

Effect of Osmopriming Sugar Beet Seed on Exudation and Subsequent Damping-off Caused by *Pythium ultimum*

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ABSTRACT

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Control of preemergence damping-off of sugar beets, caused by *Pythium ultimum*, with NaCl- or polyethylene glycol (PEG)-osmoprimed seed planted in naturally infested field soil was related to a reduction of the rate and incidence of seed colonization by the pathogen. Incidence of colonization of untreated seed was 23.3 and 86.7% by 12 and 24 hr, respectively, whereas colonization of NaCl- and PEG-osmoprimed seed was reduced to 0 and 3.3%, respectively, after 12 hr, and 13.3 and 16.7%, respectively, after 24 hr. Reduced colonization by *P. ultimum* was related to decreased exudation from osmoprimed seed upon imbibition with water. The amount of carbohydrate exuded from NaCl- and PEG-osmoprimed seed was reduced 98.1 and 91.7%, respectively, after 60 min, and 41.5 and 55.0%, respectively, after 48 hr of incubation in water compared with

untreated seed. The rate of exudation from osmoprimed seed over 48 hr was correlated with the rate of germination, whereas untreated seed did not germinate during the 48-hr incubation period. Disease reduction with NaCl-osmoprimed seed was nullified when the seeds were incubated in exudates before planting. Bacterial population densities of 10^8 – 10^9 cfu ml⁻¹ occurred in the NaCl or PEG osmopriming solutions after 6 days of seed treatment, and residual population densities on seed were 10^4 – 10^5 cfu seed⁻¹. The bacterial populations have potential to affect the incidence of damping-off. The incidence of damping-off with NaCl-osmoprimed bacteria-free seed was 20–30% less than with unprimed seed, whereas reductions ranged from 20–54% when bacteria were present on the primed seed.

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 (6,8). Osmopriming seed typically increases the rate, uniformity, and percentage of seed germination, resulting in improvement of stand and often yield (3,4,11). Osmoprimed seed is available commercially for some small-seeded vegetables and also has been produced experimentally for numerous field crops such as sugar beet (*Beta vulgaris* L.) and grain crops (2). Osmoprimed seed is usually of greatest benefit under environmental conditions that are suboptimal for seed germination and seedling emergence, such as cool, wet conditions.

Seed rot and damping-off diseases can be particularly severe in California during adverse conditions that slow germination and emergence. Under such conditions, osmopriming sugar beet seed in either NaCl or PEG markedly reduced the incidence of preemergence damping-off caused by *Pythium ultimum* Trow, and disease control was comparable to that obtained with fungicide-treated seed (18). Osmopriming seed may affect seed rot or damping-off disease by reducing the amount of nutrient exudation by the seed before germination (17). There is considerable evidence that seed or seedling diseases are affected by the level of seed exudation (5,10,14,15,19,20,22,23). However, Taylor et al (29) attributed reduced incidence of *Pythium* damping-off of osmoprimed table beet seed (*Beta vulgaris* L.) to indigenous bacteria present on the seed that multiplied in the osmoticum during seed treatment rather than to reduced exudation from the osmoprimed seed.

The objective of this study was to determine the effect of osmopriming on the rate and incidence of sugar beet seed colonization by *P. ultimum*. An examination also was made of the differences in amount of exudation from osmoprimed and untreated seed, and the role of reduced seed exudation and bacterial microflora in the osmopriming solutions in decreasing the incidence of seed colonization by *P. ultimum* and disease. A preliminary report was published previously (17).

MATERIALS AND METHODS

Soil. All experiments were done in an Oceano sandy loam soil (pH 7.2) from Moss Landing, CA. Soil was collected from the top 15 cm of the field and stored in polyethylene bags at 21 ± 2 C. For greenhouse experiments, soil was air-dried, sieved through a 5-mm-mesh screen, and mixed in a portable cement mixer. The protocol was similar for growth chamber experiments except that the soil was sieved through a 1-mm-mesh screen. Soil inoculum densities of *P. ultimum* were determined by the soil drop assay method of Stanghellini and Hancock (26) and were expressed as germinable propagules per gram of soil.

Preparation of osmoprimed seed. The sugar beet cultivar used throughout this study was USH11, seed 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 of H₂O) solutions according to Durrant et al (3) or Khan et al (11), respectively. Seeds first were subjected to six 30-min washings with tap water in an Erlenmeyer flask on a rotary shaker set at 150 rpm. Osmotica subsequently were added (1:5 w v⁻¹ of seed to osmoticum), and the seeds were shaken at 150 rpm for 6 days. On completion of osmopriming, seeds were rinsed twice with tap water, 30 min per rinse, and then dried to the original water content. Washes, osmopriming, and rinsing were done at 15 C, and seeds were air-dried at 21–24 C. After drying for 48–72 hr, seeds were placed in plastic petri dishes in polyethylene bags and stored at 21–24 C.

Effect of osmopriming on the incidence of seed colonization. Colonization of NaCl- and PEG-osmoprimed seed by *P. ultimum* was determined at 12-hr intervals over a 48-hr period and compared with untreated seed and seed treated with metalaxyl. Metalaxyl was applied to seed as a slurry at 0.31 g a.i. kg⁻¹ of seed. Ten seeds were planted 1 cm deep in a 5.5-cm-diameter \times 4.5-cm-depth brass ring containing Oceano field soil naturally infested with 17 propagules per gram of *P. ultimum*. There were three replications of each treatment per time interval. The rings were placed on a -50 kPa ceramic pressure plate, and soil matric potential was adjusted to -15 kPa with a pressure plate extraction system. Rings were transferred onto plastic petri dish lids and covered with polyethylene plastic held in place by a rubber band to maintain a constant soil moisture level. They were incubated in a growth chamber at a constant 16 C with a 12-hr diurnal fluorescent

light cycle. At the end of each time interval, seeds were recovered from the soil by wet sieving, surface-disinfested in 0.5% sodium hypochlorite for 1 min, rinsed with tap water, and plated on 2% water agar plates for identification of seed colonization by *P. ultimum*. Plates were incubated at ambient temperature for 18–24 hr, then observed for seed colonization under a dissecting microscope. Data were expressed as the percentage of seeds colonized by *P. ultimum*. After an analysis of variance, the significant differences among treatment means were determined by Duncan's multiple range test.

Effect of osmopriming on seed exudation. The amount of soluble exudate released from NaCl- and PEG-osmoprimed seed incubated in water was compared with that from untreated seed. The significant differences among treatment means were determined by Duncan's multiple range test following an analysis of variance of data. As a measure of the relative amount of exudate released, the carbohydrate fraction was assayed for various periods of time up to 60 min (1, 5, 10, 30, and 60 min) and 48 hr (6, 12, 24, 36, and 48 hr) with the anthrone colorimetric method (9). For each assay, a 2-g quantity of seed was placed in 20 ml of sterile distilled water containing 500 $\mu\text{g ml}^{-1}$ of vancomycin and polymyxin B sulfate (Sigma Chemical Co., St. Louis, MO) in a 125-ml Erlenmeyer flask and shaken at 150 rpm on a rotary shaker at 16 C. There were three replications of each treatment per time interval. Resultant exudate solutions were filtered through a single layer of Whatman No. 1 filter paper, filter-sterilized through a disposable 0.2 μm filter (Gelman Sciences Inc., Ann Arbor, MI), then assayed for carbohydrate content. Additionally, percent seed germination also was determined because additional exudates released from germinating seeds would affect the total amount of carbohydrate detected. The relatedness of seed exudation to seed germination was analyzed by measuring the coefficient of correlation.

Bacterial population density in osmopriming and on seed. The internal and external bacterial population density of untreated seed was assayed to determine the initial density before osmopriming. Population density was assayed by triturating three replicate 10-seed samples in 10 ml of 10 mM phosphate buffer (pH 7.0) and serially diluting. One-hundred-microliter aliquots of each dilution were plated on tenth-strength tryptic soy agar (Difco Laboratories, Detroit, MI) and incubated for 48 hr at 28 C. Resultant bacterial counts were expressed as colony-forming units (cfu) per seed. The density of bacteria in the NaCl and PEG osmopriming solutions then was determined at the end of 6 days of seed treatment. Three replicate 1-ml samples were removed and serially diluted in phosphate buffer. The dilutions were plated as described above, and the bacterial counts were expressed as colony-forming units per milliliter of solution. A determination also was made of the residual population density present on and in the osmoprimed seed after rinsing the seed free of osmoticum and drying. The population density was assayed as described above, and the bacterial counts were expressed as colony-forming units per seed.

Role of reduced exudation from osmoprimed seed in disease control. The effect of reduced exudation from osmoprimed seed in decreasing the incidence of damping-off by *P. ultimum* was investigated in greenhouse experiments comparing NaCl-osmoprimed seed with that of a second NaCl-osmoprimed seed treatment that was treated with seed exudate following osmopriming. The exudate used for treatment of seed was collected by placing 40 g of untreated sugar beet seed in 100 ml of water and shaking on a rotary shaker at 150 rpm for 2 hr at 16 C. The resultant exudate solution was clarified by centrifugation at 12,000 g (10,000 rpm) for 10 min, and filter-sterilized with a disposable 0.2- μm , 115-ml-capacity filter (Nalge Co., Rochester, NY). Ten grams of NaCl-osmoprimed seed was incubated in 50 ml of exudate solution and shaken at 150 rpm for 60 min at 16 C. Seeds then were redried at ambient temperature to the original water content.

The amount of carbohydrate exuded from the NaCl-osmoprimed/exudate seed treatment upon subsequent rewetting was compared with that from unamended NaCl-osmoprimed seed and untreated seed. Exudates were collected from 2-g quantities of

seed incubated in 20 ml of sterile distilled water plus antibiotics for 60 min. Other details of the experimental design and methods for assay of carbohydrate content were similar to those previously described. The bacterial population density on and in NaCl osmoprimed/exudate seed also was determined to assess whether treatment of the seed with exudate affected the population density relative to that present on and in unamended NaCl-osmoprimed seed. The methods used for determination of the bacterial population density were similar to those previously described.

Experiments relating exudation and disease were done in Oceano field soil naturally infested with 75–100 propagules per gram of *P. ultimum*. Disease control was compared with metalaxyl-treated seed. Ten seeds were planted 1 cm deep in 10-cm-diameter ceramic pots containing Oceano soil. There were five replications per treatment in a completely randomized experimental design. Pots were placed in a temperature-controlled greenhouse chamber, with a daytime temperature of 16–21 C and a nighttime temperature of 16 C, and were watered once daily. Disease incidence was determined daily for 21 days by recording the percent stand of healthy seedlings. Damped-off seedlings were collected and assayed on water agar plates to verify the presence of *P. ultimum*.

Role of indigenous bacterial microflora in disease control. To investigate the effect of osmopriming sugar beet seed on the incidence of disease in the absence of bacteria, an additional NaCl osmopriming treatment was prepared in which the antibiotics vancomycin, polymyxin B sulfate, and chlorotetracycline (Sigma Chemical Co., St. Louis, MO) were added to the priming solution at a rate of 250 $\mu\text{g ml}^{-1}$ to inhibit bacterial growth. Other details of the methods for seed treatment were similar to those previously described.

The resultant density of bacteria in the NaCl-antibiotic solution upon completion of seed treatment and the population density present on and in the osmoprimed seed were determined as previously described to assess whether the antibiotics effectively inhibited bacterial growth. Carbohydrate exudation from seeds osmoprimed in the NaCl-antibiotic solution also was assayed to determine whether presence of the antibiotics during seed treatment altered the subsequent amount of exudate released into solution on rewetting compared with that from seeds osmoprimed in their absence. Exudates were collected from 2-g quantities of seed incubated in 20 ml of sterile distilled water plus antibiotics for 60 min. Other details of the experimental design and methods for assay of carbohydrate content were similar to those previously described. The possible phytotoxic effect of the antibiotics on subsequent germination of the osmoprimed seed and seedling emergence was examined in greenhouse experiments. Seeds osmoprimed in solutions with and without antibiotics were planted in 10-cm-diameter ceramic pots containing pasteurized University of California (UC) soil mix (1). Other details of the experimental design were similar to those previously described. Seedling emergence from treated seed was compared with untreated seed by recording the percentage seedling stand daily over a 3-wk period.

The effectiveness of the NaCl-antibiotic seed treatment in reducing the incidence of damping-off by *P. ultimum* was compared with seed osmoprimed without antibiotics in greenhouse experiments. Seeds were planted in Oceano field soil infested with 75–100 propagules per gram of *P. ultimum*, and disease control was compared with metalaxyl-treated seed. The experimental design and method for assessment of disease were similar to those previously described.

The same seed treatments described above also were compared for their effectiveness in reducing the incidence of seed colonization by *P. ultimum* over a 48-hr period in growth chamber experiments. Treatment effect on seed colonization was compared with metalaxyl-treated seed. The experimental design and method for assessment of seed colonization were similar to those previously described.

RESULTS

Effect of osmopriming on incidence of seed colonization. *P.*

ultimum colonized 23.3 and 86.7% of the untreated sugar beet seeds within 12 and 24 hr, respectively, after planting in naturally infested Oceano field soil (Fig. 1). The incidence of colonization of NaCl- and PEG-osmoprimed seed was reduced to 0 and 3.3%, respectively, after 12 hr, and 13.3 and 16.7%, respectively, after 24 hr. The substantial reduction of colonization of osmoprimed seed compared with untreated seed was maintained through the 48-hr assay period. Only about 4% of the metalaxyl-treated seed was colonized by *P. ultimum* during the 48-hr period. This experiment was repeated twice with similar results.

Effect of osmopriming on seed exudation. More than 50% of the carbohydrate exuded from untreated seed during a 60-min incubation period in water occurred during the first minute (Fig. 2). Carbohydrate exudation from NaCl- and PEG-osmoprimed seed was reduced 100 and 96.4%, respectively, at 1 min, and 98.1 and 91.7%, respectively, at 60 min compared with untreated seed. The difference in carbohydrate exudation between osmoprimed seed and untreated seed decreased with time over 48 hr. However, carbohydrate exudation from NaCl- and PEG-osmoprimed seed still was reduced 41.5 and 55.0%, respectively, compared with untreated seed after 48 hr (Fig. 3).

Percent germination of NaCl-osmoprimed seed at 6, 12, 24, 36, and 48 hr was 0, 22.1, 29.2, 50.3, and 52.4%, respectively, and that of PEG-osmoprimed seed at the same time intervals was 0, 4.2, 11.3, 16.1, and 20.2%, respectively. Untreated seed did not germinate when incubated in water for 48 hr. The rate of exudation from NaCl- and PEG-osmoprimed seed over 48 hr was closely correlated with the rate of germination ($r = 0.97$ and 0.96 , respectively). Each of the assays described above was repeated once with similar results.

Bacterial population density in osmopriming solutions and on seed. Bacterial densities of 10^8 – 10^9 cfu ml⁻¹ occurred in both the NaCl and PEG osmopriming solutions after 6 days of seed treatment. Residual population densities of 10^4 – 10^5 cfu seed⁻¹ were present on and in NaCl- and PEG-osmoprimed seed after rinsing the seed free of the osmoticum and redrying to the original water content. Untreated seed harbored a bacterial population density of 10^1 – 10^2 cfu seed⁻¹.

Role of reduced exudation from osmoprimed seed in disease control. Treatment of NaCl-osmoprimed seed with seed exudate after osmopriming increased the amount of carbohydrate exuded on drying and subsequent rewetting from 40 to 150 μ g of glucose

equivalents per gram of seed without appreciably affecting the bacterial population density on and in the seed. This was compared to 550 μ g per gram of seed with untreated seed. Accordingly, there was no difference in the incidence of damping-off by *P. ultimum* when comparing the NaCl-osmoprimed/exudate seed treatment and untreated seed (Table 1). This experiment was repeated once with similar results.

Role of the indigenous bacterial microflora in disease control. The antibiotics in the osmopriming solutions effectively inhibited bacterial growth. They did not affect seed exudation nor rate of seedling emergence. No fungi were noted in the osmopriming solutions.

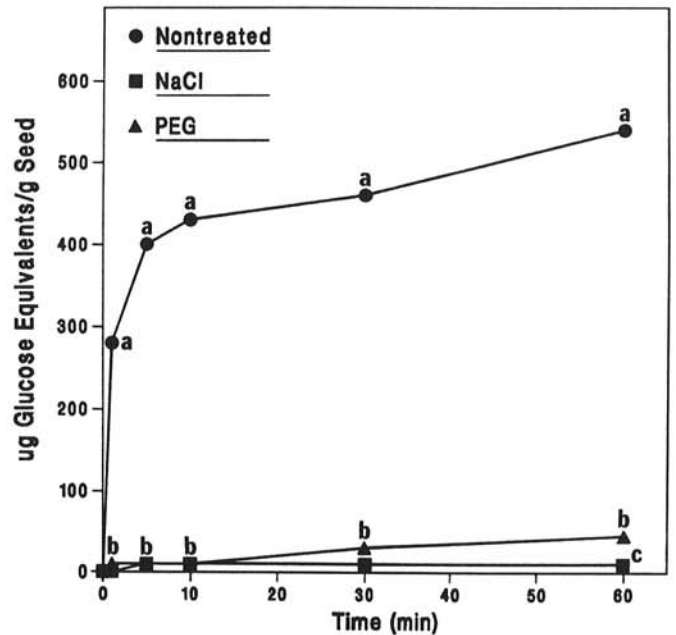


Fig. 2. Exudation of carbohydrate from NaCl- and PEG-osmoprimed seed and untreated seed over a 60-min period. Means at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

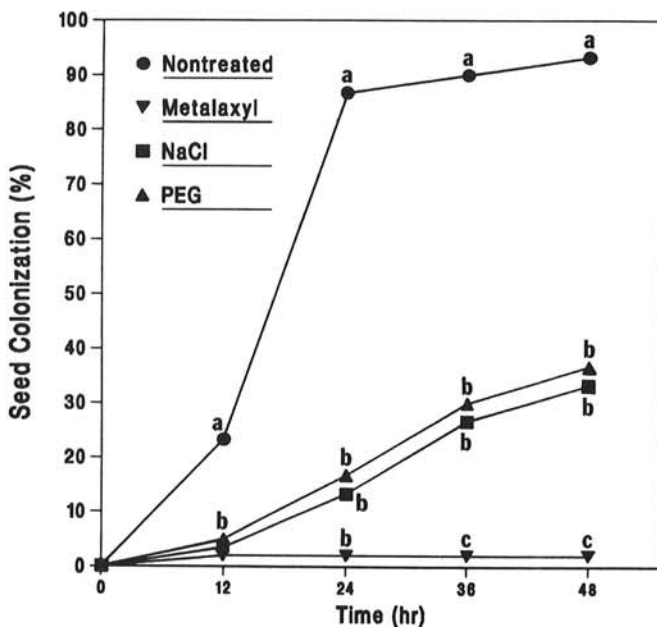


Fig. 1. Effect of osmopriming sugar beet seed on the incidence of seed colonization by *Pythium ultimum*. Means at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

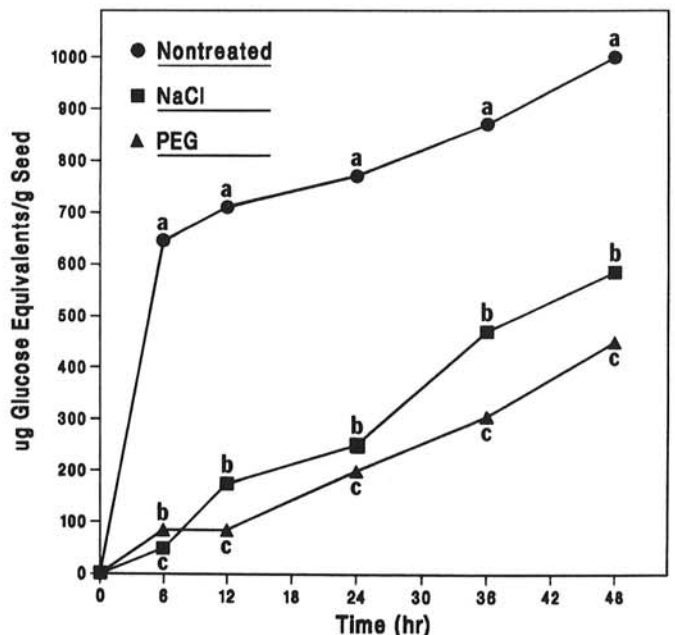


Fig. 3. Exudation of carbohydrate from NaCl and PEG osmoprimed seed and untreated seed over a 48-hr period. Means at a given sampling time with the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

The incidence of damping-off by *P. ultimum* with the NaCl-osmoprimed seed free of bacteria was 20–30% less than with untreated seed. This level of control was comparable to the effect of seed treatment with metalaxyl. When bacteria were present in the NaCl osmopriming solution, and on and in seed on subsequent drying, reduction of damping-off ranged from 20 to 54% compared with untreated seed in eight experiments. However, the results were highly variable. The reduced incidence of damping-off was better ($P = 0.05$) than with bacteria-free seed in only three of eight experiments.

The incidence of colonization by *P. ultimum* of NaCl-osmoprimed seed free of bacteria over 48 hr was greatly reduced compared with untreated seed to an extent similar to that shown in Figure 1. The reduction of colonization was comparable to that observed with NaCl-osmoprimed seed when bacteria were present on and in the seed. The experiment was repeated once with similar results.

DISCUSSION

The control of preemergence damping-off of sugar beet by *P. ultimum* with NaCl- or PEG-osmoprimed seed was related to a reduction of seed colonization by the pathogen. This is consistent with the etiology of the disease in California soils, since the seed is the primary site of initial colonization by *P. ultimum* (18). Developing seedlings usually remain healthy if the seeds escape colonization. Reduced seed colonization by the pathogen appeared to be the consequence of decreased exudation from the osmoprimed seed upon imbibition with water. This was supported by the finding that disease reduction with osmoprimed seed was nullified when they were incubated in exudates before planting.

The amount of seed exudation is particularly important because sporangia of *P. ultimum* can germinate within 1.5 hr in response to nutrients diffusing from imbibing seeds, with maximal germination occurring within 3–4 hr in soil contiguous to the seed (27). In addition, *P. ultimum* can colonize or infect seeds in as short a time as 4 hr (18), with 100% colonization or infection frequently occurring within 24 hr (12,13,16,18,27,28). Decreased exudation from osmoprimed seed should cause a reduction of the size of the spermosphere (25,30). Under such conditions, there would be less exudate to activate the fungus, thereby resulting in reduced seed colonization. Similarly, Short and Lacy (21) reported that chlamydospores of *Fusarium solani* f. sp. *pisi* germinated only in the millimeter of soil adjacent to presoaked pea seed, compared with 7 mm with untreated seed.

The greatly reduced exudation from osmoprimed seed upon incubation in water resulted from a leaching of soluble exudates

TABLE 1. Effect of exudation from osmoprimed seed on incidence of damping-off of sugar beet caused by *Pythium ultimum*

Treatment	Healthy seedling stand ^u (%)	Carbohydrate exudation (µg glucose equivalents/g seed) ^v
Untreated	28 b ^w	550
Metalaxyl ^x	72 a	550
NaCl ^y	62 a	40
NaCl + exudate ^{y,z}	30 b	150

^u Mean of five replications, 10 seeds per replication, 21 days after planting.

^v Exudates were collected from 2-g quantities of seed incubated in 20 ml of sterile distilled water containing 500 µg ml⁻¹ of vancomycin and polymyxin B sulfate for 60 min and assayed by the anthrone colorimetric method.

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

^x Metalaxyl applied at a rate of 0.31 g a.i. kg⁻¹ of seed.

^y Bacterial population density on and in NaCl-osmoprimed and NaCl-osmoprimed + exudate seed treatments were 1.1×10^5 and 9.4×10^4 cfu seed⁻¹, respectively.

^z Exudate solution used for treatment of seed prepared by placing 40 g of seed in 100 ml of water and collecting after 2 hr. Seeds were treated with exudate by incubating 10 g of seed in 50 ml of exudate solution for 1 hr, then redrying to the original water content.

from the seed during the washing and osmopriming phases of seed treatment. The original purpose of the wash phase with both sugar beet and table beet seed was to remove a germination inhibitor present in the seed coat (3,11). However, the removal of the germination inhibitor was accompanied by the loss of other soluble exudates. Simon and Harun (24) previously reported that each time a seed is dried and subsequently rewetted, the seed will release exudates. The data presented here, however, show that the amount of exudate released from a rewetted seed can be greatly reduced compared with untreated seed.

The presence of bacteria in the osmopriming solutions, and on and in the osmoprimed seed, sometimes was associated with significantly improved disease control. This inconsistency was not surprising because of the variability in nature and size of the bacterial populations. Improved disease control may have resulted from the ability of the bacteria to use nutrients exuded from the osmoprimed seed, further reducing the amount of exudate diffusing into the surrounding soil. Thus, the bacterial microflora in osmopriming solutions need to be considered, especially since it also is possible that some populations could have a deleterious effect.

Although this study shows that osmopriming seed reduces seed colonization and damping-off by *P. ultimum*, the results likely are applicable to other seed and seedling pathogens. A reduction in exudation from osmoprimed seed also should affect colonization by other pathogens of sugar beet, such as *P. aphanidermatum* or *Rhizoctonia solani*, which require a food base for germination and infection.

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