Germination of *sh2* Sweet Corn following Seed Disinfestation, Solid-matrix Priming, and Microbial Seed Treatment

T.K. Hartz and J. Caprile¹

Department of Vegetable Crops, University of California, Davis, CA 95616

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Abstract. Sweet corn (*Zea mays* L.) cultivars carrying the *sh2* mutation show poor seed vigor under stressful field conditions, requiring higher seeding rates to ensure stand establishment. The effects of sodium hypochlorite seed disinfestation, solid matrix priming (SMP), and seed-coating with *Gliocladium virens* Miller, Giddens & Foster to enhance emergence of *sh2* sweet corn in controlled-environment cold stress tests and field trials were investigated. In combination with a chemical fungicide seed treatment (captan, thiram, imazalil, and metalaxyl), SMP significantly improved the percentage and rate of seedling emergence of 'Excel' and 'Supersweet Jubilee' in a cold stress test (in soil for 7 days at 10C, then 15C until emergence) but was inconsistent under field conditions, improving emergence in only one of four field trials. Sodium hypochlorite disinfestation was ineffective. Compared to a film-coated control, coating seeds with *G. virens* strain G-6 was highly effective in increasing emergence in two of three cultivars tested in cold stress tests in two soils, while strain G-4 was generally ineffective. In field trials, G-6 treatment significantly increased emergence over that of nontreated seed but was inferior to conventional fungicide treatment and conferred no additional benefit in combination with fungicide treatment. Overall, no seed treatment evaluated was an economically viable alternative for or supplement to chemical fungicide treatment. Chemical names used: *cis-N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide* (captan); *tetramethyl-thiuram disulfide* (thiram); *1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole* (imazalil); *N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester* (metalaxyl).

Supersweet sweet corn cultivars (those carrying the *sh2* mutation) now dominate the fresh-market sweet corn industry in the United States due to extended shelf life and excellent eating quality. However, seedling emergence and vigor are negatively affected by the mutant *sh2* gene (Cantliffe et al., 1975). Stand establishment is problematic when soil conditions, particularly temperature, are suboptimal. Poor seedling vigor of *sh2* cultivars has been attributed to several factors. The small endosperm has minimal nutrient reserves (He and Burris, 1992; Wann, 1980). Rate of imbibition and electrolyte leakage are high, which may enhance susceptibility to preemergence damping-off (Parera and Cantliffe, 1991). Seed lots are frequently infected with *Fusarium moniliforme* J. Sheld. and *Penicillium oxalicum* Currie & Thom (Mohan and Wilson, 1990). Also, cracks in the pericarp of *sh2* kernels allow pathogen entry into the endosperm and embryo (Styer and Cantliffe, 1983).

Chemical, physiological, and microbial seed treatments to improve *sh2* seedling emergence have been investigated extensively. Fungicide seed treatments significantly improved

sweet corn seedling emergence (Callan et al., 1991; Parera and Cantliffe, 1990, 1994); seed treatments with a combination of broad-spectrum fungicides are used commercially. Parera and Cantliffe (1991) reported that sodium hypochlorite was an effective seed disinfestant. Presowing hydration (Bennett and Waters, 1987; Gerber and Caplan, 1989), osmopriming (Murray, 1990), and solid-matrix priming (SMP) (Harman and Taylor, 1989; Parera and Cantliffe, 1990, 1994) have improved *sh2* germination. Callan et al. (1990, 1991) reported that applying *Pseudomonas fluorescens* Migula, alone or in combination with seed priming, reduced seedling loss to *Pythium ultimum* Trow and increased field establishment of *sh2* corn. Harman and Taylor (1989) showed similar results using *Trichoderma harzianum* Rifai.

The success of biological and physiological seed treatments in improving seedling emergence has been highly variable. We conducted this study to determine the effects of SMP, sodium hypochlorite, and application of microbial protectants on *sh2* corn seedling emergence under field conditions representative of the central California sweet-corn industry. These techniques were chosen for evaluation because they showed the greatest potential for commercialization.

Materials and Methods

Experiments were performed at the Univ. of California–Davis (UCD) and in commer-

cial sweet-corn fields near Brentwood, Calif., during 1992 and 1993. Commercial seed lots of 'Excel', 'Supersweet Jubilee' (both years), and 'How Sweet It Is' (HSII) (1993 only) were used.

Seed disinfestation. Seeds were soaked for 15 min in 0.05% a.i. solution of sodium hypochlorite (NaOCl) at 22 ± 2C, then triple-rinsed with tap water (Parera and Cantliffe, 1991), and air-dried for at least 2 days before planting.

SMP. Seed, finely ground Leonardite shale (Agro-Lig, American Colloid Co., Arlington Heights, Ill.), and distilled water with 0.1% Tween 80 were thoroughly blended (Taylor et al., 1988) at a 1.0:1.5:0.85 (w/w/v) ratio and were incubated for 48 h at 20 ± 1C. After treatment, the seed was screened to remove most of the shale particles and air-dried at 22 ± 2C for at least 2 days before sowing. Where combinations of NaOCl disinfestation and SMP were used, NaOCl treatment preceded SMP.

A preliminary test in field soil showed poor emergence by SMP- and/or NaOCl-treated seed in the absence of fungicide protection; all SMP and NaOCl treatments described in this study were in conjunction with the industry standard combination fungicide treatment (captan, thiram, imazalil, and metalaxyl at 1.25, 1.25, 0.9, and 0.5 g a.i./kg seed).

Biological treatment. Conidia of strains G-4 and G-6 of *Gliocladium virens* on granulated moist millet seed were obtained from C. Howell, Texas A&M Univ., College Station. These organisms have shown promise as biological seed treatments (Howell, 1991; Howell et al., 1993). Seeds were coated with an aqueous solution of film-forming polymer (Opadry AG Blue, Colorcon, West Point, Pa.) into which the *Gliocladium* inoculum was mixed. Seed then were forced-air-dried at 25C in a rotating drum. The dried coating comprised ≈2% of seed weight. The amount of each *Gliocladium* strain added to the coating material was controlled to deliver ≈30,000 colony-forming units per coated seed.

Controlled-environment stress tests. SMP and NaOCl treatments were evaluated in Feb. 1992 by planting into moist field soil in plastic boxes; the soil (Yolo clay loam), collected from a fallow field at UCD in which corn had been grown the previous summer, was prechilled to 10C. After planting, the boxes were kept at 10C for 7 days and then transferred to 15C. Germination counts were taken

Table 1. Effect of sodium hypochlorite (NaOCl) and solid-matrix priming (SMP) on seedling emergence of *sh2* corn in a 1992 controlled-environment stress test.^z

Treatment	Emergence (%)		
	Cultivar		
	Excel	Jubilee	T ₅₀ ^y
Fungicide (F)	68 b ^x	74 b	14.9 b
F + NaOCl	73 ab	73 b	15.2 b
F + SMP	83 a	90 a	13.5 a
F + NaOCl + SMP	81 a	89 a	13.5 a

^zSeven days at 10C, then 14 days at 15C.

^yMean days to emergence, across cultivars.

^xMean separation within columns by Duncan's multiple range test at *P* ≤ 0.05.

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¹Current address: Univ. of California Cooperative Extension, Contra Costa County.

daily for 14 days at 15C. *Gliocladium* treatments were similarly evaluated in Apr. 1993 using soil from two UCD fields in which stand establishment tests were being conducted concurrently.

Field trials. Four field trials evaluating SMP and NaOCl were conducted in 1992 and 1993, two at UCD and two in commercial sweet-corn fields near Brentwood, Calif. Planting dates were 3 and 15 Mar. (Brentwood) and 31 and 22 Mar. (UCD) in 1992 and 1993, respectively. March is the beginning of the traditional planting season for *sh2* cultivars in central California. Replicates of 100 seeds were planted in a clay loam (Brentwood) or a sandy loam (UCD) soil in a randomized complete-block design, with four (Brentwood) or five (UCD) replications per treatment/cultivar. Sprinkler irrigation was applied at UCD; no irrigation was used in Brentwood.

Two trials for evaluating *G. virens* treatments were conducted at UCD in 1993. Replicates of 100 seeds were planted into a clay loam (soil 1; 2 Apr.) and a sandy loam (soil 2; 12 Apr.). Sprinkler irrigation was applied in both trials. The design was a randomized complete block with five replications per treatment/cultivar. Stand counts were taken at 2- to 4-day intervals until emergence was complete. All statistical analyses of emergence data were conducted following arcsine-transformation. Emergence rate was determined by the time required for 50% (T_{50}) of the seedlings to emerge (Alvarado et al., 1987).

Results and Discussion

The controlled-environment stress test showed that SMP, with or without NaOCl, improved the percentage and rate of seedling emergence compared to the fungicide-only control (Table 1). The NaOCl treatment did not improve emergence over fungicide alone.

In 1992 field trials, SMP treatment improved emergence in both cultivars tested in the Brentwood trial but was ineffective at UCD (Table 2). NaOCl alone was effective only on 'Excel' at Brentwood, while NaOCl disinfestation before SMP gave no benefit beyond that shown by SMP alone. Percent emergence in both field trials was substantially lower than in the stress test, despite a less severe temperature regime. Only small differences were measured in T_{50} due to treatment. In 1993 field trials, emergence from seed without fungicide was lower than for all other treatments (Table 2). SMP improved emergence of 'Supersweet Jubilee' compared to the fungicide control in Brentwood but had no effect at UCD. At either site, NaOCl was ineffective.

Gliocladium strain G-6, applied in a film coating (FC), significantly improved emergence compared to the film-coated control in two of three cultivars tested in both soils in the stress tests (Table 3). G-4 did not improve emergence over film-coating alone. All samples treated with fungicides had high emergence and neither G-6 nor G-4 improved emergence further. In field trials, both strains were inferior to the fungicide combination in in-

creasing emergence (Table 4). The film coating had a marginal effect on emergence, significantly decreasing the stand of HSII only in sandy loam. Neither strain improved emergence of fungicide-treated seed.

Parera and Cantliffe (1990, 1994) reported that the SMP and NaOCl combination was a viable alternative to fungicide treatment for enhancing *sh2* sweet-corn germination. Our preliminary results of the cold stress test suggest that SMP + NaOCl is not a useful treatment without the addition of fungicides. This discrepancy could relate to the difference in SMP media and technique or to the more

extreme temperature conditions of our stress test. Therefore, we concentrated on evaluating SMP and NaOCl in conjunction with the commercial standard combination fungicide treatment. Also, based on the experience in other crops, commercialization of these treatments most likely would be in conjunction with chemical fungicide treatments.

SMP was effective in increasing seedling emergence in the laboratory cold stress test but showed inconsistent results in field trials, with statistically significant benefit across cultivars only in Brentwood in 1992. That trial also had the earliest planting date, resulting in the

Table 2. Effect of sodium hypochlorite (NaOCl) and solid matrix priming (SMP) on seedling emergence of *sh2* corn in field trials.

Treatment	Emergence (%)						T_{50} ^y	
	Location						Location	
	Brentwood, Calif.			UCD ^z				
	Cultivar			Cultivar				
	Excel	HSII ^x	Jubilee	Excel	HSII ^x	Jubilee	Brentwood	UCD
1992								
Fungicide (F)	15 c ^w		28 b	33 a		43 a	17.9 b	11.1 ab
F + NaOCl	32 b		32 b	32 a		38 a	17.5 b	11.4 b
F + SMP	55 a		43 a	43 a		35 a	16.9 a	10.8 a
F + NaOCl + SMP	43 a		43 a	37 a		39 a	16.9 a	11.1 ab
1993								
Nontreated	16 c	5 b	10 d	21 b	8 b	16 b	12.8 c	14.1 b
F	32 ab	25 a	29 bc	35 a	28 a	38 a	12.8 c	13.7 b
F + NaOCl	30 b	28 a	25 c	37 a	25 a	38 a	12.3 b	13.8 a
F + SMP	32 ab	28 a	38 a	33 a	26 a	37 a	12.2 b	12.9 a
F + NaOCl + SMP	37 a	30 a	34 ab	32 a	30 a	40 a	11.8 a	12.3 a

^zUCD = Univ. of California-Davis.

^yMean days to emergence, across cultivars.

^xHSII = 'How Sweet It Is'.

^wMean separation within columns and years by Duncan's multiple range test at $P \leq 0.05$.

Table 3. Effect of coating seeds with *Gliocladium virens* strains G-4 and G-6 on seedling emergence of *sh2* corn in a 1992 controlled-environment stress test.^z

Treatment	Emergence (%)					
	Soil type					
	Clay loam			Sandy loam		
	Cultivar			Cultivar		
	Excel	HSII ^y	Jubilee	Excel	HSII ^y	Jubilee
Nontreated	33 c ^x	11 c	23 b	48 d	11 c	13 c
Fungicide (F)	79 a	88 a	93 a	86 a	88 a	88 a
Film-coated (FC)	55 bc	13 c	18 b	50 cd	10 c	21 bc
G-4 + FC	31 c	14 c	28 b	65 b	10 c	31 b
G-4 + FC + F	89 a	78 ab	85 a			
G-6 + FC	76 ab	65 b	85 a	63 bc	75 b	90 a
G-6 + FC + F	85 a	81 a	85 a			

^zSeven days at 10C, then 14 days at 15C.

^yHSII = 'How Sweet It Is'.

^xMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 4. Effect of coating seeds with *Gliocladium virens* on seedling emergence of *sh2* corn in 1992 field trials.

Treatment	Emergence (%)			
	Clay loam		Sandy loam	
	Cultivars		Cultivars	
	Excel	HSII ^z	Excel	HSII ^z
Nontreated	31 c ^y	18 c	26 de	9 c
Fungicide (F)	49 a	44 a	42 ab	37 a
Film-coated (FC)	32 bc	13 c	28 cd	4 d
G-4 + FC	33 bc	18 c	21 e	8 c
G-4 + FC + F	51 a	41 a	40 ab	36 a
G-6 + FC	36 b	29 b	33 bc	19 b
G-6 + FC + F	49 a	41 a	44 a	34 a

^zHSII = 'How Sweet It Is'.

^yMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

slowest emergence rate. These results agree with those of Parera and Cantliffe (1994) who found SMP ineffective in improving field performance of fungicide-treated *sh2* seed. Where SMP did improve emergence in our study, it may have been related to the increased speed of emergence of SMP-treated seed, which limited time of exposure to soil or seedcoat pathogens. Enhanced speed of germination with SMP or other priming techniques has been consistently reported. The bulky nature of and high seeding rate typical for sweet corn would make SMP an expensive treatment in relation to seed cost. Given the inconsistent field performance, SMP does not appear to be a commercially viable treatment as long as chemical fungicides remain available.

The NaOCl treatment generally was ineffective in improving seedling emergence. The NaOCl treatment we used had significantly reduced fungal pathogen infection of *sh2* seedcoats (Parera and Cantliffe, 1991). The lack of effectiveness of NaOCl suggested that seedcoat infection was not a significant factor in limiting emergence in our study. Other investigators (Headrick and Pataky, 1989; Schoen and Kulik, 1977) reported that *F. moniliforme*, the most common pathogen infesting *sh2* corn seedcoats, was a minor factor in stand establishment.

Gliocladium virens strain G-6 generally provided some seedling protection in laboratory stress tests and field trials, while G-4 was generally ineffective. Both strains are effective biocontrol agents but with different mechanisms of action (Howell, 1991; Howell et al., 1993). G-4 produced the antibiotic gliovirin and was effective in suppressing *P. ultimum*, while G-6 produced gliotoxin and suppressed *Rhizoctonia solani* Kuehn. While G-6-treated seed performed at or near the level of fungicide-treated seed in the laboratory trials, it was relatively less effective in the field trials. Howell (1991) reported synergism between *G. virens* and metalaxyl treatment. We did not find synergism, undoubtedly due to the broad-spectrum action of the fungicide combination used.

Other investigators reported significant improvement of *sh2* corn stand establishment with biological seed treatments. Harman and Taylor (1989) found *T. harzianum* suppressed *P. ultimum*-induced damping-off. Callan et al.

(1990, 1991) showed similar activity with *P. fluorescens*. The weakness in biological seed treatment has been that no agent has been identified that has activity against the range of soil pathogens commonly encountered. Conceptually, a combination of organisms may provide the broad-spectrum suppression of damping-off organisms provided by the fungicide combination treatment, but this suppression has not been demonstrated under representative field conditions.

In field trials, seedling emergence was consistently lower than that achieved in the laboratory cold stress tests, even though field temperature regime was less extreme than the laboratory test. Factors other than temperature and soil pathogen population, notably soil crusting, aeration, and moisture content, appear to play an important role in limiting *sh2* corn stand establishment. Presowing seed treatments are unlikely to consistently reduce the effects of these factors.

SMP and NaOCl were not consistently effective in improving *sh2* corn seedling emergence, either alone or in combination with a combination fungicide treatment. *Gliocladium virens* strain G-6 showed promise as a biological seed treatment but was less effective than the chemical fungicide combination under field conditions.

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