Effect of Osmopriming Sugar Beet Seed on Germination Rate and Incidence of *Pythium ultimum* Damping-off

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ABSTRACT

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Osmopriming sugar beet seed in NaCl or polyethylene glycol (PEG) solutions reduced *Pythium ultimum* preemergence damping-off by 50 and 65%, respectively, compared with nontreated seed when planted in naturally infested field soil. Disease control was comparable to or better than that provided by metalaxyl-treated seed. Addition of metalaxyl to NaCl or PEG osmoprimed seed resulted in an additive increase of disease control greater than any of the treatments alone. Damping-off was reduced 24.8 and 12.6% with NaCl and PEG osmoprimed seed treatments, respectively, compared with nontreated seed in one of two field trials. Seedling fresh weight was increased an average of 57.1 and 39.2%, respectively, in the two trials. The germination rate of both osmoprimed seed treatments was greater than that of nontreated seed at temperatures ranging from 9 to 27 C in petri dish assay. Similarly, seedling emergence rate was improved at 16–21 C in fumigated field soil. The viability and faster germination rate of osmoprimed seed were maintained at least 6 mo when stored at 21–24 C.

Sugar beet seed germination and seedling emergence are often slow and nonuniform, particularly under suboptimal environmental conditions such as low temperature or excessive soil moisture (4,14). Seed rot and damping-off caused by *Pythium ultimum* Trow occurs wherever the crop is grown in the western United States and can be particularly severe at suboptimal temperatures, causing reduced plant stands.

Osmoprimed seed treatments have been used with a variety of crops, including sugar beets and table beets (*Beta vulgaris* L.), to improve seed germination rate and to provide more uniform germination and emergence under suboptimal conditions (3–5,12). This typically results in better plant stands and yields under unfavorable conditions. Osmopriming is a presowing treatment where seeds are allowed to imbibe in an aerated osmotic solution such as polyethylene glycol (PEG) or various salts. The osmotic potential of the solution regulates the amount of water uptake by the seeds, enabling germination processes to proceed but preventing radicle emergence (6,9,10).

Durrant et al (5) found that osmopriming sugar beet seed in NaCl resulted in an increased seedling emergence rate and often an increased stand and yield in field trials. Khan et al (12) reported that osmopriming table beet seed in PEG also resulted in an increased seedling emergence rate, stand, and yield in the field. With the exception of the study by Taylor et al (18), however, the effect of osmopriming sugar beet, table beet, or seed of other crops on the incidence of seed or seedling diseases has not been reported. They found that osmopriming table beet seed in PEG or $MgSO_4$ resulted in the reduced incidence of damping-off by *Pythium* spp.

The objective of this study was to investigate the effect of osmopriming sugar beet seed in NaCl or PEG on the incidence of damping-off by *P. ultimum*. An examination was also made of the effect of osmopriming on seed germination and seedling emergence rates under varying environmental conditions.

MATERIALS AND METHODS

Preparation of osmoprimed seed. The sugar beet seed used throughout this study was cv. USH11, size 8–9, from Union Sugar Co., Santa Maria, CA. Seeds were osmoprimed in −1.5 MPa NaCl (0.34 M) or −1.2 MPa PEG 8000 (30.2 g 100 ml^{-1} H_2O) solutions according to Durrant et al (4) and Khan et al (12), respectively.

Seeds were initially washed with tap water before osmopriming to remove a germination inhibitor present in the sugar beet seed coat (1,2,8) and to remove soluble exudates. This consisted of six 30-min-cycle washings in an Erlenmeyer flask on a rotary shaker set at 150 rpm. The osmotica were subsequently added (1:5, w/v, of seed to osmoticum), and the seeds were shaken at 150 rpm for 6 days. Upon completion of osmopriming, the seeds were rinsed twice with tap water, 30 min per rinse cycle, then dried to the original water content. The wash, osmoprime, and rinse cycles were done at 15 C, and the seeds were dried at 21–24 C. After drying for 48–72 hr, the seeds were placed in plastic petri dishes in polyethylene bags and stored at 21–24 C.

Laboratory seed germination assays. The germination rate and final percentage germination of NaCl and PEG osmoprimed seed were assayed at 9, 15, 21, and 27 C and compared with those of nontreated seed. Fifty seeds were placed on three layers of Whatman No. 1 filter paper moistened with 3 ml of sterile distilled water in 100-mm-diameter glass petri dishes (7,16). Each treatment was replicated three times. The dishes were stacked in metal racks and sealed in polyethylene bags to prevent moisture loss. The seeds were incubated in the dark in growth chambers, and germination was assayed daily over 21 days. Seeds were considered to have germinated when the radicle protruded through the seed coat. The seed germination rate was expressed as T_{50} and T_{90}, the time (days) necessary to attain 50 and 90% of final germination.

The germination rate and final percentage germination of stored NaCl and PEG osmoprimed seed were assayed at monthly intervals over 6 mo. In each monthly assay, the seeds were incubated at 21 C on moistened filter paper in petri dishes previously described, and germination was assessed over 5 days. Other details of the experiment also were similar to those described above.

Greenhouse seedling emergence assay. The seedling emergence rate and final percentage emergence of NaCl and PEG osmoprimed seed were assayed in fumigated field soil at 15 and 21 C and compared with nontreated seed. The soil used in these experiments was an Oceano loamy sand (pH 7.2) from Moss Landing, CA. The soil was collected from the top 15 cm of the field and stored in polyethylene bags at 21 ± 2 C. The soil was air-dried, screened through a 5-mm-mesh screen, then fumigated with metham-sodium (Vapam) to control any potential diseases caused by soilborne fungal pathogens that would complicate assessment of the seedling emergence rate. The fumigant was applied to the soil in a portable cement mixer at a rate of 10 ml L^{-1} H_2O per 16 kg of soil. After application of the fumigant, the soil was stored in polyethylene bags for 1 wk, then air-dried on an open bench for an

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additional week before use.

Twenty-five seeds were planted 1 cm deep in 20 × 15 × 7 cm wood boxes, with three replications per treatment in a completely randomized design. Seedling emergence was recorded daily over 21 days. Seedlings were considered to have emerged once the hypocotyls protruded above the soil surface. The seedling emergence rate was expressed at T_{50} and T_{90} and as the mean rate of emergence (MRE) [MRE = (N) + T_2N_2 + . . . + T_nN_n/ total number of seedlings emerged, where N = number of seedlings emerged and T = day number], as adapted from Kotowski (13).

Greenhouse disease control assays. The effectiveness of NaCl and PEG osmoprimed seed treatments in reducing the incidence of *P. ultimum* damping-off was assayed in Oceano loamy sand field soil naturally infested with 75–100 germinable propagules per gram of soil (p g^{-1}) of the pathogen, as determined by the soil drop assay method of Stanghellini and Hancock (17). The incidence of damping-off was compared with that of nontreated seed and seed treated with metalaxyl. Metalaxyl was applied to seed as a slurry at a rate of 0.31 g a.i. kg^{-1} seed.

The soil was air-dried, screened through a 5-mm-mesh screen, then mixed in a portable cement mixer prior to use. Ten seeds were planted 1 cm deep in 10-cm-diameter ceramic pots with five replications per treatment in a completely randomized design. The pots were placed in a temperature-controlled greenhouse chamber with a daytime temperature of 15–21 C and nighttime temperature of 15 C. Disease was assessed by recording the percentage seedling stand daily over 21 days. Damped-off seedlings were collected and assayed on 2% water agar plates to verify that *P. ultimum* was the cause of disease.

Subsequent experiments were conducted under similar conditions to determine whether or not the combination of osmoprimed and metalaxyl seed treatments results in additive disease control compared with the individual treatments alone. Metalaxyl was applied to osmoprimed seed at the same rate as nonosmoprimed seed. Data were collected as previously described.

Field studies. The effectiveness of NaCl and PEG osmoprimed seed treatments in reducing the incidence of *P. ultimum* damping-off was further studied in two field trials in Brentwood silty clay loam soil. Other treatments included nontreated seed, metalaxyl-treated seed, and combined osmoprimed-metalaxyl seed treatments. The effect on seedling growth, measured in terms of fresh top weight, was also evaluated. The soil was naturally infested with 75–120 and 50–75 p g^{-1} of *P. ultimum* in the two trials, respectively. The soil inoculum densities of *P. ultimum* were determined from six soil samples collected from the top 15 cm of soil in each trial and assayed at the time of planting.

One hundred seeds were planted at a depth of 0.5–1.0 cm per 6.7-m single row plot, with a seed spacing of 2.5 cm. There were five replications per treatment in a randomized complete block design. The trials were furrow-irrigated the day after planting, then 5 and 22 days later. Seedling stand was assayed at weekly intervals for 4 wk. Damped-off seedlings were collected and assayed on 2% water agar plates to identify the cause of disease. The trials were terminated after 4 wk, and plant fresh weight was determined.

Table 1. Effect of osmopriming sugar beet seed on germination rate and final germination percentage over a range of temperatures

Treatment	Temperature (C)	Germination[x] (%)	T_{50}[x,y] (days)	T_{90}[x,y] (days)
Nontreated	27	88.0 a[z]	4.0 a	5.7 a
NaCl		93.3 a	2.0 b	2.0 b
PE		91.3 a	2.0 b	2.0 b
Nontreated	21	96.7 a	4.0 a	5.3 a
NaCl		92.0 a	2.0 c	3.0 b
PEG		90.0 a	3.0 b	3.3 b
Nontreated	15	95.3 a	5.0 a	7.0 a
NaCl		88.0 a	3.0 b	4.0 b
PEG		88.7 a	3.3 b	5.0 b
Nontreated	9	74.0 a	11.3 a	14.3 a
NaCl		77.3 a	6.0 c	8.0 b
PEG		77.3 a	7.0 b	8.0 b

[x] Mean of three replications, 50 seeds per replication, after 21 days of incubation on three layers of 9.0-cm Whatman No. 1 filter paper moistened with 3 ml of water in 10-cm-diameter glass petri dishes.
[y] T_{50} and T_{90} refer to the time necessary to attain 50 and 90% of final germination, respectively.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Effect of osmopriming sugar beet seed on seedling emergence rate and final percentage emergence at 16 and 21 C in fumigated soil[v]

Treatment	Temperature (C)	Seedling emergence[w] (%)	T_{50}[w,x] (days)	T_{90}[w,x] (days)	Mean rate of emergence[w,y] (days)
Nontreated	21	81.6 a[z]	4.6 a	12.4 a	6.6 a
NaCl		84.0 a	4.2 a	7.0 b	5.0 b
PEG		90.0 a	4.0 a	6.2 b	4.6 b
Nontreated	16	87.2 a	6.0 a	11.6 a	7.2 a
NaCl		84.0 a	4.8 b	6.6 b	5.5 b
PEG		88.0 a	4.6 b	5.6 b	4.9 b

[v] Experiment planted in Oceano loamy sand field soil fumigated with metham-sodium (Vapam). The fumigant was applied at a rate of 10 ml L^{-1} H_2O per 16 kg of soil.
[w] Mean of three replications, 25 seeds per replication, 21 days after planting.
[x] T_{50} and T_{90} refer to the time necessary to attain 50 and 90% of final emergence, respectively.
[y] Mean rate of emergence = (N) + T_2N_2 + . . . + T_nN_n/ total number of seedlings emerged, where N = number of seedlings emerged and T = day number.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

Laboratory seed germination assays. The T_{50} and T_{90} of seeds osmoprimed in either NaCl or PEG and incubated in optimal moisture conditions were significantly reduced 50 and 65%, respectively, compared with nontreated seed at 27 C (Table 1). Significant reductions of the T_{50} and T_{90} of NaCl or PEG osmoprimed seed were also observed when seed were assayed at 21, 15, and 9 C, ranging between 25 and 50% and 29 and 44%, respectively. There were no differences in final percentage germination between nontreated seed and osmoprimed seed at any of the four temperatures.

There was no detectable loss of viability or performance of NaCl or PEG osmoprimed seed over a 6-mo period. The reduction of T_{50} and T_{90} and the percentage germination of the osmoprimed seed treatments compared with nontreated seed assayed at 21 C in optimal moisture conditions were almost identical when seed was stored at 21–24 C and tested monthly. Each of the experiments above were repeated at least twice, with similar results.

Greenhouse seedling emergence assay. The T_{50}, T_{90}, and MRE of NaCl and PEG osmoprimed seed were significantly reduced 20–23, 43–52, and 25–32%,

respectively, compared with nontreated seed when planted in fumigated field soil at 15 C (Table 2). At 21 C, the T_{90} and MRE were also significantly reduced 44–50 and 24–30%, respectively, compared with nontreated seed. However, there was no difference in T_{50} between osmoprimed seed and nontreated seed at 21 C. There was no significant difference in percentage germination between osmoprimed seed and nontreated seed at either 15 or 21 C. This experiment was repeated twice with similar results.

Greenhouse disease control assays. Damping-off by *P. ultimum* was significantly reduced 33 and 38% with NaCl and PEG osmoprimed seed treatments, respectively, compared with nontreated seed when planted in naturally infested field soil (Table 3). The reduction of disease was comparable to that obtained with metalaxyl-treated seed. The improvement of the healthy seedling stand with NaCl and PEG osmoprimed seed resulted from a significant reduction of preemergence damping-off, which was the principal form of the disease caused by *P. ultimum*. Disease control with metalaxyl-treated seed, however, resulted from a reduction of both preemergence and postemergence damping-off. Addition of metalaxyl to both NaCl and PEG osmoprimed seed resulted in an additive increase in disease control significantly greater than metalaxyl or either osmoprimed seed treatment alone (Fig. 1). Disease control with the combined treatments resulted from an even greater reduction of preemergence damping-off than that observed with the individual treatments and from control of postemergence damping-off. Both of the experiments above were repeated at least twice with similar results.

Field studies. The percentage stand of healthy seedlings was significantly increased 24.8 and 12.6% with the NaCl and PEG osmoprimed seed treatments, respectively, compared with nontreated seed in the first of two field trials (Table 4). The reduction of disease with the osmoprimed seed treatments resulted from a significant decrease of preemergence damping-off, which was the principal form of disease that occurred. Damping-off of seedlings from metalaxyl-treated seed, however, was not significantly different from damping-off of nontreated seed. The addition of metalaxyl to osmoprimed seed did not significantly improve disease control compared with osmoprimed seed alone. *P. ultimum* was isolated from over 75% of 100 damped-off seedlings that were assayed, and *Rhizoctonia solani* Kühn was isolated from nearly 20%. None of the seed treatments significantly increased the percentage stand compared with nontreated seed in the second trial, in which less disease occurred as a result of the lower *P. ultimum* inoculum density (Table 4).

Seedling fresh weight was significantly increased 50.0 and 34.9% with the NaCl and PEG osmoprimed seed treatments, respectively, compared with nontreated seed in the first field trial and 64.1 and 43.5%, respectively, in the second trial. Treatment of seed with metalaxyl did not significantly affect plant growth. The increased seedling fresh weight with PEG osmoprimed seed also was not significantly changed by the addition of metalaxyl. However, addition of metalaxyl to NaCl osmoprimed seed resulted in a significant reduction of plant growth compared with NaCl osmoprimed seed alone in one of two field trials.

DISCUSSION

P. ultimum preemergence damping-off of sugar beets was markedly reduced when either NaCl or PEG osmoprimed seed were used, and disease control was comparable to or better than that provided by metalaxyl-treated seed. When combined, the osmoprimed and fungicide seed treatments were complementary and resulted in even greater reduction of disease. The only time that metalaxyl-treated seed was not comparable to osmoprimed seed in disease control occurred in the field tests. This apparently was due to *R. solani*, which causes damping-off and is not affected by metalaxyl. This also indicates an advantage from using osmoprimed seed in that it reduces disease by both *Pythium* and *Rhizoctonia*.

The substantial increase of seedling vigor that occurred from osmopriming was an additional benefit. In the field, seedling fresh weight in two tests averaged 57.1 and 39.2% greater with NaCl and PEG osmoprimed seed, respectively, than nontreated seed. This supports the findings of Durrant et al (4,5), who reported that osmopriming sugar beet seed in NaCl increased plant growth and final sugar yield. They

Table 3. Effect of osmopriming sugar beet seed on incidence of *Pythium ultimum* damping-off[w]

Treatment	Healthy seedling stand[x] (%)	Preemergence damping-off[x] (%)	Postemergence damping-off[x] (%)
Nontreated	20 b[y]	60 a	20 a
Metalaxyl[z]	60 a	40 b	0 c
NaCl	52 a	32 b	16 ab
PEG	54 a	38 b	8 bc

[w] Experiment planted in Oceano loamy sand field soil naturally infested with 75–100 propagules g^{-1} soil of *P. ultimum*.
[x] Mean of five replications, 10 seeds per replication, 21 days after planting.
[y] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Metalaxyl (Apron 25W) applied at a rate of 0.31 g a.i. kg^{-1} seed.

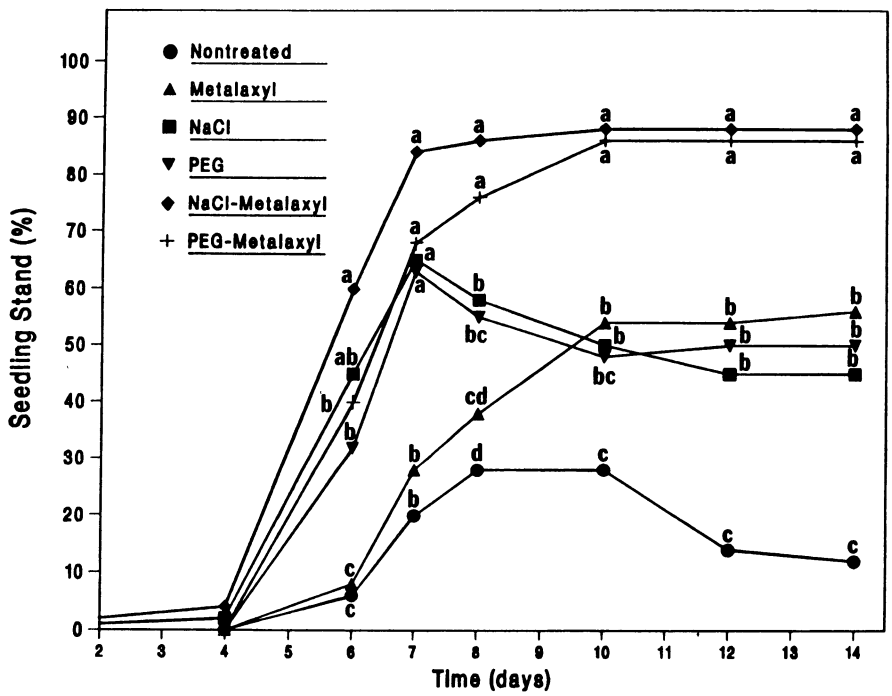

Fig. 1. The effect of combined osmoprimed-fungicide seed treatments on the incidence of *Pythium ultimum* damping-off of sugar beets. Means at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Effect of osmopriming sugar beet seed on incidence of *Pythium ultimum* damping-off and plant growth under field conditions[u]

Treatment	Healthy seedling stand[v] (%)	Increase in plant growth vs. control[v,w] (%)
Trial 1[x]		
Nontreated	42.2 c[y]	··· b
Metalaxyl	41.8 c	−13.2 b
NaCl	67.0 ab	+50.0 a
NaCl/metalaxyl[z]	71.2 a	+39.6 a
PEG	54.8 b	+34.9 a
PEG/metalaxyl	56.2 b	−32.1 a
Trial 2[x]		
Nontreated	54.8 ab	··· c
Metalaxyl	47.2 b	−1.1 c
NaCl	63.8 a	+64.1 a
NaCl/metalaxyl	66.4 a	+34.8 b
PEG	55.2 ab	+43.5 ab
PEG/metalaxyl	59.2 ab	+23.9 bc

[u] Both trials were planted in Brentwood silty clay loam soil at the University of California, Davis.
[v] Mean of five replications, with 100 seeds planted per 6.7-m single row plot, 4 wk after planting.
[w] Plant growth measured in terms of fresh top weight after 4 wk.
[x] *P. ultimum* inoculum densities were 75–120 and 50–75 propagules g^{-1} soil in trials 1 and 2, respectively.
[y] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Metalaxyl (Apron 24W) applied at a rate of 0.31 g a.i. kg^{-1} seed.

attributed the increased plant growth and yield to increased seedling emergence rate. The data presented here, however, suggest that it may have also resulted in part from disease control. Subsequent studies (15) revealed that control of preemergence damping-off of osmoprimed seed was related to reduction of the rate and incidence of seed colonization by *P. ultimum*. This in turn was related to reduced exudation of nutrients from the primed seeds upon imbibition of water.

The germination rate of both osmoprimed seed treatments was markedly improved over a broad temperature range, and both the rate and percentage germination of NaCl osmoprimed seed were better in excess moisture conditions compared with nontreated seed. With the exception of the superior performance of NaCl osmoprimed seed in excess moisture conditions, the osmoprimed seed treatments did not differ significantly from each other in their effect on seed germination or seedling emergence rates, seedling growth, or disease control. The greatly increased germination rate of osmoprimed seed under suboptimal environmental conditions should allow their planting under conditions that would not normally be conducive for rapid stand establishment. This is important with sugar beets in the western United States because early plantings are generally related to higher yields (11).

Data showing that osmopriming sugar beet seed results in a markedly reduced incidence of damping-off, in addition to its effect on seed germination rate and plant growth, provide further reasons for exploring the use of osmoprimed seed in agriculture. The prospects of obtaining additive disease control with a combined osmoprimed-fungicide seed treatment is also attractive, particularly where a crop is planted to stand. The additive disease control offered by the combined seed treatment could potentially result in improved disease control over a broader range of environmental conditions than currently possible with fungicide seed treatment alone.

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