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Cold Test and Saturated Cold Test Reliability for Testing Carryover Corn Seed Treated with Seed-applied Insecticides

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A. Susana Goggi,* Daniel Curry, and Jeff Daniels

ABSTRACT

The cold test germination percentage of carryover seed corn lots treated with a seed-applied insecticide (SAI) can be below the seed industry's sale standard. However, the same seed lots have good emergence (80 to 90%) when planted in the field. The objectives of this study were to 1) evaluate the extent of cold test germination differences between carryover seed lots treated with fungicide + SAI or fungicide-only; 2) determine if an alternative preparation can be made to a seed lot prior to the cold test and the saturated cold test; and 3) address the accuracy of the conventional cold versus the saturated cold testing method in predicting field emergence. Nineteen seed lots treated with fungicide-only or fungicide + SAI were tested in the laboratory and the field. The cold test germination percentage of carryover seed lots treated with fungicide + SAI was lower than fungicide-only treated seed. When the treatments were removed with Tween 20, the cold test germination of the fungicide + SAI-treated seed was not significantly different from the fungicide-only treated control. The cold test of fungicide-only treated and fungicide + SAI-treated seed correctly estimated emergence under all field conditions. After the fungicide + SAI seed treatment was removed, the saturated cold test accurately predicted field emergence under "poor" field conditions but underestimated field emergence under "average" or "good" field conditions. Removing the fungicide + SAI treatment before conducting the cold test may help seed companies better predict field emergence of the seed lots.

INTRODUCTION

Seed corn (*Zea mays* L.) companies are providing extended protection to the seed by applying varying rates of seed-applied insecticide (SAI). Applying the insecticide directly to the seed [e.g., Poncho 1250 (Bayer CropScience, NC)] simplifies planting procedures and provides insect protection to the seed and seedling far into the growing season. These SAIs are always applied on fungicide treated seed. As seed companies treat an increasing number of corn seed lots with high rates of SAI, the likelihood of having carryover seed lots treated with SAI also increases.

Throughout the seed industry, the cold test and the saturated cold test are

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popular methods for testing seed corn vigor (Hampton and TeKrony, 1995). Seed companies sell or discard carryover seed corn based on cold test or saturated cold test results. Most seed companies set a minimum cold or saturated cold test threshold value below which a seed lot is discarded. In some cases, cold test results of SAI-treated carryover seed lots can be lower than field emergence (Gustafson Seed Treatment Specialist, personal communication). These lower cold test results could prompt a seed company to discard a good seed lot, when in fact the seed vigor of the lot is sufficiently high to permit sale. As a result, seed companies are searching for alternative cold test methods that more accurately estimate seed quality and better predict stand establishment.

The objectives of this study were to 1) evaluate the relationship between cold test and saturated cold test results among carryover seed lots treated with fungicide [Maxim/Apron XL (Syngenta Corporation, DE) or Captan/Allegiance (Bayer CropScience, NC)] + SAI (Poncho 1250) or fungicide-only; 2) determine if an alternative preparation can be made to the seed lot prior to the cold test and the saturated cold test to assess the vigor of SAI-treated carryover seed corn; and 3) evaluate the accuracy of the conventional versus the saturated cold test methods in predicting seedling emergence under field conditions.

MATERIALS AND METHODS

Seed supply

Three Iowa seed corn companies supplied the hybrid seed used in the experiment representing a random sample of carryover seed lots and fungicide treatments. Seed lots were produced in 2002 and 2003, and within three months of production they were cleaned, treated with fungicide-only and bagged by the seed companies. The size and shape of seeds in all lots used in these experiments was medium flat. Samples of untreated seeds from these lots were not available to use as an additional control because the standard procedure is to store carryover seed lots already treated with fungicide. Table 1 indicates the year of production for each seed lot, the year the lot was used in the experiments, and the names and rates of the fungicides applied to each seed lot. Half of the seed from each seed lot was then treated with a high rate of Poncho 1250 (1.25 mg active ingredient Clothianidin/kernel) at the Gustafson/Bayer CropScience research facility (Redfield, IA) and both halves of the seed lot were returned bagged to the seed company for one-year storage at 10 °C constant temperature. After a year, the stored seed was subdivided into 454 g samples at Gustafson/Bayer CropScience research facility (Redfield, IA) in January. One 454 g sample of each seed lot remained stored at the Gustafson/Bayer CropScience research facility (Redfield, IA) for subsequent field planting in the spring. The remaining seed was sent to the Iowa State University (ISU) Seed Testing Laboratory (Ames, IA) for testing. Seed was stored at 10 °C and 50% relative humidity, and all tests and field plantings were completed within 6 months.

Prewash procedure

In 2004, three prewash treatments were tested: a deionized water prewash, a surfactant prewash of 0.05% Tween 20 solution (polyoxyethylene sorbitan monolaurate) (ICI Americas, Inc., Wilmington, DE) prewash and a non-pre-

TABLE 1. Seed lot numbers, year of production, year of experimentation, and rate of fungicide application for seed lots used in 2004 and 2005.

Seed lot no.	Year of production	Seed lots included in experiments of year	Rate of application of each fungicide			
			Maxim†	Apron XL	Captan	Allegiance
			g of active ingredient/100 kg seed			
1	2002	2004	2.5	1.0	–	–
2	2002	2004/2005	–	–	65	2
3	2002	2004/2005	–	–	65	2
4	2002	2004/2005	–	–	65	2
5	2002	2004/2005	–	–	65	2
6	2002	2004	–	–	65	2
7	2002	2004/2005	2.5	1.0	–	–
8	2002	2004/2005	2.5	1.0	–	–
9	2003	2005	5.0	2.0	–	–
10	2003	2005	2.5	2.0	–	–
11	2003	2005	2.5	2.0	–	–
12	2003	2005	2.5	2.0	–	–
13	2003	2005	2.5	2.0	–	–

†Fungicide labels are trademarks of the respective chemical suppliers
Seed size for all seed lots is medium flat

washed control. The prewash steps included: 1) 105 seeds were soaked in 105 mL of water or a 0.05% solution of Tween 20 for 10 min in a 250-mL beaker; 2) seeds were poured into a 10-cm strainer and then rinsed under a stream of running tap water for 45 s (rinse water was collected for proper disposal); 3) seeds were air-dried for 90 to 120 min; and 4) after the seed had dried, it was placed back in the original envelope. The process was repeated four times for each test to obtain four 100-seed replicates.

Seeds were tested using the cold test. Based on the results of the 2004 cold test experiments, only control and Tween prewash were used in the saturated cold test in 2004 and all experiments in 2005.

Laboratory tests

SAI recovery analysis – In 2004, the fungicide + SAI-treated seeds were prewashed using the water or Tween prewash procedures. Two replications of water prewashed, Tween prewashed, and non-prewashed control seed were sent to the Gustafson Seed Technology Center (McKinney, TX) for active ingredient analysis. The percentage of Poncho 1250-active ingredient on individual seeds was determined by high-performance liquid chromatography (HPLC). A bulk sample of 50 sound (unbroken) seeds were imbibed in 100 mL of 50% acetonitrile in water and agitated to stimulate the extraction process. The HPLC system was an Agilent HP 1100 Series (Agilent Technologies, Inc., Santa Clara, CA) with degasser, quadratic pump, automated loading system, Peltier's thermostatted column compartment, and a diode-array detector. A Water's Symmetry C-18 capillary column (Waters Corporation, Beverly, MA) (250 × 4.6 mm, particle size 5 μm, pore size 100 Å, and a mobile phase consisting of 1/999 mL acetic acid buffer and acetonitrile) was used for the analysis. The software used to capture the data was Agilent's Chemstation. Results from the bulk samples were compared to the HPLC analysis results from external standard solutions. Mean separation according to the Tukey-Kramer method was performed to determine the effectiveness of the pre-wash treatment at removing the treatment.

Cold test – Samples were completely randomized within trays and carts. One-hundred seed replicates per seed lot were planted on fiberglass trays (Hoffman Manufacturing Company, Albany, OR) for a total of four seed lots per tray. Seeds were planted on a sheet of Kimpak (Kimberly Clark Corp., Neenah, WI), watered with 1000 mL of water, and prechilled to 10 °C overnight (approximately 16 h). One cm of dry 80% sand:20% soil mixture was applied over the top of the seeds. The trays were placed inside enclosed carts. The tests were incubated at 10 °C in a dark chamber for 7 d and then moved to a 25 °C growth chamber with 24 h light for 7 d. Seedlings were evaluated by ISU Certified Seed Analysts following AOSA Rules for Testing Seeds (2006). All tests were repeated four times.

Saturated cold test – Based on the seed-applied insecticide recovery analysis and the results of the cold test in 2004, samples for the remaining experiments were only prewashed with Tween solution. Samples were completely randomized within saturated cold test trays and carts. A plastic grid rack of 60 × 40 cm was placed in a 61 × 41 × 5 cm tray (Hoffman Manufacturing Company, Albany, OR). A 60 × 30 cm single germination paper towel (Anchor Paper Company, St. Paul) was wrapped over the plastic grid rack serving as a wick with two additional paper towels placed on top. Tap water (1.0 L) was poured on the paper towels and allowed to soak through into the tray. Excess water was sufficient to keep the paper towels and soil saturated throughout the test. Sandy loam soil sifted through a 70-mm sieve was sprinkled over the paper towels to form a thin layer. The trays with media were chilled for 24 h at 10 °C before planting. Four 100-seeds replicates were planted with their embryos down following the saturated cold procedure outlined in the AOSA Vigor Testing Handbook (2002). The seed trays were placed inside carts in a 10 °C chamber for 7 d. After the cold incubation, carts were moved to a 25 °C growth chamber with 24 h light for 3 d. The seedlings were evaluated following the saturated cold evaluation protocol (AOSA, 2002).

Field emergence

A subset of seed samples treated with fungicide-only and fungicide + SAI were stored at the Gustafson Research Farm near Redfield, IA in 2004 and 2005. Seeds were not prewashed for the field experiments. Fifty seeds per row were planted in two 10.67 m long rows/plot using a four-row plot planter. Plots were replicated four times at each planting location. There were four planting locations in 2004: river bottom A, river bottom B, building C, and river bottom D; and two in 2005: building C and river bottom D. A description of the field conditions and planting dates are given in Table 2. Weather data during planting and emergence are presented in Table 3. GDD_{10}, growing degree days in Table 3 are expressed in degree Celsius and calculated by the formula [(minimum temperature + maximum temperature) $\times 2^{-1}$] – 10 °C accumulated per days; where

TABLE 2. Field characteristics and classification based on planting dates, soil type and drainage, climate, and average field emergence for fields planted at the Gustafson Research Farm near Redfield, IA in 2004 and 2005.

Field Name	Planting date	Soil type	Soil conditions	Climatic conditions	Field classification
2004					
River bottom A	7 April 2004	Nodaway Silt Loam	Loam, moderately well drained‡	Cool temperatures first seven days after planting (mean air temperature was 8 °C) and no rain†	Average
River bottom B	15 April 2004	Nodaway Silt Loam	Loam, moderately well drained	Mild temperatures (mean air temperature of 17 °C) and abundant rain (89 mm)	Good
Building C	15 April 2004	Ladoga Clay Loam	Silty Clay Loam, gently sloping on slightly convex upland. Fine textured, heavy soil, with moderately slow permeability	Mild temperature (mean air temperature of 17 °C) and abundant rain (89 mm)	Poor
River bottom D	7 May 2004	Nodaway Silt Loam	Silt Loam, good drainage	Ideal temperature (mean air temperature of 18 °C) and moderate rain (25 mm)	Good
2005					
Building C	18 April 2005	Ladoga Clay Loam	Silty Clay Loam, gently sloping on slightly convex upland. Fine textured, heavy soil, with moderately slow permeability	Cold temperature (mean air temperature of 13 °C) and abundant rain (61 mm)	Poor
River bottom D	3 May 2005	Nodaway Silt Loam	Silt Loam, good drainage	Ideal temperature (mean air temperature of 13 °C) and no rain	Average

† Mean of the average daily air temperatures and total rain fall for the 7 days after planting; from 7 – 15 April 2004 the mean air temperature was 8°C and 0.0 mm of rain; from 15 – 22 April 2004 was 16°C and 89 mm; 7 – 15 May 2004 was 18°C and 25 mm, respectively. In 2005, from 18 – 25 April 2005 the mean air temperature was 13°C and 61 mm of rain; from 3 – 10 May 2005 was 13°F and 0.0 mm of rain.

‡ USDA Dallas County, IA Soils Survey, October 1983.

if maximum temperature is > 30 °C, then maximum temperature = 30 °C. If minimum temperature is < 10 °C, then minimum temperature = 10 °C.

Stand counts on all of the field plantings were performed at the V2 stage (Ritchie et al., 2005). Based on planting date, soil characteristics, air temperature and rain during the first 7 d after planting, fields were classified into "poor," "average," and "good" fields for seed emergence in 2004, and "poor" and "average" in 2005 (Table 2).

TABLE 3. Precipitation, maximum, minimum and average temperature, and growing degree days (GDD) for Redfield, IA, in 2004 and 2005.

Parameter	Month: April	May	Total
2004			
Precip. (mm)	107	230	337
Temp. max. (°C)	18	23	
Temp. min. (°C)	6	12	
Temp. avg. (°C)	12	18	
GDD_{10}	127	213	340
2005			
Precip. (mm)	120	131	251
Temp. max. (°C)	19	22	
Temp. min. (°C)	7	10	
Temp. avg. (°C)	13	16	
GDD_{10}	135	189	324

Precip. (mm), precipitation in mm; temp. max. (°C), and temp. min. (°C), average maximum and minimum temperature in °C; Temp. avg. (°C), average temperature in °C; GDD_{10}, growing degree days in °C.

Statistical Analysis – Laboratory tests were conducted in a completely randomized design and all tests were repeated four times. Data were recorded in germination percentage. Both the equal variance and normality assumptions were validated; thus the analysis was performed on untransformed data. Data were analyzed as a factorial using a proc mixed procedure in Statistical Analysis System (SAS Institute, Cary, NC). Seed treatment (fungicide + SAI, fungicide-only), and prewash (water, Tween 20, and control) were the main effects in 2004. Based on the statistical analysis of the laboratory results of the first year, water prewash was not used in the remaining experiments. Thus, data were analyzed by year due to the slightly different setup of the experiments. In 2005, seed treatment (fungicide + SAI, fungicide-only), prewash (Tween 20, and control) and age of the seed (16 or 28 months) were main effects. Seed lots were treated as random effects and seed treatment, prewash, and age of the seed in 2005 were treated as fixed effects both years. Mean comparisons between control and prewashed seed samples within laboratory tests or field emergence were conducted according to unadjusted t-test of least square means. Simple linear regression equations were calculated between laboratory test results (cold test, saturated cold test from control and Tween prewashed seed samples) and field emergence data collected in fields of "poor" and "average" field plantings in 2004 and 2005.

RESULTS AND DISCUSSION

Laboratory tests

Efficacy of the prewash treatments – Table 4 shows the results from SAI (1.25 mg active ingredient Clothianidin) recovery analysis conducted in 2004. The average active ingredient on the seed before washing was approximately 90% (1.12 mg) of the target rate of 1.25 mg active ingredient Clothianidin/ kernel applied to the seed. After washing with Tween, an average of 20% of the original active ingredient remained on the seed. The water prewash seed had 23% of the original active ingredient. The prewash treatments significantly ($P < 0.001$) reduced the amount of active ingredient in the seed and these treatments were not significantly different ($P < 0.05$).

The amount of SAI recovered from each seed lot ranged from 1.06 to 1.22

TABLE 4. Results from the seed-applied insecticide active ingredient (AI) Clothianidin target rate recovery analysis in the seed before and after Tween 20 and water prewash in 2004.

	Target rate recovered analysis [target rate of 1.25 mg (AI) seed-applied insecticide (Clothianidin)/kernel]		
	Control	Tween 20 prewash	Water prewash
	mg active ingredient/kernel		
1	1.07	0.27	0.29
2	1.09	0.22	0.27
3	1.12	0.22	0.29
4	1.13	0.32	0.31
5	1.06	0.28	0.28
6	1.06	0.20	0.23
7	1.19	0.30	0.35
8	1.22	0.25	0.26
Means	1.12 a	0.26 b	0.29 b

Means followed by a different letter are significantly different ($P < 0.001$).

(86 to 98%) of the 1.25 mg target rate (Table 4). This variation is expected due to several factors, which include: application rate variation inherent to small-batch application equipment; seed count variability between the seed companies and analytical recovery count; and chemical recovery efficiency. For every seed lot, seed size, and genotype analyzed, the expected chemical recovery efficiency is below 100% of the target rate (Jeff Daniels, Bayer Crop Science, personal communication).

Based on the active ingredient recovery analysis (Table 4) and the results from the cold test in 2004 (Table 5), only one prewash treatment (Tween prewash) was used in the saturated cold test in 2004 and all experiments in 2005.

Cold test and saturated cold test germination percentage differences – The cold and saturated cold test germination in the fungicide-only were significantly higher ($P < 0.05$) than the fungicide + SAI in 2004 and 2005 (Tables 5 and 6). The age of the carryover seed lot (16 or 28 months) and the interaction between age and seed treatment were not significant in 2005 for both tests (data not shown). Although the 2004 and 2005 experiments were analyzed separately due to the slightly different experimental design, the general conclusions from 2005 did not differ from those of 2004. The cold test germination percentages of seed lots treated with fungicide + SAI were significantly lower (2% in 2004 and 4% in 2005) than the fungicide-only treated seed lots in both years (Tables 5 and 6). Seed lots treated with fungicide + SAI also had significantly lower saturated cold test germination percentages (19% in 2004 and 20% in 2005) for the control (CO). These results indicate that, for some

TABLE 5. Mean cold test and saturated cold test germination percentages of control (not prewashed), prewashed with Tween 20, and field emergence percentages by seed lot and overall means of samples treated with fungicide and fungicide + seed-applied insecticide (SAI) in 2004. LOT, seed lot number; CO, control (no prewash); WA, Tween 20 prewash; poor, average, and good field conditions as defined in Table 2.

	Cold test				Saturated cold test					
	Fungicide-only		Fungicide + SAI		Fungicide-only	Fungicide + SAI		Field planting		
LOT	CO	WA	CO	WA	CO	CO	WA	Poor	Average	Good†
	%									
1	97	95	91	91	94	60	72	83	96	95
2	87	92	86	89	76	63	79	87	85	92
3	92	93	90	92	92	85	87	80	94	92
4	95	91	93	93	92	91	92	79	90	94
5	87	91	86	87	80	52	65	77	91	93
6	86	86	77	83	74	37	47	79	85	90
7	95	95	87	96	95	70	81	76	95	96
8	98	97	95	96	96	87	87	79	96	97
Overall means	92 a*	92 a	90 b	90 b	87 a	68 c	76 b	77 c	87 b	93 a

*Overall means within a test followed by the same letter are not significantly different at the 0.05 level of probability.
†Average of two locations (river bottoms B and D).

TABLE 6. Mean cold test and saturated cold test germination percentages of control (not prewashed), prewashed with Tween 20, and field emergence percentages by seed lot and overall means of samples treated with fungicide, and fungicide + seed-applied insecticide (SAI) in 2005. LOT, seed lot number; CO, control (no prewash); WA, Tween 20 prewash; poor, and average field conditions as defined in Table 2.

	Cold test				Saturated cold test					
	Fungicide-only		Fungicide + SAI		Fungicide-only		Fungicide + SAI		Field planting	
LOT	CO	WA	CO	WA	CO	WA	CO	WA	Poor	Average
	%									
2	93	94	84	87	81	77	62	63	77	94
3	92	94	94	95	91	90	83	91	77	95
4	96	96	95	93	93	95	91	83	76	93
5	91	89	88	85	81	81	39	53	56	90
7	96	98	88	94	97	95	74	81	71	95
8	98	98	98	97	98	96	84	92	87	97
9	97	97	97	98	97	98	91	94	76	96
10	95	95	98	97	96	93	93	93	75	93
11	97	97	95	95	99	94	88	79	71	93
12	96	99	82	90	86	88	48	77	67	94
13	92	94	76	81	92	85	44	61	62	92
Overall means	95 a*	95 a	91 b	91 b	92 a	90 a	72 c	79 b	64 b	93 a

*Overall means within a test followed by the same letter are not significantly different at the 0.05 level of probability.

seed lots, the cold and saturated cold test germination percentages of carryover seed corn treated with fungicide + SAI are lower than the same seed lots treated with fungicide-only.

The mean cold test results in fungicide-only and fungicide + SAI treated seeds were not significantly different before and after prewash ($P < 0.05$) in 2004 and 2005 (Tables 5 and 6). Thus, the pre-wash treatment had no effect or was unnecessary when seed lots were tested using the cold test tray method. The response of the different seed lots to prewash in the fungicide + SAI treated seed varied among the carryover seed lots (Tables 5 and 6). For some seed lots, there was an increase in cold test germination percentage after prewash that was equivalent to the cold test germination percentage of fungicide-only control. One possible explanation is that genetics influence a seed's response to high levels of fungicide + SAI or prewash treatment. Further research should be conducted to test this hypothesis.

Tween prewash treatment significantly ($P < 0.05$) increased the saturated cold test germination percentage of fungicide + SAI-treated seed in 2004 and 2005 (Tables 5 and 6). The prewash treatment did not significantly affect the saturated cold test germination percentage of seeds treated with fungicide-only (Table 6). These results indicate that prewashing seeds with Tween does not negatively affect seed quality as determined by the cold and saturated cold tests. The results also indicate that the lower saturated cold test germination values of the fungicide + SAI seed lots are likely the result of an interaction between the seed treatment and seed lots, as well as saturated cold test conditions. Once the seed treatment is removed with a Tween prewash, the saturated cold test germination percentage of the fungicide + SAI-treated seed increased. However, this increase in saturated cold test germination percentage is significantly lower than the germination percentage value for control, fungicide-only treated seed of the same lots. These results suggest that the saturated cold test is not reliable for assessing cold germination percentage of carryover seed lots treated with fungicide + SAI.

The cold test and the saturated cold test are used by most United States seed corn companies to assess seed vigor. The seed companies have internal cold and saturated cold tests standards below which they will discard a seed lot. A two percentage increase in cold test values over all seed lots tested is very important when seed companies are making seed marketing decisions.

Field emergence trials

Field conditions were classified as poor, average, and good in 2004, and poor and average in 2005 according to soil characteristics and climate records obtained from the Iowa Environmental Mesonet (2001–2007) (Tables 2 and 3). The field conditions were classified as "poor" when seeds were planted on heavy, moderately slow drained soils. The soil temperatures at 10-cm depth were 12 and 10 °C and followed by intense rains of 89 and 61 mm within the first 7 d after planting in 2004 and 2005, respectively. The field conditions were classified as "average" and "good" when seeds were planted on moderately well to well drained soils, and both years the 10-cm depth soil temperatures at planting were between 12 and 15 °C. The field conditions for the early planting in 2004 were classified as "average" because the soils were well drained and the weather was dry, the 10-cm depth temperature was 10 °C.

Simple regression equations were calculated between laboratory test results of fungicide + SAI-treated control and prewash treatments, and field emergence of fungicide + SAI-treated control seed under "poor" and "average" field plantings (Fig. 1). The prewash treatment improved the prediction capability of the tests under "average" but not under "poor" field planting, as indicated

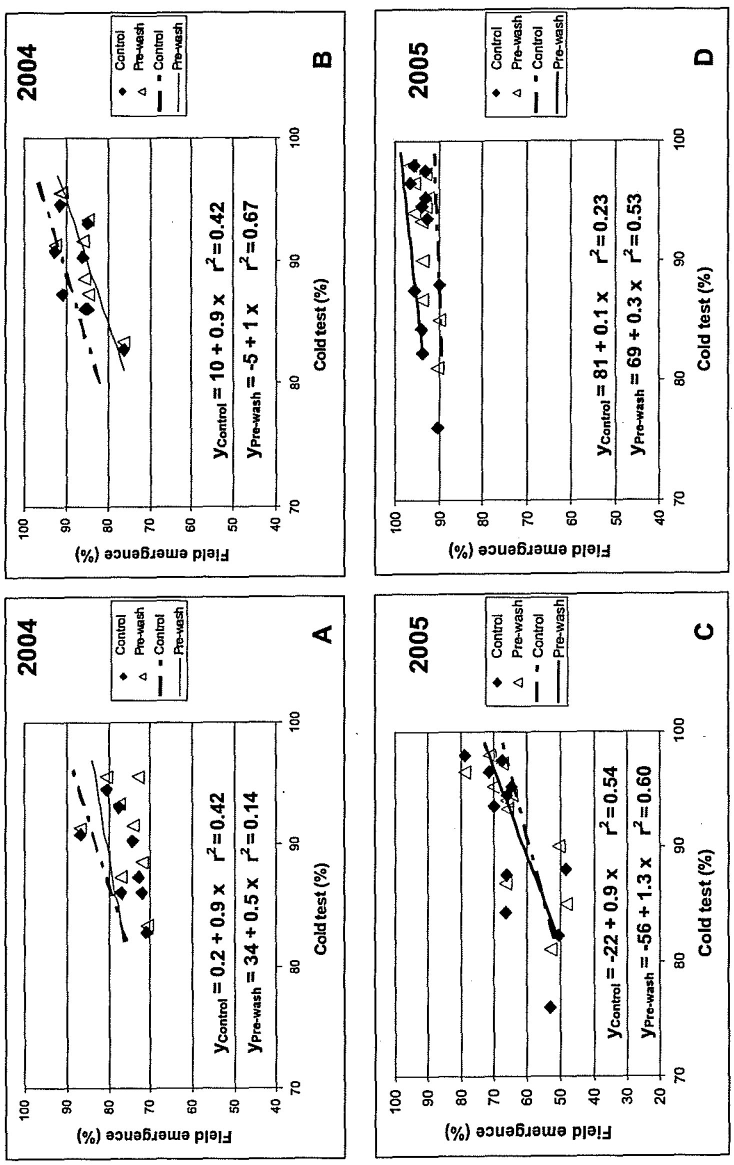
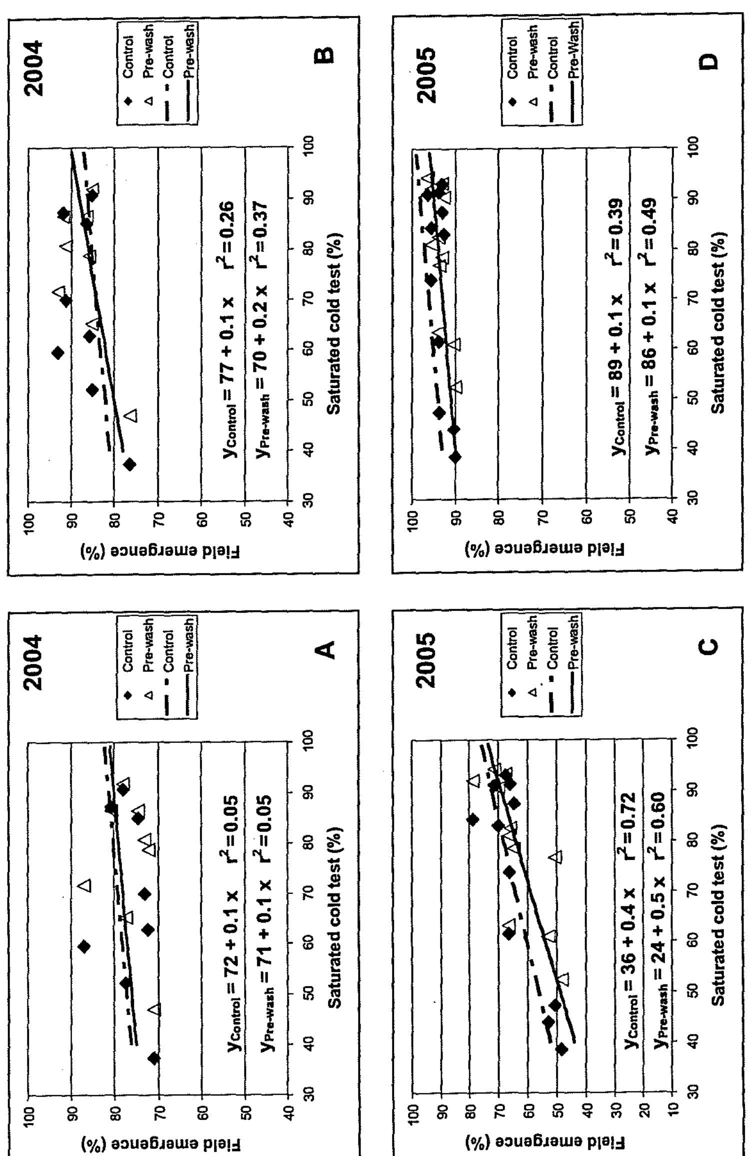


FIGURE 1. Simple linear regression models fitted to emergence percentage data from seed lots planted in poor (A, C) and average (B, D) field conditions, and cold test germination percentages of control (◆) and Tween prewash treatment treated with fungicide + SAI (△) in 2004 (A, B) and 2005 (C, D).

by the R^2 values of the regression equations. The exception was the cold test in 2005 where it also improved (Fig. 1). The R^2 values represent how much of the variation in the data set is explained by the regression model. The R^2 values for the cold tests ranged from 0.14 to 0.67 and from 0.05 to 0.72 for the saturated cold test (Fig. 2). These values were similar to those reported in the literature (Martin et al., 1988, DeVries et al., 2007).

The slopes for a regression line between a laboratory test results and field emergence should be 1.0 when the percentage germination in the laboratory

FIGURE 2. Simple linear regression models fitted to emergence percentage data from seed lots planted in poor (A, C) and average (B, D) field conditions, and saturated cold germination percentages of control (◆) and Tween prewash treatment treated with fungicide + SAI (Δ) in 2004 (A, B) and 2005 (C, D).



test and field emergence are identical. The slopes of the regression between cold test and field emergence under all plantings in 2004 and "poor" field emergence in 2005 were close to 1 (data not shown). The slopes of the regression equations for the "average" field in 2005 were close to 0 (data not shown). This lack of relationship between cold test and field emergence could be attributed to close to ideal growing conditions in the field. Thus, all seed lots had high field emergence percentage values regardless of their cold test percentage. The slopes of the regression lines between saturated cold test and field emergence under all plantings (0.1 to 0.5) indicated the saturated cold test failed to predict field emergence, or that field conditions were too variable for obtaining a strong relationship between field emergence and laboratory test results. Other authors have reported correlation coefficients between vigor tests and field emergence in the range of 0.35 and 0.75 (Martin et al., 1988; DeVries et al., 2007). These values indicated that seed vigor tests only correlated to field emergence in 35 to 75% of the cases depending on field conditions. For example, when field conditions were close to ideal, the standard germination test was a better predictor of field emergence than the vigor tests (Woltz and TeKrony, 2001). Other authors also have reported a poor correlation between vigor tests and field emergence under severely stressful conditions (TeKrony et al., 1989; Goggi et al., 2008). In our experiments, the cold test predicted field emergence of fungicide + SAI-treated seeds under all field conditions while the saturated cold test predicted better "poor" field conditions in 2005 but not in 2004.

CONCLUSIONS

The cold test germination percentages of carryover seed lots treated with fungicide + seed-applied insecticide (SAI) were lower than those treated with fungicide-only. A prewash with a Tween 20 solution effectively reduced the amount of fungicide + SAI treatment on the seed. The cold test germination values of the fungicide + SAI seed lots increased in Tween 20-washed seeds indicating the likely interaction between the chemical seed treatment and the seed lot. Once the fungicide + SAI treatment was removed using a prewash with Tween 20 solution, the cold test germination percentage of fungicide + SAI-treated seeds increased, but the overall germination mean was still below the mean from fungicide-only treated controls. The prewash treatment did not affect the cold and saturated cold test germination percentages in fungicide-only treated seed, indicating the prewash had no detrimental effect on seed vigor of the seed lots as determined by these tests. The cold test correlated well to field emergence in fungicide + SAI-treated seed lot, but the saturated cold test underestimated field emergence under "average" field conditions. The saturated cold test correlated poorly with "poor" field planting conditions in 2004.

The cold test and the saturated cold test are the vigor tests most widely used by the US seed corn industry. In our study, the cold test tray method was a more reliable predictor of field emergence of fungicide + SAI-treated seed than the saturated cold test. Thus, the saturated cold test should probably be avoided when testing carryover seed lots. In carryover seed lots treated with SAI, a prewash with Tween 20 improved the prediction capability of the tests under average field plantings. Even for those carryover seed lots where the cold test was not impacted by the fungicide + SAI seed treatment, prewashing had no negative effect.

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