Beneficial Effects of Solid Matrix Chemo-priming in Okra

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ABSTRACT

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Two field trials at Stillwater and Bixby, OK, evaluated the efficacy of solid matrix priming techniques, alone or in combination with fungicide seed treatment on seedling emergence and reduction of damping-off of okra in field soil naturally infested with *Pythium ultimum*. The following treatments were evaluated: thiram + carboxin (chemo-primed) (commercially applied), biological seed treatment (bio-primed) (*Trichoderma harzianum* isolate OK-110, 1 g suspended in 1% carboxymethylcellulose [CMC]), untreated seed (control), and a 1% CMC control. Chemo-primed seeds had a more uniform and faster emergence compared with untreated seeds at both field sites. Within 3 days, 92 and 78% of chemo-primed seeds had emerged at Stillwater and Bixby, respectively, compared with 84 and 71% emergence in the untreated control. Mean emergence of chemo-primed seeds was lower ($P \leq 0.05$) than the untreated control. Chemo-primed seeds had greater vigor ($P \leq 0.05$) at both locations compared with either fungicide-treated or priming alone, at both locations. There were no differences ($P \leq 0.05$) in yield among treatments at both locations. *P. ultimum* was consistently isolated from damped-off seedlings and surrounding soil at both locations. Isolates of *P. ultimum* were more pathogenic on okra in laboratory tests than isolates of *Rhizoctonia* spp., *Fusarium* spp., and other *Pythium* spp. also isolated from seed or soil.

Additional keywords: bio-priming, emergence rate, seed vigor

Okra is planted on about 260 ha (650 acres) in Oklahoma and has a value of \$2 million annually (J. E. Motes, Department of Horticulture & Landscape Architecture, Oklahoma State University, Stillwater, *personal communication*). Delayed and erratic germination creates problems with fertilizer utilization, postemergence weed control, and uniform harvesting (16). The hard seed coat of okra is a major physiological constraint to uniform stand establishment and performance (12,16). Environmental factors such as inadequate or excessive soil moisture, low soil temperatures, crusting of soil, and preemergence damping-off caused by *Pythium* spp. also contribute to erratic germination of okra seed.

Since environmental factors often cannot be controlled, modification of the seed by either biological, chemical, or physiological techniques should be evaluated to improve the vigor of the seedling and uniformity of stand. Solid matrix priming (SMP) is a technique developed by John Eastin (7). In SMP, a solid matrix is used to regulate water imbibition by seeds. SMP

alone (15) or in combination with fungicides (2,9) or biological agents (4,8) has improved the rate and uniformity of emergence of vegetable seeds and reduced damping-off diseases (8). We evaluated 10 solid carriers as priming agents to improve seedling vigor of okra (13) and pine (18). We found that clay carriers consistently improve seedling vigor compared with humic acid or cellulosic formulations. In this study, we evaluated combinations of seed priming with either fungicide or biological treatments to reduce damping-off disease in field-planted okra seed. The term chemo-priming is used to indicate that fungicide-treated seeds rather than untreated seeds were used in the priming process. In bio-priming, untreated seeds were primed and 1 g of biological formulation was applied to the seeds using 1% carboxymethylcellulose (CMC).

MATERIALS AND METHODS

Chemo-priming. Fifty okra seeds cv. Clemson Spineless with the standard fungicide treatment consisting of thiram plus carboxin, 113.4 g per 45.4 kg (4 oz per 100 lb) of seed (commercially applied by Abbott & Cobb Seed Co., 4120 West Pecan St., McAllen, TX) were placed in a self-sealing polyethylene bag containing the SMP agent clay-carri-all (CCA) (crystalline quartz, pH 7.4; American Colloid Co., Arlington Heights, IL). Water had been added to the SMP to provide 50% water content (-0.7 mPa water potential). The

ratio of SMP to seeds was 3:1 (wt/wt). Treatments were replicated four times for a total of 200 seeds. The contents of the bag were thoroughly mixed, and the bags were incubated at room temperature for 3 days. These amounts and conditions had been established in previous research (13). Seeds were separated from the SMP agent by sieving, allowed to dry overnight, and placed in paper bags for transport to the field.

Bio-priming and seed coating. Fifty untreated okra seeds cv. Clemson Spineless (Abbott and Cobb Seed Co.) were primed with heat-treated CCA (140°C , 24 h) at 50% water content (-0.7 mPa water content). After priming, seeds were spread on aluminum foil and dried overnight on a lab bench and then coated with a formulation of *Trichoderma harzianum* isolate OK-110 from soil in Stillwater, OK, by suspending 1 g of the formulation in 1% CMC. A dry formulation of *T. harzianum* (6) was used which contained a mixture of conidia, hyphal fragments, and chlamydospores and provided approximately 10^6 CFU/ml. Four replications of 50 seeds each were prepared and dried overnight. The CMC control was prepared by coating untreated seeds in 1% CMC and drying overnight.

Field trials. Planting was conducted at two sites to evaluate six seed treatments: 1, untreated seed; 2, fungicide-treated seed (thiram + carboxin); 3, primed seed with no fungicide; 4, chemo-primed seed; 5, bio-primed seed; and 6, primed seed coated with 1% CMC. The first planting site was at the plant pathology research farm in Stillwater, and the second site was at the Department of Horticulture & Landscape Architecture Vegetable Research Experiment Station at Bixby, OK. Each treatment had 50 seeds with four replications, and treatments were arranged in a completely randomized design. The plots were disked, and fertilizer was applied according to standard agricultural practices for okra as recommended by the Oklahoma State University soil testing laboratory. Plots consisted of 24 rows 5 m long separated by 2-m alleys. At both locations, seeds were planted with a manually operated belt seeder calibrated to place the seed at a depth of 4 cm. Seeds were planted on 17 May at Stillwater and on 19 May at Bixby. Emerging seeds were marked with plastic coffee stirrers. Seeds were considered emerged when hypocotyls were above the soil surface. Subplots, 3 m long, were established in each row 3 to 4 weeks after

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emergence by thinning plants to leave approximately 15 plants per row. These subplots were harvested repeatedly by hand for yield data (number and weight of pods per subplot). Thus, any difference in yield after thinning could not be attributed to stand variation. There were 14 to 15 picks for harvest at each location. Okra was harvested by hand at intervals of 2 to 3 days at both locations, and the number and weight of Okra was recorded from subplots.

Isolation of fungi from seedlings and soil. Diseased okra seedlings and surrounding soil were collected randomly from all plots. Seedlings were washed under running tap water for 5 min to remove all soil debris, rinsed with sterile water, blotted dry with sterile paper towels, and roots were plated onto potato dextrose agar (PDA). Representative fungal colonies were hyphal-tipped to selective media for identification. Pathogenicity of isolates was tested in growth chambers (16/8 h, light/dark cycle, 25°C). Plastic pots, 7.6 cm (3 in), were filled with soilless mix (Fafard Potting Soil Mix, Conrad Fafard, Inc., Aquawam, MA) with 15% moisture. A centrally located hole was made with a

dibble, and a 0.5-cm agar plug of selected fungi was placed at the bottom of the hole and covered with a layer of mix. One untreated okra seed was placed into the hole and covered. Additional watering was from the bottom as needed.

Soil samples were air-dried before use and processed separately. For isolation of *Pythium*, 2 g from each sample was added to 38 ml of tepid (0.3%) water agar amended with calcium chloride (0.368 g per 100 ml of agar adjusted to pH 5.5 with phosphoric acid). The suspension was thoroughly mixed, and 1 ml was spread onto the surface of *Pythium*-selective agar (5). After 24 h of incubation at 25°C in the dark, the surface of the agar in each petri dish was gently washed in a stream of tap water to remove the soil suspension and bacterial growth. Representative colonies were transferred to PDA for verification of *Pythium*. Species determination was accomplished by removing plugs from actively growing cultures and transferring to water containing sterilized blades of grass (17) to induce sporangia and oospore formation. The key to species by Van der Plaats-Niterink (17) was used for species determination.

Emergence. Seedling emergence was expressed as the mean of emergence (ME) (14).

$$ME = (N) + T_2N_2 + \dots + T_nN_n / \text{total number of seedlings emerged}$$

where N = total number of seedlings emerged on T = day number.

An index of seed vigor (11) expressed as germination vigor was also calculated.

$$VI = \frac{G1 + G2 + \dots + GL}{D1 + D2 + \dots + DL}$$

$G1$ = number of germinants (first count)

$D1$ = number of days to first count

$G2$ = number of germinants (second count)

$D2$ = number of days to second count

GL = number of germinants (last count)

DL = number of days to last count

All data from the experiments were converted to vigor index and ME values and subjected to an analysis of variance. Where appropriate, means were separated with a Duncan's multiple range test ($P \leq 0.05$). Higher values of the vigor index indicate better vigor and lower values for ME indicate faster emergence.

RESULTS

Field trials. In general, stand at both field locations was excellent because of favorable growing conditions and little disease pressure. Chemo-primed seeds, at both locations, had faster emergence and greater seedling vigor compared to the other treatments (Tables 1 and 2).

Seedling establishment occurred during an 8-day period. Seedlings of chemo-primed seeds emerged more uniformly at both locations, and at Stillwater 92% of the stand from these seeds was established within the first 3 days. Chemo-primed seeds had greater ($P \leq 0.05$) stand at the 3-day count compared with untreated seeds at Stillwater (Table 1). There were no differences in stand among primed, unprimed, bio-primed, CMC treated, or untreated control seeds at either location. ME values for the chemo-primed treatment were lower ($P \leq 0.05$) than for untreated seeds. At both sites, okra was hand harvested at 2- to 3-day intervals (14 to 15 picks) from subplots of each treatment. There were no differences ($P \leq 0.05$) in yield among treatments.

Pythium ultimum was consistently isolated from seedlings and soil and was the only fungus tested that was pathogenic on okra in growth chamber inoculations. Isolates of *Rhizoctonia solani* and *Fusarium* spp. from soil were weakly pathogenic in growth chamber tests. Other *Pythium* spp. and miscellaneous fungi (*Trichoderma* spp., *Mucor* spp., *Rhizopus* spp., and *Chaetomium* spp.) isolated from seed or soil were not pathogenic.

DISCUSSION

Seed hydration techniques were developed as early as 1919 (10) to improve the

Table 1. Effects of seed treatment on mean emergence (ME), vigor index, and stand of okra at Stillwater, OK

Treatment ^y	Stand (%)		Vigor index ^z	ME (days)
	3 days ^z	8 days ^z		
Chemo-primed	91.5 a	88.5 a	54.3 a	1.9
Primed	84.5 b	80.5 b	45.8 bc	2.1
Treated	91.5 a	87.5 a	48.6 b	2.0
Untreated	84.1 b	87.5 a	44.8 bc	2.2
CMC	81.5 b	86.0 a	43.4 bc	2.2
Bio-primed	80.0 b	77.0 b	42.5 c	2.2
LSD ($P \leq 0.05$)	...	...	...	0.2

^y Except for untreated control, all others were primed with the solid matrix clay-carri-all (CCA) at 25°C. CMC = carboxymethylcellulose. Treatments included: 1, chemo-primed, primed commercially applied fungicide-treated seed, fungicides (thiram + carboxin); 2, primed, no fungicide applied to seed; 3, treated, fungicide-treated seeds, no priming; 4, untreated, seed with neither fungicide treatment nor priming; 5, CMC, primed seeds coated with 1% CMC; 6, bio-primed seeds coated with a formulation of *Trichoderma harzianum* (1 g) suspended in 1% CMC.

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

Table 2. Effects of seed treatment on mean emergence (ME), vigor index, and stand of okra at Bixby, OK

Treatment ^y	Stand (%)		Vigor index ^z	ME (days)
	3 day ^z	8 day ^z		
Chemo-primed	77.5 a	85.0 a	48.4 a	2.4
Primed	72.0 ab	81.0 ab	38.5 b	2.6
Treated	67.0 abc	83.5 a	33.0 bc	3.2
Untreated	71.0 abc	77.0 ab	33.1 bc	3.1
CMC	60.0 bc	69.5 b	37.0 bc	2.7
Bio-primed	59.0 c	70.0 b	30.4 c	3.2
LSD ($P \leq 0.05$)	...	...	...	0.6

^y Except for the untreated control, all others were primed in clay-carri-all (CCA) at 25°C. CMC = carboxymethylcellulose. Treatments included: 1, chemo-primed, primed commercially applied fungicide-treated seed, fungicides, (thiram + carboxin); 2, primed, no fungicide applied to seed; 3, treated, fungicide-treated seeds, no priming; 4, untreated, seed with neither fungicide treatment nor priming; 5, CMC, primed seeds coated with 1% CMC; 6, bio-primed seeds coated with a formulation of *Trichoderma harzianum* suspended (1 g) in 1% CMC.

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

rate and uniformity of seed germination. Osmotic or liquid priming was developed (3) using an osmotic solution such as potassium nitrate or polyethylene glycol (PEG) to regulate imbibition of water. The water potential of the osmoticum is adjusted in such a manner to allow enough water to be imbibed by the seeds to complete pregermination metabolic activities, but to prevent the emergence of the radicle. Osmotic priming has improved the rate and uniformity of seed germination and reduced damping-off (3,16). However, its utility is limited by aeration problems with seeds due to the viscous nature of PEG, phytotoxicity of some salts, large volumes of solutions needed, and problems of temperature control of the osmoticum. In order to ameliorate these problems, a new method of priming termed solid matrix priming using lignite was developed by John Eastin (7). Problems with this lignite system: Patent protection and solubility of water lead to our research to evaluate additional solid matrix substrates.

Erratic germination of okra has been reported since 1960 (1). It has been suggested (12,16) that the problem of a hard seed coat of okra was reversible and an increase in the moisture content of okra seed had beneficial effects in emergence and uniformity in the field. Erratic germination and emergence of okra seed can be ameliorated by chemo-priming. Within 3 days after planting, 78 and 92% of seeds had emerged at Stillwater and Bixby, respectively, compared with 71 and 84% of the untreated controls. Chemo-primed seeds had higher vigor indices and lower mean emergence values than all other treatments. Bio-primed seeds were not as effective as chemo-primed seeds, perhaps indicating that our isolate of *T. harzianum* was not an effective biocontrol agent for *P. ultimum*.

The term bio-priming has been used by at least three research groups, and each group has used a different technique to achieve bio-priming. Harman and Taylor (8) added their biocontrol agents directly to the SMP, which allowed the *T. harzianum* to colonize the seeds during the priming process. They also achieved improved stand of tomato in *Pythium*-infested soil. Callan et al. (4) added a suspension of the

bacterium *Pseudomonas fluorescens* to 1.5% methylcellulose coated, surface-sterilized sweet corn seed prior to hydrating the seeds between moistened paper towels or germination blotters. Seeds were hydrated for 20 h, which allowed the bacterium to colonize the seed coat. This treatment protected seeds from *P. ultimum* damping-off at a level comparable to treatment with the fungicide metalaxyl. Our bio-priming treatment consisted of two steps—SMP followed by the addition of our biocontrol agent suspended in 1% CMC—and did not include a period of time for colonization before drying the seed. Harman and Taylor (8) noted that there was a four- to six-fold decrease in CFU per seed during the drying process.

We had noted that several SMP sources had fungal saprophytes associated with them that interfered with germination of seed after the priming process, but we found that we could eliminate these contaminating organisms by sterilizing the SMP at high temperature (140°C, 24 h). We believe that one of the benefits of chemo-priming is the prevention of colonization of seed coats by saprophytic organisms which reduce seed performance during and after the priming process.

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