

Techniques

Comparison of Seed Priming Techniques with Regard to Seedling Emergence and Pythium Damping-Off in Sugar Beet

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ABSTRACT

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Three seed priming techniques were compared for their effects on earliness, rate, and uniformity of seedling emergence of sugar beet in infested and uninfested soils. Seed was osmoprimed with -1.5 MPa NaCl or -1.2 MPa PEG 8000, or solid-matrix-primed (SMP) with water and a hydrous silicate clay as the solid substrate. Washed and untreated seeds were included as controls. Seed was planted in soil infested with *Pythium ultimum* or in uninfested soil, and stand data were recorded for approximately 15 days. Three days after planting in uninfested soil, SMP- and NaCl-treated seed produced greater stands than the untreated control, and SMP-treated seed produced a greater stand and faster, more uniform emergence than all other treatments. Eight days after emergence, only

the stand of the washed treatment was greater than the untreated control. Stands in all other treatments emerged at a faster rate than in the untreated control, and SMP induced faster emergence than any other treatment. In infested soil, primed seed also gave significantly better stands than washed or untreated seed 8 and 15 days after planting. Primed seed also had less preemergence damping-off, but there was no difference in postemergence damping-off. SMP was better than both osmoprimed treatments in promoting early emergence, suppressing preemergence damping-off, and in producing a greater final stand. SMP-treated seed still maintained a "primed condition" 7 mo after treatment.

Stand establishment problems are one of the most frequently experienced difficulties encountered by sugar beet (*Beta vulgaris* L.) producers in the United States. In the Texas Panhandle, where environmental conditions at planting time are unpredictable, stand establishment in sugar beet is an annual challenge. After planting, fields are irrigated and sudden drops in temperature often result in cold, wet soil, predisposing seed and seedlings to Pythium damping-off. Hard driving rains and high winds can produce soil crusting, which can prevent seedlings from emerging, or if seedlings already have emerged, winds can easily break delicate hypocotyls at soil level. Any technique that could accelerate emergence or increase seedling vigor would greatly benefit sugar beet producers. Seed priming is potentially such a technique (7,8,15,20).

Seed priming is a method of controlled hydration in which the physiological process of germination is initiated but stopped before radicle emergence (7). Historically, hydration during priming has been controlled with inorganic salts, such as KNO_3 and NaCl, or high molecular weight compounds, such as polyethylene glycol (PEG) dissolved in water (8,12). Using these methods, numerous researchers have increased stand establishment, disease resistance, and seedling vigor in a variety of crops (2-4,21,24,25). However, with PEG, adequate aeration is frequently a problem and inorganic salts may be phytotoxic (1,11,23).

Recently, a new method of priming, termed solid matrix priming (SMP), has been developed (23). This method, conceived by John Easton (Kamterter, Inc., Lincoln, NE), controls hydration through matrix potential, as opposed to traditional priming methods that employ osmotic potential. SMP has increased stand establish-

ment, disease resistance, and seedling vigor of several crops (6,9,10), but how SMP compares with traditional osmopriming is unknown. Therefore, the objective for this study was to compare different priming techniques with regard to sugar beet seedling emergence and disease susceptibility. Preliminary reports have been published (5,6).

MATERIALS AND METHODS

Seed treatments. Five seed treatments, including SMP, two osmopriming treatments, washed seed, and an untreated control, were compared. A dry, hydrous silicate clay (supplied by Kamterter Inc.), which passed through a 1.4-mm² sieve but was retained on a 1.0-mm² sieve, was used as the solid matrix material in the SMP treatment. In preliminary studies, varied ratios of matrix, water, and seed were evaluated. In these studies, 22.7 g of sugar beet seed (Hilleshög Mono-hy cultivar Tx18) were mixed with 22.7 g of matrix and 18, 20, 22, 24, 26, 28, or 30 ml of H₂O. The mixtures were placed in 15- × 10-cm diameter polystyrene tubes and covered on both ends with plastic caps. Tubes were placed on a roller, programmed to activate four times per day for 15 min, and incubated for 2 days at 15 C. After the 2-day prime, the plastic caps were replaced with vented caps and the seeds were allowed to slowly dry for 3 days. Seeds were separated from the dried matrix by sieving, evaluated for damage (cracked or germinated seeds), planted in soil contained in 10-cm² plastic pots, and incubated at 15 C. Ten seeds were planted per pot, and there were four replicates arranged in a randomized complete block design for each water rate. Stand counts were initiated when the first seedlings appeared and were continued daily for approximately 1 wk. The rate of 22 ml of H₂O was determined to be safe (no seeds cracked or germinated) and effective, and subsequently was used in all SMP treatments.

Osmopriming was achieved with NaCl or PEG 8000 as described by Osburn and Schroth (19,20). Approximately 22 g of seed was washed six times for 30 min in tap water and then primed in 100 ml of -1.5 MPa NaCl (19.87 g/L) or -1.2 MPa PEG 8000 (302 g/L) for 6 days in flasks on a rotary shaker. Seeds then were washed, dried, and stored in a plastic bag at room temperature until used, usually no longer than 1 wk.

Seeds for the washed treatment were washed with tap water six times for 30 min per wash on a rotary shaker, dried, and stored until used. The same seed lot was used in all studies, and all treatments were conducted simultaneously.

Soil mix and inoculum preparation of *Pythium*. The soil mix used in all studies was prepared from a nonsterile silt loam pasture soil mixed with peat (5:2, v/v). For pathogen-infested soils, an oatmeal broth-vermiculite inoculum was prepared by mixing 50 g of oatmeal and 2 L of H₂O in a blender at high speed for 5

min. The mixture then was heated for 5-10 min at 50 C and filtered through cheesecloth. Two hundred milliliters of the oatmeal broth was added to 100 g of vermiculite in widemouth flasks, mixed thoroughly, and autoclaved. A mycelial plug from a 3-day-old culture of *Pythium ultimum* Trow growing on potato-dextrose agar was transferred to each flask after cooling. Flasks were stored on a shelf at room temperature for 4 wk before use. Inoculum was added to the soil mix at a rate of 2.5% (w/w) and mixed in a cement mixer before use.

Effects of seed treatments on sugar beet seedling emergence and disease incidence. Studies were conducted in a greenhouse where temperatures ranged from 20 to 30 C. Plastic flats were filled with infested or uninfested soil, and 15 seeds from each of the five seed treatments were planted in each flat. Flats were initially subirrigated so as not to disturb seed placement, and no further water was added until after seedlings began to emerge. Stand counts were started 3 days after planting and continued for 15 days. Counts included the number of plants that emerged or damped-off each day, and results were expressed as percentages. There were six replicates of each seed treatment-soil combination arranged in a randomized complete block design on greenhouse benches. The study was repeated once.

Longevity of seed treatment effects. Seven months after initial treatment, seeds, which had been stored at room temperature, were planted in uninfested soil mix and incubated at 15 C. Ten seeds from each of the original five treatments were planted in 10-cm² pots. There were four replicates arranged in a randomized complete block design. Emergence was compared among treatments, and the test was repeated once.

Data analysis. Percentage data were subjected to analysis of variance (ANOVA) after arcsine-square root transformation, but actual percentages are presented in the tables. Treatment means were separated with Duncan's multiple range test or an LSD test at $P \leq 0.05$. When the results from repeated tests were analyzed by ANOVA, either there were no differences between tests or, if tests were different, no significant test × seed treatment interaction. Therefore, data from repeated studies were combined unless otherwise noted. The effect of seed treatment on seedling emergence rate in uninfested soil was evaluated by determining the mean rate of emergence as previously described (14,20).

RESULTS

Seed priming in preliminary tests. Priming sugar beet seed with the SMP technique in 26-30 ml of H₂O damaged seed; seed caps

TABLE 1. Effect of seed priming on seedling emergence and mean rate of emergence of sugar beet cultivar Tx18 in uninfested soil

Treatment ^a	Emergence (%)		MER ^b (days)
	3 days	8 days	
Solid matrix priming	55.5 a ^c	73.9 b	3.4 a
NaCl	12.2 b	76.7 b	4.6 b
Polyethylene glycol (PEG)	6.1 bc	68.9 b	5.1 c
Washed	5.0 bc	87.8 a	4.6 b
Control	1.7 c	76.7 b	5.6 d

^aOsmoprimed seeds were incubated in -1.5 MPa NaCl or -1.2 PEG 8000 for 6 days at 15 C. Solid matrix priming was achieved by mixing 22.7 g of seed with 22.7 g of solid matrix and 22 ml of H₂O, incubating for 2 days at 15 C, and then drying for 3 days at 15 C. The washed treatment entailed washing seed in distilled water six times for 30 min each on a rotary shaker at 15 C.

^bMER = mean emergence rate, which is $N + T_2N_2 + \dots + T_nN_n$ /total number of seedlings emerged, in which N = number of seedlings emerged and T = number of days.

^cMeans followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

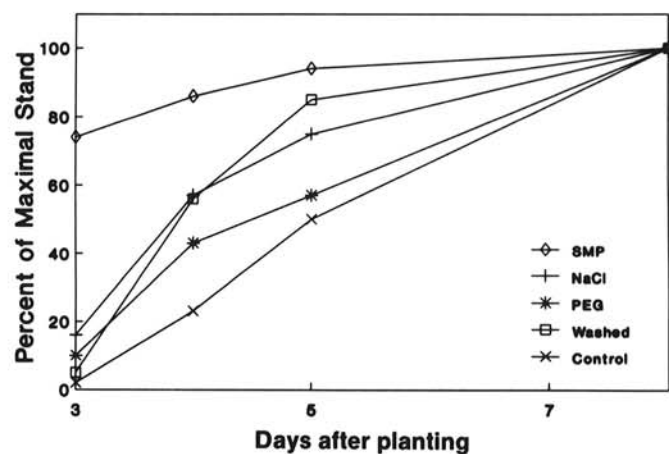


Fig. 1. Effect of various seed priming treatments on uniformity of sugar beet seedling emergence in uninfested soil. Maximal stand was the total number of seedlings that emerged within 8 days after planting. Uniformity of emergence was evaluated by determining the percentage of maximal stand that had emerged 3, 4, or 5 days after planting. Treatments included osmopriming with -1.5 MPa NaCl and -1.2 MPa polyethylene glycol (PEG) 8000, solid matrix priming (SMP), washing with tap water, and an untreated control. LSD ($P = 0.05$) for days 3, 4, and 5 were 12, 13, and 14%, respectively, and should be used only for pairwise comparisons.

(pericarps) cracked and seed began to germinate. Germinated seed died during drying. Seed viability of cracked but ungerminated seed was not affected, but such seeds are unacceptable to industry. Less than 1% of seed caps cracked when seeds were primed with 18–24 ml of H₂O, and seed primed with 22 ml of H₂O emerged as early as seed primed with 24–30 ml. Osmoprimered seed showed no sign of damage from either the NaCl or PEG 8000 treatment.

Emergence and seedling disease. In uninfested soil, SMP was superior to all other treatments with regard to 3-day stand establishment and rate of emergence (Table 1). Three days after planting, more than four times the seedlings emerged from SMP-treated than from NaCl-treated seed, which had the second highest emergence. The mean emergence rate of SMP-treated seed was significantly lower, signifying faster emergence, than all other treatments, and seedlings from all seed treatments emerged significantly faster over an 8-day period than the untreated control.

TABLE 2. Effect of seed priming on seedling emergence and disease incidence of sugar beet cultivar Tx18 in soil infested with *Pythium ultimum*

Treatment ^x	Emergence (%)			Damping-off ^y (%)	
	3 days	8 days	15 days	Pre-emergence	Post-emergence
Solid matrix priming	25.0 a ^z	63.3 a	47.2 a	36.7 a	25.9 a
NaCl	6.1 b	51.1 b	36.1 b	48.9 b	32.6 a
Polyethylene glycol (PEG)	3.3 b	48.9 b	33.9 b	51.1 b	32.9 a
Washed	0.6 b	15.6 c	9.4 c	84.4 c	42.0 a
Control	0.6 b	16.6 c	8.9 c	83.3 c	40.1 a

^xOsmoprimered seeds were incubated in -1.5 MPa NaCl or -1.2 PEG 8000 for 6 days at 15 C. Solid matrix priming was achieved by mixing 22.7 g of seed with 22.7 g of solid matrix and 22 ml of H₂O, incubating for 2 days at 15 C, and then drying for 3 days at 15 C. The washed treatment entailed washing seed in distilled water six times for 30 min per wash on a rotary shaker at 15 C.

^yPreemergence damping-off is based on 15 seeds planted in each of six replicates. Postemergence damping-off, calculated 15 days postplanting, is based on the total number of plants that emerged within 8 days after planting.

^zMeans followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

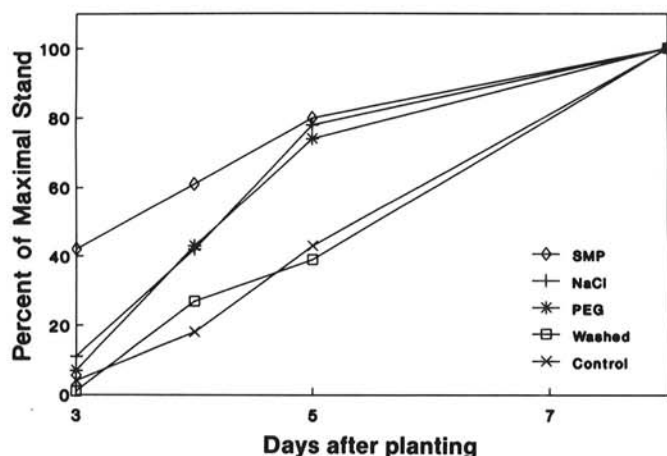


Fig. 2. Effect of various seed priming treatments on uniformity of sugar beet seedling emergence in soil infested with *Pythium ultimum*. Maximal stand was the total number of seedlings that emerged within 8 days after planting. Uniformity of emergence was evaluated by determining the percentage of maximal stand that had emerged 3, 4, or 5 days after planting. Treatments included osmoprimering with -1.5 MPa NaCl and -1.2 MPa polyethylene glycol (PEG) 8000, solid matrix priming (SMP), washing with tap water, and an untreated control. LSD ($P = 0.05$) for days 3, 4, and 5 were 12, 23, and 26%, respectively, and should be used only for pairwise comparisons.

Eight days after planting, there were few differences in stand, with only the washed treatment significantly greater than the untreated control.

In addition to earlier stand establishment, seedlings from SMP-treated seed also emerged more uniformly (Fig. 1). Three days after planting, 74% of the maximal stand achieved by the SMP treatment had emerged. There was no significant difference among the washed and osmoprimered treatments, and, other than SMP, only the NaCl treatment was significantly different from the control. Four days after planting, all treated seed had a significantly greater percentage of maximal stand emerged than the untreated control, but the SMP treatment still was significantly better than all others. There were no statistical differences among the washed treatment and SMP or NaCl by day 5, but these three were significantly better than PEG or the untreated control, which were not different from each other.

In infested soil, overall trends in stand establishment and uniformity of emergence were similar to those in uninfested soil. Again, seedlings from SMP-treated seed emerged earlier and more uniformly than seedlings from all other treatments. Three days after planting, 25% of seedlings from SMP-treated seed had emerged compared with only 6% in the next closest treatment (NaCl) (Table 2). Eight days after planting, SMP still had a significantly greater stand than all other treatments. Both osmoprimered treatments were better than the control, but the washed treatment, which had the greatest stand at day 8 in uninfested soil, was no better than the control. All priming treatments provided significantly greater stands 15 days after planting than either the washed treatment or untreated control, which were not significantly different from each other.

Most seedling disease was a result of preemergence damping-off (Table 2). All priming treatments significantly decreased the amount of preemergence damping-off compared with the untreated control, and the SMP treatment was best. Post-emergence damping-off ranged from 26% in the SMP treatment to 40% in the untreated control, but differences were not significant.

With regard to uniformity of emergence in infested soil, SMP-treated seed, with 42% of its maximal stand emerged by day 3, was significantly better than all other treatments (Fig. 2). However, this was significantly lower than the 74% of maximal stand at day 3 in uninfested soil, indicating a slowed rate of initial emergence in infested soil. There was no difference in the percentage of maximal stand emerged among any of the priming treatments by day 5, but all were greater than the washed treatment or untreated control. The difference in uniformity of emergence in infested and uninfested soils was greatest with the washed treatment. At day 5 in uninfested soil, emergence from the washed treatment had reached 85% of its maximal stand, but, in infested

TABLE 3. Emergence of sugar beet seedlings in uninfested soil 7 mo after initial priming treatment

Treatment ^x	Emergence (%)		MER ^y (days)
	5 days	8 days	
Solid matrix priming	50.0 a ^z	92.7 ab	5.5 a
NaCl	28.3 b	89.2 ab	5.9 b
Polyethylene glycol (PEG)	11.7 c	82.5 b	6.3 c
Washed	0.0 d	94.2 a	6.5 c
Control	0.0 d	82.5 b	6.9 d

^xOsmoprimered seeds were incubated in -1.5 MPa NaCl or -1.2 PEG 8000 for 6 days at 15 C. Solid matrix priming was achieved by mixing 22.7 g of seed with 22.7 g of solid matrix and 22 ml of H₂O, incubating for 2 days at 15 C, and then drying for 3 days at 15 C. The washed treatment entailed washing seed in distilled water six times for 30 min per wash on a rotary shaker at 15 C.

^yMER = mean emergence rate, which is $N + T_2N_2 + \dots + T_nN_n$ /total number of seedlings emerged, in which N = number of seedlings emerged and T = number of days.

^zMeans followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

soil, only 39% of maximal stand had emerged after the same amount of time. Only the washed treatment provided a significant difference in percentage of maximum stand emerged at day 5 in infested versus uninfested soil.

Longevity of priming. Seven months after treating, primed seed, stored at room temperature, still maintained its primed condition (Table 3). Five days after planting, all priming treatments provided significantly greater stands than the other two treatments, and SMP provided the greatest stand and fastest emergence of all. However, 8 days after planting, only washed seed had a significantly greater stand than the untreated control, but seedlings from all treatments emerged faster.

DISCUSSION

Seed priming has potential to improve stand establishment in sugar beet, and, in this study, SMP was the most effective treatment. In uninfested soil, seedlings from SMP-treated seed emerged earlier and more uniformly than seedlings from other treatments. In infested soil, SMP-treated seed had less preemergence damping-off and a greater final stand. Taylor et al (23) reported similar results with vegetable seed given SMP or osmopriming. Although SMP did not increase final stands, it increased the rate of emergence of carrot and onion compared to osmopriming with either PEG 8000 or KNO_3 . Also, tomato seedlings from seed primed with SMP or KNO_3 emerged faster than seed primed with PEG.

In Taylor's study, Agro-Lig, a ground Leonordite shale, was used as the solid component in the SMP process. After priming, the water potential of the Agro-Lig was determined, and >97% of the total water potential was attributed to osmotic potential due to solutes from the Agro-Lig and seed exudates (23). The clay mineral used in this study was an inorganic hydrous silicate, and the predominant water potential component was matric as opposed to osmotic (J. Easton, *personal communication*). Despite this difference, the basic methodology of SMP was similar between this study and Taylor's, and SMP was superior to osmopriming with either PEG 8000 or salt solutions in both studies.

A possible explanation for the superiority of SMP over osmopriming with regard to emergence variables may be improved aeration with SMP. When seeds are mixed with a solid material, such as the clay mineral used in this study, Agro-Lig, soft coal, sand, or vermiculite, the resultant mixture is friable and readily permeable to oxygen. However, when seeds are added to a viscous PEG solution, aeration is a major problem, and numerous researchers have reported unsatisfactory results when priming with PEG (1,11,16,17). Others have avoided the problem by designing special equipment in which seeds and PEG solution are vigorously aerated (1). Still, from industry's viewpoint, the logistical problems of traditional osmopriming methods are formidable (12,23).

A second possible reason SMP out-performed other priming treatments in this study could relate to the precision of treatment. Considerable time was spent in preliminary studies to determine the best combination of variables (i.e., time, water, temperature) to use with SMP. When osmopriming with NaCl or PEG 8000, the methods of Osburn and Schroth (19,20) were followed, and, possibly, these were not the best techniques with the cultivar used in this study. Murray et al (16-18) reported extreme variation in results when priming sugar beet seed with PEG of varied concentrations and for different durations. They determined that 3-7 days were optimal, but that variation could exist among cultivars and possibly seed lots, so preliminary testing would always be required (18). Thus, the comparatively poor performance of PEG-treated seed in this study in uninfested soil was not unexpected.

One of the more interesting aspects of this study was the variation in seed treatment effects on emergence variables in infested and uninfested soils. In both infested and uninfested soils, SMP-treated seed emerged earlier and more uniformly than seedlings from all other treatments. In uninfested soil, 74% of

the maximal stand already had emerged in 3 days, but only 41% of the maximal stand had emerged after 3 days in infested soil. The reduction in stand in infested soil was expected, but the negative effect on uniformity of emergence was not. Possibly, nonlethal infection of seedlings reduced vigor and rate of emergence, which, in turn, could have prolonged the emergence period.

The washed treatment exhibited the greatest difference between infested and uninfested soil. Sugar beet seeds contain germination inhibitors, which are leached out when seed is washed, and washing increases seedling emergence compared with untreated seed (16,17). In this study, in uninfested soil, washed seed provided a significantly greater stand after 8 days than all other treatments. However, in infested soil, the washed treatment was no better than the untreated control at any time.

Osburn and Schroth (19,20) reported that seedling infection by *P. ultimum* was related to exudation of carbohydrates during seed germination, and that rate of germination also was related to seed exudation. They found that sugar beet seed osmoprimed with NaCl or PEG 8000 germinated faster and had less infection by *P. ultimum* than untreated controls, and that both osmopriming treatments greatly reduced the amount and rate of carbohydrate exudation. In the study reported herein, SMP, osmopriming, and washing all resulted in increased rates of emergence compared with the untreated control. However, in infested soil, only seeds given the three priming treatments had significantly less preemergence damping-off than the untreated control. Conceivably, the washing treatment may have removed germination inhibitors and reduced carbohydrate exudation sufficiently to result in an increased rate of emergence in uninfested soil but not enough to prevent severe infection and damping-off in infested soil.

Because of the nature of the SMP treatment and its short duration compared with osmopriming, it seems unlikely that SMP treatment would reduce subsequent carbohydrate exudation more than osmopriming. However, seedlings from SMP-treated seed emerged faster in uninfested soil and had significantly less preemergence damping-off in infested soil than seedlings from other priming treatments. Probably, factors other than carbohydrate exudation affected performance of SMP-treated seed in infested soils.

Taylor et al (22) suggested that populations of indigenous bacteria on beet seed reduced damping-off caused by *P. ultimum*. Later, others (9,10) showed that the addition of biocontrol agents to seed during SMP with Agro-Lig resulted in increased stand establishment of several crop species in pathogen-infested soils. The combination gave better disease suppression than only SMP or seed treatment with biocontrol agents. These studies could lead to speculation that SMP enhances populations of indigenous microflora on sugar beet seed, resulting in reduced disease severity. Although this may have occurred, no data was obtained to support or reject the hypothesis. The effects of various priming methods on populations of indigenous microflora on sugar beet seed would be a reasonable research subject to pursue.

A third possible explanation for reduced preemergence damping-off in primed treatments is that, due to the increased rate of emergence, seedlings may have been able to escape lethal infection. Leach (13), using several host-pathogen combinations, found that "other factors being constant, the relative growth rates of host and pathogen determine to a considerable degree the severity of preemergence infection at different temperatures." The results of the present study definitely support Leach's conclusions.

Despite our lack of understanding of the mechanisms involved, seed priming, and SMP in particular, is effective in reducing disease incidence and improving stand establishment in soils infested with *P. ultimum*. SMP is a simple process, economically feasible, and environmentally sound. In addition, the longevity of the "primed condition" satisfies a key criterion of industry. Although SMP appears to be an effective technique for improving seedling vigor of numerous crops (6,23), the relative newness of the technique and the many questions concerning its mode of action and variability warrant continued research.

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