4 and −51 bars, and was lowest at 0 bars (Fig. 2A). Carboxin-thiram seed treatment quadrupled establishment at 0 bars and raised it substantially at other ψ_m tested. In contrast, establishment for untreated seeds of the lot with low incidence of *Phomopsis* sp. exceeded 85% at all ψ_m except saturation, and carboxin-thiram raised establishment substantially only at 0 bars. In untreated soil, untreated seeds of the lot with high incidence of *Phomopsis* sp. had poorer establishment than in pasteurized soil, particularly at −0.008, −0.01, and −51 bars (Fig. 2B). Establishment from untreated seeds of the lot with low incidence of *Phomopsis* sp. was similar in pasteurized and untreated soils. Results for emergence closely resembled those for establishment, except that emergence was typically 3–7% higher than establishment. Time from planting to emergence of 20% of seeds (T_{20}) was longest at 0 bars, shortest at −0.008 to −0.1 bar, and increased for each successive treatment drier than −0.1 bar (Table 2). At unsaturated ψ_m, T_{20} had significant ($P \leq 0.001$) negative correlations with emergence and establishment for the lot with high incidence of *Phomopsis* sp. but not for the lot with low incidence of *Phomopsis* sp. (Table 3).

TABLE 1. Results of germination and vigor tests and percent incidence of seedborne fungi for soybean seed lots used in experiments

	Germination and vigor tests					Percent incidence of seedborne fungi[a]									
	Germination (%)[c]		Accelerated aging[d]		Cold test[f]	*Phomopsis* sp.		*Cercospora* spp.		*Alternaria* spp.		Other fungi[g]		Uninfected by fungi	
Seed lot[b]	Standard	3-day	(%)	Conductivity[e]	(%)	Coat	Coty	Coat	Coty	Coat	Coty	Coat	Coty	Coat	Coty
1	66	55	46	81	49	29	18	42	20	14	10	6	3	2	29
2	55	46	62	91	37	30	18	33	10	20	7	10	13	18	50
3	83	68	3	75	12	0	0	1	0	44	26	45	8	6	43
4	92	79	86	49	74	1	1	9	0	42	18	30	4	2	66
5	42	35	45	ND[h]	26	42	24	18	2	8	0	1	1	26	70
6	71	65	56	ND	ND	40	20	19	4	8	4	37	6	0	63
7	95	ND	0	54	2	2	0	0	0	4	0	8	2	46	94
7a inoc.[i]	73	30	1	53	1	100	2	0	0	0	0	2	6	0	84
7b control[j]	89	48	5	56	1	0	0	2	0	10	0	0	2	20	80
8	95	ND	70	51	50	0	0	2	0	4	0	18	0	40	88
8a inoc.[i]	95	82	55	37	65	100	0	0	0	0	0	0	0	0	98
8b control[j]	97	87	77	37	61	2	0	18	0	2	0	2	0	26	98

[a] Percent seed coats (Coat) or cotyledons (Coty) yielding each fungus after incubation on amended potato-dextrose agar. Only fungal genera with incidence ≥20% in any seed lot are listed.
[b] Lot 6 is cv. Cumberland; all others are cv. Williams.
[c] Data are means of four replicates of 50 seeds each.
[d] Standard germination after 72 hr at 41 C and 100% relative humidity, then 5 days at 25 C. Data are means of four replicates of 50 seeds each.
[e] Units are (Siemens $m^{-1}g^{-1}$ seed wet weight) × 10^4. Data are means of four replicates of 50 seeds each.
[f] Standard germination after incubation for 7 days in moist untreated soil at 10 C, then 5 days at 25 C; $n = 100$.
[g] *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Talaromyces* spp., *Rhizopus* spp., *Epicoccum* spp., and *Chaetomium* spp.
[h] ND = not determined.
[i] Seeds were raised to 37% water content, sprayed with a suspension of mycelial fragments of *Phomopsis* sp., incubated 18 hr at 25 C, then air-dried to 12% water content.
[j] Seeds were raised to 37% water content, sprayed with autoclaved deionized water, incubated 18 hr at 25 C, then air-dried to 12% water content.

TABLE 2. Effects of soil ψ_m, incidence of soybean seed lot infection by *Phomopsis* sp., and seed and soil treatments on mean values of T_{20}[a]

	Low incidence of *Phomopsis* sp. infection				High incidence of *Phomopsis* sp. infection			
	Pasteurized soil		Untreated soil		Pasteurized soil		Untreated soil	
Soil ψ_m (− bars)	Treated seeds[b]	Untreated seeds	Treated seeds	Untreated seeds	Treated seeds	Untreated seeds	Treated seeds	Untreated seeds
0	7.6	9.9	9.4	9.7	8.5	11.0	9.3	10.5
−0.008	1.5	1.4	1.5	1.5	1.5	1.6	1.6	1.9
−0.01	1.5	1.4	1.5	1.5	1.5	1.7	1.6	2.1
−0.1	2.3	1.5	1.7	1.5	1.8	2.1	2.0	2.1
−4	3.5	3.5	3.5	3.5	3.6	3.8	3.6	3.7
−17	5.1	4.8	5.4	5.2	5.2	4.9	5.1	5.0
−51	5.6	5.8	5.7	5.5	5.8	6.1	5.7	6.6

[a] T_{20} = duration of period from planting until emergence from 20% of seeds. Data are from experiment shown in Figure 2.
[b] Data are means of six replicates of 20 seeds each per treatment.

Of the seedborne fungi present in five Williams seed lots (lots 1–5), only *Phomopsis* sp. had a significant negative correlation of percent incidence with emergence in pasteurized soil (Table 4). The correlation coefficients for *Phomopsis* sp. were significant at all ψ_m except saturation.

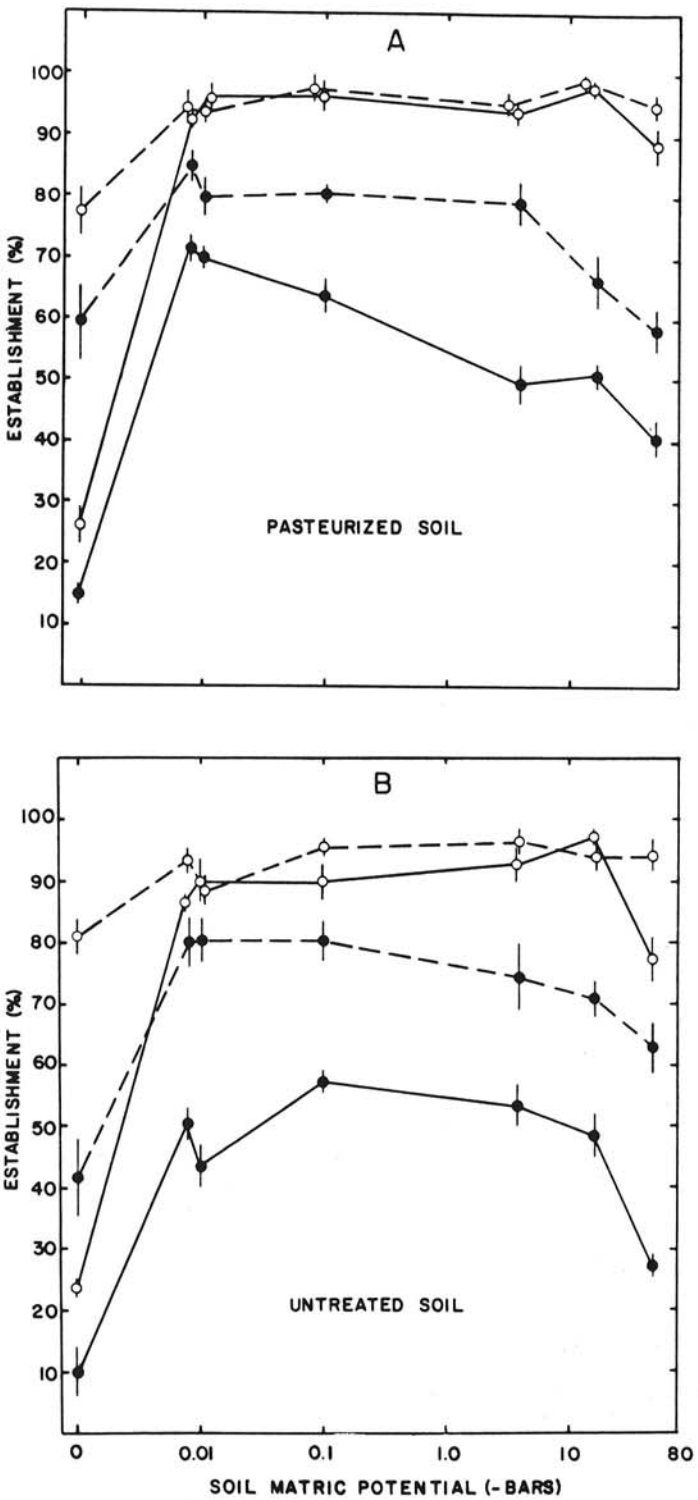

Fig. 2. Effects of initial soil moisture on mean establishment of soybean seedlings at 21 days from carboxin-thiram–treated (dashed lines) and autoclaved deionized water–treated (solid lines) seeds of a lot with 42% incidence of *Phomopsis* sp. infection (•) and a lot with 1% incidence of *Phomopsis* sp. (o). Seeds were incubated in soil for 3 days at the ψ_m values indicated. Soil ψ_m was then equalized to −0.008 bar after 3 days and maintained between −0.008 and −2.2 bars until day 21. **A,** Pasteurized soil; **B,** untreated soil. Values are the means of six replicate samples, 20 seeds per replicate. Error bars = ±1 standard error of the mean.

Growth of *Phomopsis* sp. from seed coats to cotyledons. A 3-day incubation of a naturally infected seed lot (lot 6) with a 40% incidence of *Phomopsis* sp. produced a sharp rise in infection of cotyledons by *Phomopsis* sp. at −0.1 bar but not at ≥−0.008 bar ψ_m (Fig. 3A). As noted for other experiments (Fig. 2), establishment

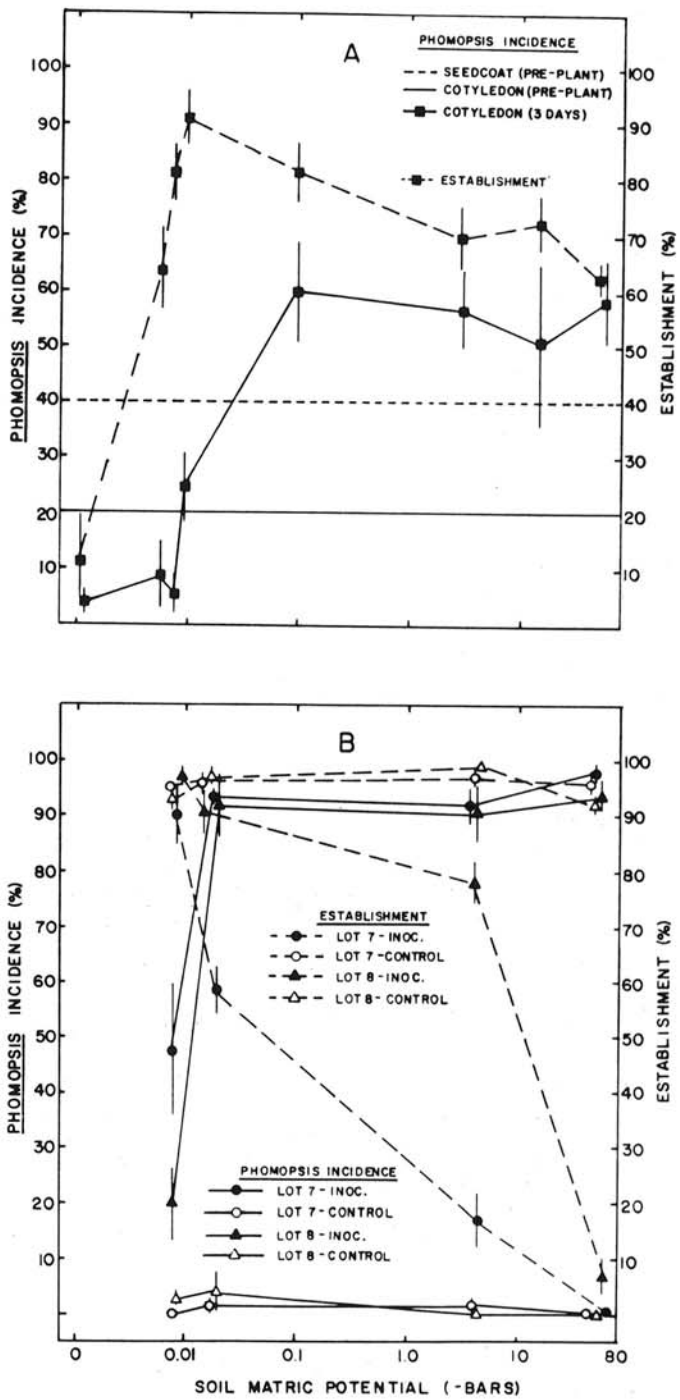

Fig. 3. Effect of soil ψ_m during an initial 3-day incubation of soybean seeds in pasteurized soil on incidence of *Phomopsis* sp. and on establishment of seedlings 20 days after planting. After incubation for 3 days at the soil ψ_m values indicated, some seeds were sieved from soil, separated into seed coats and cotyledons, and plated on amended PDA. For other seeds, soil ψ_m was equalized after 3 days and maintained between −0.008 and −2.2 bars until day 20. **A,** Naturally infected seed lot with mean incidence of 40% seed coat infection and 20% cotyledon infection before planting. **B,** Artificially inoculated (inoc.) and control seed lots (lots 7a, 7b, 8a, 8b, Table 1). Percent incidence of *Phomopsis* sp. on cotyledons was determined after 3 days in soil at the indicated ψ_m levels. Values are means of 200 seeds for the preplant infection assay (A) and five replicate samples of 15 seeds each for all other measures. Error bars = ±1 standard error of the mean.

was highest at −0.008 to −0.1 bar and lower at $\psi_m > -0.008$ or ≤ -4 bars. Similarly, percent cotyledon infection of high-vigor and low-vigor seed lots inoculated with *Phomopsis* sp. before planting (lots 7a and 8a, respectively) increased much more at $\psi_m < -0.01$ bar than at −0.008 bar (Fig. 3B). The high levels of cotyledon infection were associated with reduced establishment at $\psi_m < -0.01$ bar. The progressive decline in establishment with declining $\psi_m < -0.01$ bar was more rapid for the low-vigor lot than for the high-vigor lot. The controls (lots 7b and 8b) had low infection of cotyledons by *Phomopsis* sp. after incubation in soil and almost no decline in establishment at $\psi_m < -0.01$ bar.

DISCUSSION

These experiments show that soil moisture can influence seed lot performance by regulating the activity of seedborne *Phomopsis* sp. Seed lots with a high incidence of seeds naturally infected by *Phomopsis* sp. had much lower establishment when planted in dry soil than when planted in moist pasteurized soil (Figs. 2 and 3). This was not true for lots with a low incidence of *Phomopsis* sp. The fact that only *Phomopsis* sp. had significant negative correlations with emergence indicates that *Phomopsis* sp. was the most damaging seedborne fungus at $\psi_m < 0$ bars. Poorer establishment from seeds infected by *Phomopsis* sp. in drier treatments coincided with vigorous growth of the fungus from seed coats into cotyledons (Fig. 3) and longer duration of the preemergence period (Table 2).

In saturated soil, seeds treated with carboxin-thiram had considerably higher establishment than untreated seeds (Fig. 2), suggesting that the severe seed mortality at $\psi_m = 0$ was at least partially microbially mediated. However, three observations suggest that seedborne fungi were not directly responsible for this result. First, establishment of untreated seeds at $\psi_m = 0$ was almost as poor for a lot with low incidence of *Phomopsis* sp. as for a lot with high incidence of this fungus (Fig. 2). Second, percent incidence of *Phomopsis* sp. declined after 3 days in pasteurized, saturated soil (Fig. 3A). Third, none of the seedborne fungi had significant negative correlations of percent incidence in seed lots with emergence at $\psi_m = 0$ (Table 4). Some mechanism(s) other than activity of seedborne fungi is probably responsible for poor emergence after flooding.

The activity of soilborne microorganisms was apparently responsible for the relatively low establishment of a lot with high incidence of *Phomopsis* sp. at −0.01 and −0.008 bar ψ_m in untreated soil compared with that in pasteurized soil (Fig. 2). These results support the suggestion that infection with *Phomopsis* sp. may increase vulnerability of seeds to attack by soilborne microorganisms (37).

Our findings underscore both the value and the limitations of seed vigor tests. The fact that seed lots with high incidence of *Phomopsis* sp. and relatively low vigor test ratings (lots 5 and 7a) had much poorer establishment in several ψ_m treatments (Figs. 2 and 3B) than lots with high vigor test ratings (lots 4 and 8) confirms that vigor test scores can be general indicators of ability to tolerate stress. However, the performance of all lots with high incidence of *Phomopsis* sp. was strongly influenced by soil ψ_m (Figs. 2 and 3). Widely used standardized laboratory vigor tests in which seeds are supplied with abundant moisture for seedling development (1) thus did not reliably estimate performance of such lots over the range of soil moisture conditions tested here. Moreover, the vigor tests did not discriminate consistently between control lots and lots inoculated with *Phomopsis* sp. (Table 1) even though their response to soil ψ_m treatments was vastly different (Fig. 3B). These observations suggest that although results of vigor tests for soybean seed sometimes correlate with incidence of *Phomopsis* sp. in seed lots (16), infection with *Phomopsis* sp. and vigor test ratings have different implications for seed lot performance. Consequently, both infection with *Phomopsis* sp. and seed vigor should be assayed as part of soybean seed lot quality testing. Some investigators (18,38) have proposed incorporating both optimal and suboptimal conditions of important environmental variables into routine vigor tests to improve the ability of these tests to anticipate field performance. There is a need for further studies to determine the usefulness of adding a "low water potential" component to vigor testing of seed lots.

Two related factors may contribute to poor performance of seeds infected by *Phomopsis* sp. at low water potentials: a growth rate advantage of *Phomopsis* over host seeds and a prolonged preemergence period. If increased percent incidence of cotyledon infection after incubation in soil is used as an index of growth of *Phomopsis* sp., then our results indicate that soil ψ_m as low as −51 bars has little inhibitory effect on this fungus. The fact that growth rate of *Phomopsis* sp. on agar osmotically adjusted with sucrose or KCl is still 50–80% of the maximum at −50 bars (26) supports this interpretation. For a naturally infected seed lot (Fig. 3A), incidence of *Phomopsis* sp. on cotyledons rose sharply after incubation at $\psi_m \leq -0.1$ bar but increased much less or even declined at $\psi_m \geq -0.01$ bar. This pattern may have resulted from more intense competition by other microorganisms in wet soil than in drier soil, from differential effects of soil water potential on host and

TABLE 3. Effects of *Phomopsis* sp. infection, soil treatment, and soybean seed treatment on correlation coefficients (r) of T_{20} with emergence and establishment[a]

Soil	Low incidence of *Phomopsis* sp. infection		High incidence of *Phomopsis* sp. infection	
	Treated[b] seeds	Untreated seeds	Treated seeds	Untreated seeds
Pasteurized				
Emergence	0.07	−0.22	−0.60***[c]	−0.85***
Establishment	0.21	−0.21	−0.76***	−0.84***
Untreated				
Emergence	0.07	0.02	−0.62***	−0.61***
Establishment	0.09	−0.10	−0.62***	−0.63***

[a] T_{20} = duration of period from planting until emergence from 20% of seeds. Data are from experiment shown in Figure 2 (0 bars treatment deleted; n = 36, six moisture treatments × six replicates per treatment).
[b] Carboxin-thiram.
[c] *** = Significant at $P \leq 0.001$; values not starred are not significant at $P \leq 0.05$.

TABLE 4. Correlation coefficients (r) of percent incidence of naturally occurring fungal infections of five soybean seed lots (lots 1–5, cv. Williams, Table 1) with percent emergence for ψ_m treatments in pasteurized soil[a]

Soil ψ_m (− bars)	*Phomopsis* sp.		*Fusarium* spp.		*Cercospora* spp.		*Alternaria* spp.		*Aspergillus* spp.		*Penicillium* spp.	
	Coat	Coty	Coat	Coty	Coat	Coty	Coat	Coty	Coat	Coty	Coat	Coty
0	−0.65	−0.62	0.61	0.48	−0.01	−0.07	0.63	0.54	0.55	0.43	0.56	0.37
−0.008	−0.95*[b]	−0.96*	0.82	0.97**	−0.72	−0.59	0.95*	0.84	0.98**	0.66	0.88*	0.19
−0.01	−0.93*	−0.93*	0.73	0.90*	−0.63	−0.52	0.94*	0.81	0.94*	0.72	0.84	0.29
−0.1	−0.96**	−0.98**	0.75	0.99**	−0.79	−0.65	0.98**	0.88	0.98**	0.76	0.90*	0.28
−4	−0.92*	−0.93*	0.58	0.95*	−0.84	−0.75	0.96**	0.83	0.95*	0.84	0.83	0.33
−17	−0.98**	−0.97**	0.75	0.92*	−0.62	−0.46	0.98**	0.90*	0.91*	0.80	0.91*	0.44
−51	−0.96*	−0.95*	0.75	0.90*	−0.60	−0.46	0.96**	0.86	0.92*	0.75	0.80	0.38

[a] Data are from two greenhouse experiments. Soil was maintained at indicated ψ_m values for first 3 days after planting, then brought to −0.008 bar and maintained between −0.008 and −2.2 bars until day 21. Coat = seed coats, Coty = cotyledons. n = 5.
[b] * = Significant at $0.05 \geq P > 0.01$; ** = significant at $P \leq 0.01$.

pathogen growth rates, or from a combination of these factors. The longer duration of the preemergence period in dry soil treatments may have allowed individual *Phomopsis* sp. infections to damage host seeds more severely than in moist soil treatments; T_{20} was more than three times longer at −51 bars than at −0.008 bar (Table 2) and was correlated with reduced establishment of a seed lot with high incidence of *Phomopsis* sp. (Table 3).

Leach (17) found that emergence of vegetable seedlings from soil infested with various pathogenic fungi was proportional to the ratio of emergence rate without pathogens to pathogen growth rate in vitro and that this ratio varied with temperature. More recently it has been suggested that soil moisture, like temperature, can affect disease progress by controlling the balance between host and pathogen activity (2,19,29). Our finding that the impact of soil water potential on seed lot performance varies in accordance with relative growth rates of *Phomopsis* sp. and host seeds supports this generalization. Confirmation of this hypothesis for the *Phomopsis*-soybean system, however, will require a more detailed investigation of the effects of soil moisture on host and pathogen activities.

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