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THE EFFECTS OF SEED SIZE AND SEED DENSITY ON GERMINATION AND VIGOR IN SOYBEAN (*Glycine max* (L.) Merr.)

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The germinability and vigor of seeds of different sizes and densities from each of 18 soybean seedlots were tested using the standard rolled towel germination test, the single seed conductivity test, and the bulk leachate conductivity test. The largest seeds and the low density seeds performed worst in the standard germination test, although there was an indication that the viability of extremely small seeds may also be low. Single seed leachate conductivity levels were highest for large seeds and low density seeds, indicating low vigor. Bulk conductivity tests showed high levels of leakage in large seeds, but did not detect differences between seeds of high and low density. Seed size and seed density effects were usually less pronounced or non-existent in seedlots of extremely high or extremely low vigor. Furthermore, the effects of seed size and density on viability and vigor do not appear to be of sufficient magnitude to allow for significant seedlot improvement through conditioning unless cleanout percentages are prohibitively high.

Key words: Soybean, seed size, seed density, germination, vigor

[Effets de la taille et de la densité des semences sur la germination et la vigueur du soja (*Glycine max* (L.) Merr.).]
Titre abrégé: Effets de la taille et de la densité des semences chez le soja.
Nous avons testé le pouvoir germinatif et la vigueur de semences de soja de tailles et de densités diverses provenant de 18 lots au moyen de la méthode habituelle de germination sur serviettes de papier roulées, de l'essai de conductivité sur une seule graine et de l'essai de conductivité sur le percolat mélangé. Les graines les plus grosses et les moins denses sont celles qui ont donné les plus piètres résultats au test de germination. Il semble en outre que la viabilité des graines de très petite taille ne soit pas très bonne. Les graines de grande taille et de faible densité ont donné les valeurs les plus élevées au test sur une seule graine, ce qui dénote un manque de vigueur. Par ailleurs, les essais de conductivité sur percolat mélangé ont montré une perte importante d'électrolytes chez les graines de grande taille mais n'ont pas laissé voir de différence entre les graines de densité faible et élevée. Les effets de la taille et de la densité sont habituellement moins prononcés ou inexistants chez les lots de semences de vigueur très grande ou très faible. De plus, les effets de la taille et de la densité sur la viabilité et la vigueur ne semblent pas suffisamment importants pour permettre une amélioration sensible des lots par conditionnement sauf dans les cas où les pertes au nettoyage sont extrêmement élevées.

Mots clés: Soja, taille des semences, densité des semences, germination, vigueur

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The effects of seed size and seed density on seedlot vigor have been of interest to researchers because of the potential for vigor improvement through seed conditioning. Knowledge of the effects of these two physical seed parameters on vigor in soybean (*Glycine max* (L.) Merr.) would be useful, since low seed vigor is a common soybean production problem.

A positive correlation between seed size and seed performance has been reported in several species (McDonald 1975); however, studies on the effects of soybean seed size on germination, vigor, and field performance have produced inconclusive and contradictory results. Burris et al. (1971, 1973) observed that larger soybean seeds produced larger embryos, exhibited higher respiratory rates, and possessed greater field emergence potential than did small seeds. Physiologically large seeds of Lee soybean were found by Gupta (1976) to have higher respiratory rates, greater shoot length, and less leakage of sugars than small seeds. More rapid germination and greater field emergence was noted for large seeds than for small seeds by Hopper et al. (1979). On the other hand, Edwards and Hartwig (1971) reported faster emergence and better root development from small soybean seeds. A small-seeded variant of Lee had better germination, greater early hypocotyl development, and lower leakage of sugars than the large-seeded type (Gupta 1976). In several studies, genetically small seeds have been shown to have superior viability (Calero et al. 1981; Nangju 1979; Singh et al. 1978). Green et al. (1965) compared seedlots of different 100-seed weight and found a negative correlation between seed size and germination. Finally, there are other researchers who have been unable to detect any relationship between soybean seed size and germination or field emergence (Fontes and Ohlrogge 1972; Johnson and Luedders 1974; Johnson and Wax 1978; Singh et al. 1972; Smith and Camper 1975).

No published reports on the effects of soybean seed density on viability or vigor have been found; however, evidence from other crops including rice (*Oryza sativa* L.), alfalfa (*Medicago sativa* L.), crimson (*Trifolium incarnatum* L.), red (*Trifolium pratense* L.) and white (*Trifolium repens* L.) clovers (McDonald 1975), cotton (*Gossypium hirsutum* L.) (Krieg and Bartee 1975), and sorghum (*Sorghum bicolor* L. Moench) (Maranville and Clegg 1977) suggests that low density seeds are generally low in vigor.

Laboratory tests of vigor are often employed to predict the performance of seeds under field stress. The standard germination test is the most common, but appears to be incapable of detecting subtle differences in field emergence potential (Johnson and Wax 1978). Among the vigor tests which have been used to predict field emergence are the seedling classification test (Woodstock 1973; McDonald 1975), and the conductivity test (McDonald 1975). The Automatic Seed Analyzer (ASA-610) has been used to predict the germinability of soybean seeds based on the current level of the seed steep water of individual seeds (McDonald and Wilson 1980; Steere et al. 1981).

The purpose of this study were to assess the effects of seed size and seed density on soybean viability and vigor through laboratory testing, to determine whether or not these effects were constant across seedlots and to ascertain whether or not seed size and seed density act independently.

MATERIALS AND METHODS

Four separate experiments were conducted in 1980 and 1981 to assess the effects of seed size and seed density on germination and vigor using the following sets of soybean seedlots or seeds:

Experiment 1: unconditioned (unprocessed) commercial seedlots from the 1979 production year;

Experiment 2: eight conditioned pedigreed seedlots from the 1979 production year;

Experiment 3: four conditioned pedigreed seeds from 1980 production year.

Seed Size and Density Conditioning

In exp. 1, three unconditioned seedlots from each of two cultivars, Harcor (Maturity Group II) and Maple Arrow (Group 00), were collected in the autumn of 1979 from various locations in southwestern Ontario, Canada. Each seedlot was divided into four size classes by passing the seeds over round-holed screens in a Clipper M-2B fanning mill, such that each size class differed in diameter from the preceding size class by 0.4 mm (1/64 inch). Size classes were numbered S1 (smallest) through S4 (largest). It should be noted that S1 and S4 were open-ended size classes, and that all seeds from the separations were retained for further separation and evaluation. The absolute size of seeds in each size class depended on the cultivar and seedlot; therefore, comparisons of size classes between seedlots can be made on a relative basis only. After size separation, the total weight of seeds in each size class was recorded.

Seeds of each size class were then further subdivided, using flotation on sucrose solutions of $1.24\ g\cdot cm^{-3}$ for Harcor seedlots and $1.21\ g\cdot cm^{-3}$ for Maple Arrow seedlots, into two density classes, low density seeds (LD) and high density seeds (HD). Solution densities were chosen to given an approximate 1:1 ratio of LD to HD seeds. Seeds were immediately rinsed with deionized water and air-dried to approximately 11% moisture. Visibly diseased seeds, immature seeds, seeds with damaged or lifted seedcoats, and seeds with cracked cotyledons were then discarded. The proportion of LD and HD seeds by weight was recorded, and two samples of 100 seeds each were drawn from each size-density subclass for 100-seed weight determinations.

In exp. 2, in the spring of 1980, four commercially conditioned pedigreed seedlots from each of two Group 00 cultivars, McCall and Maple Arrow, were obtained. The seedlots were divided into four size classes, using appropriate screen sizes, as in exp. 1. Seeds from each size class of each seedlot, as well as an unsized control, were separated into two density classes by the method of exp. 1. Solution densities were chosen to give an approximate 1:2 split between LD and HD seeds. The proportion by weight of LD and HD seeds was recorded. Two 100-seed samples for 100-seed weight determination were drawn from each size-density subclass.

Seedlots for exp. 3 were collected in 1980. Two commercially conditioned pedigreed seedlots from each of McCall and Maple Arrow cultivars were obtained. Size and density classes were obtained as in exp. 2; however, 100-seed weights were not determined.

Germination and Vigor Evaluation

In the standard germination tests for exps. 1 and 2, 25 sound, unbroken seeds were placed within three sheets of standard weight Anchor germination paper moistened with deionized water. The germination paper was rolled and placed upright in a germinating cabinet at $25 \pm 1°C$ and 95–100% relative humidity. After 7 days seeds were scored as germinated or ungerminated according to criteria in Rules for Testing Seeds (Association of Official Seed Analysts, 1970). Four replicates were evaluated for each entry. For exp. 3, 50 seeds were used per replicate. Results were reported as percent germinated seeds.

The single seed conductivity test was performed using the Automatic Seed Analyzer, Model 610 (ASA-610). Tests were conducted using the method of McDonald and Wilson (1980). Results were reported as average microamps per seed over 100 seeds in each of two replicates. In the bulk conductivity test, bulk samples of 50 sound, unbroken seeds representing each size-density subclassfrom each seedlot of exp. 1 were weighed, then placed in 250 mL of deionized water in a 500-mL Erlenmeyer flask. Flasks were then sealed with Parafilm and kept at a controlled temperature of $25 + 1°C$ for 24 h. The conductivity of the leachate was then measured, after agitation, using a SYNBRON/Barnstead Model PM-70 C B conductivity bridge. Results were converted to micromhos per gram of seed for each of two replicates.

The laboratory tests of exps. 1, 2 and 3 were treated by analysis of variance using split-split-split plot designs with cultivar as the main plot, seedlot within cultivar as the second factor, seed size as the third factor, and seed density as the fourth factor. All percentage data (standard germination tests) were analyzed using both percent data and data transformed by arcsine square root. The transformation did not alter results of the statistical analyses, thus percent data only are presented. Seed size × seed density interactions did not occur; therefore, seed size and seed density effects were considered separately. When seedlot × seed size and seedlot × seed density interactions occurred, tests were statistically reanalyzed on a seedlot by seedlot basis. Space did not permit presentation of individual seedlot data; thus, only experiment-wise means are reported.

RESULTS

The 100-seed weights for the various size and density classes in the six unconditioned seedlots of exp. 1 and the eight conditioned seedlots of exp. 2 showed that seed size class had a significant effect on seed weight. In each seedlot, the S1 seeds weighed approximately 60% as much as the S4 seeds. The density separation produced a relatively small (2–3%) average weight difference between LD and HD seeds which was significant for each individual seedlot.

The primary objective of these experiments was to assess the effects of seed size and density on germination and vigor; however, the performance of the individual seedlots should be noted. The standard germination results of the individual seedlots (Table 1) indicate that in exp. 1 only Maple Arrow seedlot 3 was of high seed quality. All of the conditioned seedlots of exp. 2 were labelled either Canada Registered or Certified Grade No. 1 seed (minimum 85% germination), but the low germinability of Maple Arrow seedlots 4 and 5 suggests that these two seedlots may have been of low vigor. The germination results reported for exps. 1 and 2 were conducted in September, after planting. Germination tests conducted earlier that spring did not differ significantly. The germination tests for the conditioned pedigreed seedlots of exp. 3 suggest that Maple Arrow seedlot 8 may have been of marginal seed quality. It should be noted that in exps. 1 and 2 seedlot 100-seed weight had little relationship to seedlot germination or vigor.

The standard germination tests found viability differences among different seed populations within seedlots (Table 2). The S4 size class in each of the six unconditioned seedlots of exp. 1 had lower viability than the other three size classes, and lower viability was noted for LD seeds compared to HD seeds. In the eight conditioned seedlots of exp. 2, size had no effect on ger-

Table 1. Germination, vigor, and seed weight means, averaged over seed classes, in 18 soybean seedlots

Seedlot	Standard germination (%)	Single seed conductivity (μamps·seed^{-1})	Bulk conductivity (μmhos·g^{-1})	100-seed weight (g)
Experiment 1 (1980)				
Harcor 1	42.9	130.9	47.5	17.6
Harcor 2	68.3	89.5	38.5	14.1
Harcor 3	70.2	90.4	41.9	14.5
Maple Arrow 1	61.0	74.8	25.6	15.9
Maple Arrow 2	78.3	77.2	28.3	17.5
Maple Arrow 3	85.0	63.9	21.1	17.5
LSD ($P \leq 0.05$)	5.8	6.7	4.7	0.4
Experiment 2 (1980)				
Maple Arrow 4	42.3	93.8	—	19.7
Maple Arrow 5	47.5	94.7	—	19.6
Maple Arrow 6	63.5	91.9	—	19.8
Maple Arrow 7	79.9	73.7	—	20.4
McCall 1	82.9	63.4	—	14.1
McCall 2	89.9	59.4	—	16.6
McCall 3	91.9	51.1	—	14.4
McCall 4	93.2	55.3	—	16.6
LSD ($P \leq 0.05$)	5.6	8.6	—	0.3
Experiment 3 (1981)				
Maple Arrow 8	76.8	78.7	—	—
Maple Arrow 9	92.1	63.7	—	—
McCall 5	89.6	54.9	—	—
McCall 6	93.8	49.9	—	—
LSD ($P \leq 0.05$)	2.3	1.6	—	—

Table 2. Effects of seed size and seed density on percent germination in 18 soybean seedlots

Size density	Standard germination (%)		
	Experiment 1	Experiment 2	Experiment 3
S1	68.5*b*	75.4*a*	93.5*d*
S2	71.3*b*	74.0*a*	92.1*cd*
S3	69.1*b*	74.6*a*	87.8*b*
S4	62.0*a*	71.0*a*	76.3*a*
Unsized	—	75.5*a*	90.7*c*
LD	63.7*a*	69.7*a*	86.4*a*
HD	71.8*b*	76.0*b*	90.1*b*
Unseparated	—	76.6*b*	87.8*a*

a-d Means within the size classes or density of the same column followed by the same letter do not differ significantly according to Duncan's new multiple range test ($P \leq 0.05$).

mination, despite a trend toward lower germinability in the S4 class. Analysis of a seedlot × seed size interaction for percent germination revealed that two seedlots of intermediate viability did show a size effect which contributed to much of the trend found in the overall statistical analysis. The LD seeds of exp. 2 were, on average, less viable than HD seeds, although a seedlot × seed density interaction demonstrated that the seedlot of lowest viability did not show a density effect. On average, high density seeds were not superior to the unseparated control. The four conditioned seedlot studies in the 1981 season (exp. 3) demonstrate the inferior germinative capacities of large sized seeds. In this experiment, the use of 50 seeds per replicate rather than 25 contributed to a lowering of the experimental CV to 5.4% as opposed to an average of approximately 12% in exps. 1 and 2. Partly as a result of this, finer differences in percent germination due to seed size were detectable. Although all seedlots showed a size effect, the seedlot of marginal viability, Maple Arrow seedlot 8, had the greatest difference in percent germination between S1 and S4 seeds (84.0 and 59.5%, respectively). Only size class S1 was superior to the unsized control. The HD seeds were superior to LD seeds and the unseparated control in all seedlots.

In an experiment (data not presented) to determine whether or not further decreases in seed size would eventually lead to declines in vigor, an additional size class, smaller than S1, was screened from S1 seeds of exp. 3. The standard germination of these seeds was not different from the S1 class; however, the seedlings were generally shorter and this small size class had a higher percentage of seedlings with stunted hypocotyls or radicles or both.

In exps. 1, 2, and 3 the current level per seed, as measured in the single seed conductivity test (ASA-610) (Table 3), was found to increase as seed size increased. Generally, each size class differed from each of the others in every seedlot studied, although the absolute differences between size classes varied somewhat from seedlot to seedlot. Consequently, there were several seedlots for which current levels differed among sizes even when differences in germinability did not.

The ASA-610 results for seed density were not as straightforward. In exp. 1 the HD seeds generally leaked less than LD seeds; however, no differences in current level due to seed density occurred in Harcor seedlot 1 (the lowest viability) or in the high viability seedlot, Maple Arrow seedlot 3. In the conditioned seedlots of exp. 2, the HD seeds displayed lower leakage, although the HD seeds were not superior to the unseparated control. Further analysis of a seedlot × seed density interaction indicated that the two seedlots of highest viability showed no density effect. In exp. 3, HD seeds leaked less electrolytes than the

Table 3. Effects of seed size and seed density on seed leachate conductivity in 18 soybean seedlots

	Single seed conductivity (μamps·seed^{-1})			Bulk conductivity (μmhos·g^{-1})
Size density	Experiment 1	Experiment 2	Experiment 3	Experiment 1
S1	66.3*a*	60.1*a*	51.3*a*	31.7*a*
S2	78.9*b*	69.3*b*	54.8*b*	32.3*a*
S3	98.1*c*	76.0*c*	62.8*c*	36.0*b*
S4	107.9*d*	88.0*d*	81.8*d*	35.4*b*
Unsized	—	71.0*b*	57.9*c*	—
LD	92.3*b*	78.0*b*	63.3*c*	34.1*a*
HD	83.3*a*	69.8*a*	60.1*a*	33.5*a*
Unseparated	—	70.9*a*	61.8*b*	—

a-d Means within the size classes or density classes of the same column followed by the same letter do not differ significantly according to Duncan's new multiple range test ($P \leq 0.05$).

LD seeds and the unseparated control in all seedlots.

ASA-610 current level differences were found among all size classes of exps. 1, 2, and 3, regardless of viability differences. ASA-610 results are reported on a per seed basis, and large seeds might be expected to leak more electrolytes and metabolites, simply on the basis of a greater weight of seed. Bulk conductivity results of exp. 1 seeds (Table 3) indicated that when conductivity measurements were made on a weight basis, large seeds leaked more than small seeds. Contrary to the ASA-610 technique, however, size differences were not noted for each size class within a seedlot, and two seedlots of lower viability did not show a size effect. Although the mean conductivity per gram of seed was higher for LD seeds than HD seeds in five of six seedlots, these differences were not statistically significant.

DISCUSSION

The major objective of this study was to determine the effects of seed size and seed density on germination and vigor in soybean. Where differences were detected, the viability of seeds, as measured by the standard germination test, was found to be lower in the largest sized seeds. Inferior germinative capacity for large soybean seeds has been reported by other researchers (Calero et al. 1981; Edwards and Hartwig 1971; Green at al. 1965; Nangju 1979; Singh et al. 1978), although they were not comparing seeds of different sizes within the same seedlot. The indication that extremely small seeds may also be of lower vigor supports the findings of other researchers (Burris et al. 1971, 1973; Hopper et al. 1979). The magnitude and frequency of size effects were greater in the conductivity tests, suggesting that vigor is affected more by seed size than is viability. Low density seeds were lower in viability and vigor in many of the seedlots studied. A similar relationship between seed density and viability has been reported in several species (Krieg and Bartee 1975; Maranville and Clegg 1977; McDonald 1975).

Although seed size and seed density affect viability and vigor, these two physical seed parameters appear to act independently, so that maximum vigor improvement could only be accomplished through separations based both on seed size and seed density. The responses of seed vigor to seed size and seed density were apparent in conditioned and unconditioned seedlots alike, suggesting that current conditioning techniques do not maximally improve seedlot vigor.

The presence of seedlot × seed size and seedlot × seed density interactions demonstrated that seedlots of very high or very low viability often showed no viability or vigor responses to seed size or seed density; moreover, seed size and density effects were of greater magnitude in the intermediate viability seedlots. Even in these intermediate viability seedlots, only the S1 size class was of significantly higher vigor than the unsized control, and this size class represented, on average, only 6% of the original seedlot by weight. High density seeds were in some instances of greater vigor than the unseparated seedlots, but this advantage was not large and was only accomplished through removal of 50% or 33% of the seeds in these seedlots.

Thus, it appears unlikely that seedlot vigor improvement in soybean can be accomplished by conditioning based on seed size or density unless prohibitively large cleanout percentages are employed. However, this study deals with the effects of seed size and density per se on laboratory performance. The immature and diseased seeds common in low density seeds, and the mechanically damaged seeds common in large sized seeds were removed prior to testing. Had they not been removed, conclusions might have been different. Smaller seeds were of higher or, at least, equivalent vigor to large seeds. Because soybean seed is sold by weight, it would be possible for a farmer to reduce his seed cost by using the small seeds from a seedlot.

The tests of viability and vigor used in this study varied in their abilities to detect performance differences between seed sizes and seed densities. The standard germination test, conducted under optimum environmental conditions, detected performance differences least often. This suggests that viability differences due to seed size and density are not often large. Tests of seed leakage have been used for vigor evaluation in several species including soybean (McDonald 1975). High levels of electrolyte leakage during seed imbibition have been associated with mechanical damage, cryptic damage, poor organellar and cellular membrane integrity, and low seed vigor. This leakage may result in the unavailability of metabolites for the embryo and the accumulation of metabolites in the soil surrounding the seed, which creates an environment favorable for the growth of fungal seed pathogens (Woodstock 1973). The single seed conductivity test revealed vigor differences among all seed sizes in each seedlot whether these differences were apparent in other tests or not. The bias introduced by seed weight in the ASA-610 technique (i.e. results are reported on a per seed basis) has also been addressed by McDonald and Wilson (1980). Nonetheless, the bulk conductivity test indicated less leakage and thus higher vigor in smaller seed when results were reported on a weight basis, suggesting that at least part of the effect apparent in the ASA-610 technique is real. The bulk conductivity test is more difficult to perform and was less accurate than the ASA-610 technique. If a correlation factor for seed weight can be developed, the ASA-610 technique has distinct advantages of speed, simplicity and accuracy.

The effects of seed size and density on field performance have not been addressed here. Subsequent testing is determining whether the seed size and density effects of viability and vigor predicted by these laboratory tests are expressed in the field.

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