DATCP Agricultural Development & Diversification (ADD) Grant Final Report

January 11, 2005

Project Title: Streptomyces Biocontrol of Common Scab: Phase II Field Trials and Formulation

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Description of Project Aim and Anticipated Benefits

Common scab of potato can adversely affect potato quality resulting in significant economic losses in the state of Wisconsin. Potatoes are the Wisconsin's 3rd leading crop (behind corn and soybeans) in terms of total value: in 2003 Wisconsin's potato harvest was valued at $187 million (Wisconsin Ag. Statistics Service). USDA estimates that annual U.S. potato crop losses due to disease are approximately 8% or $250M. Common scab is the 4th most important disease of potato, according to the World Potato Congress. Scab is especially virulent in the Wisconsin due to the prevalence of sandy soils.

Heartland Farms in Waushara and Adams counties estimate that more than 20% of their potato production is negatively affected by a high level of potato scab, resulting in more than 50% of the tubers showing scab symptoms ranging from superficial blemishes to deep-pitted scab. Heartland Farms has annual contracts with processors, such as Frito-Lay, Inc. Frito-Lay docks growers and thereby reducing their revenues if scab defects exceed 6%.

Potato growers have experienced inconsistent control of common scab through the use of chemical controls, such as the fungicide Mancozeb. Fumigation is expensive, produces limited control and may kill beneficial indigenous bacteria that help minimize disease. Additionally, chemical controls can pollute groundwater and is not permitted for use on certified organic potatoes. The development of an effective biocontrol to prevent common scab could save Wisconsin potato farmers hundreds of thousands of dollars in lost yield and reduce reliance on chemicals.

TFX Bioscience and others have previously demonstrated that biocontrol strains (= suppressive strains) of *Streptomyces scabies* isolated from Minnesota soils can protect against common scab of potato. These tests were accomplished with the biocontrol strain(s) prepared in sterile vermiculite soaked in oatmeal broth. Although this wet vermiculite inoculum can be tested in small, replicated trials, it is not amenable to large-scale testing or to commercial methods of application. It is noteworthy that to produce a viable commercial product, the carrier of the biocontrol strain plus the strain (s) must be defined in for the EPA biopesticide approval process, Also, the method of applying biocontrol inoculants must fit into low-cost grower practices (e.g., a potato seed treatment dusted on seed prior to planting).

The aim of this project was to provide statistical evidence of efficacy of common scab biocontrols. Biocontrol strains isolated in Minnesota soils have provided control in small replicated trials (Heartland Farms, 2004). Not all replicates of this trial had sufficient scab pressure to facilitate statistical analysis. Therefore, withy Heartland farms cooperation, two sites were selected for the 2005 trial to better ensure that at least one site would have significant scab pressure.

In addition, we sought to determine which of three commercial formulations and which application method (applied to seed pieces or incorporated in furrow) is optimal. These formulations were prepared as follows: a) basic vermiculite and oatmeal broth provided by Dr. Linda Kinkel (positive control), b) a dry, milled product produced by grinding air-dried vermiculite-containing inoculum, and, c) a dry whey powder-containing inoculum prepared by Natural Industries Inc., Houston, Texas. Each dry inoculant formulation was mixed with fir bark to aid in coating the seed. Fir bark is organic and is used by growers to help heal cut seed and to protect seed from soft rot. In addition to testing the efficacy of these carrier preparations, we also captured qualitative data such as the ease of mixing and application.

Anticipated benefits were to find the optimal carrier type in order to start the EPA registration process. Secondly, we wanted to provide the grower with a statistically valid conclusion that the biocontrol strain (s) provided meaningful control in order to enlist their cooperation in 2006 for a large-scale test with the optimal carrier/strain type.

Summary of Tangible Accomplishments

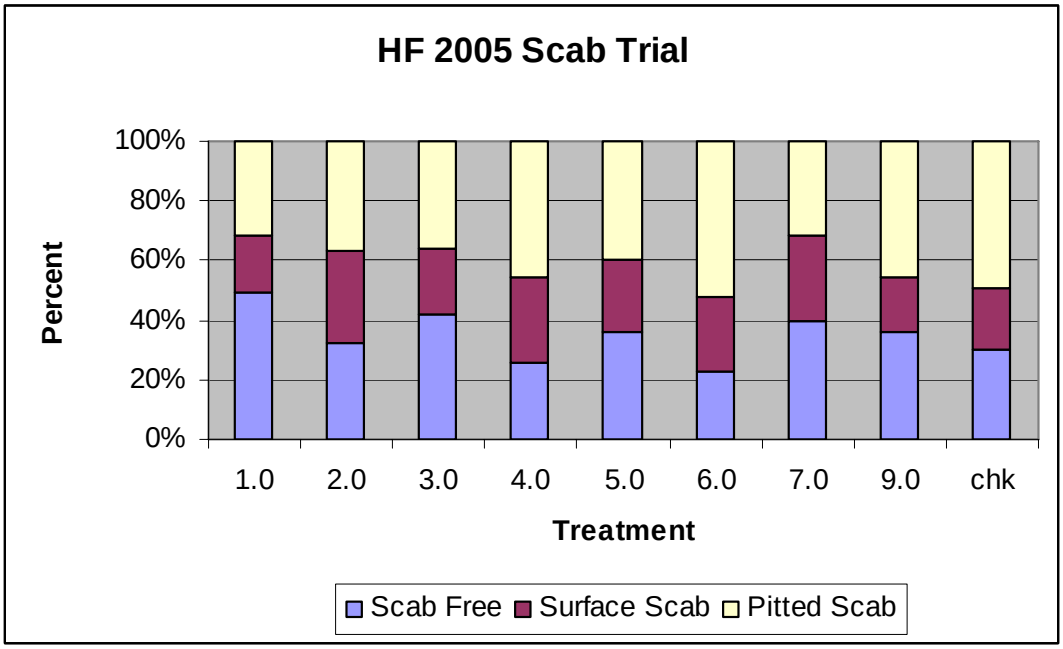
A good working relationship with Heartland Farms was established. Excellent cooperation was provided to TFX in planting the trial and in its harvest.

Application of the whey powder inoculants on the seeds gave most complete surface coverage. It was also noted that if seed had free moisture on them, the whey powder inoculum would ‘gum up’ and then become difficult to completely

cover the seed. Dry vermiculite powder covered adequately when combined with fir bark. It was not possible to determine if this coverage was better or worse than whey powder as the vermiculite powder was brown and blended in with the color of the seed periderm. In contrast, the whey powder inoculum was white and contrasted with the periderm.

High scab pressure was obtained through selecting trial sites known to have a lot of indigenous potato scab; controls (untreated) on average resulted in about 50% pitted scab by weight (Fig. 1). We used Heartland Farms input to set a goal of suppressive strain scab reduction to < 15% by weight in order to significantly impact a quality advantage in chip manufacture. Although treatment 1 (Strain 93 prepared in moistened vermiculite) resulted in 31% pitted scab (Fig. 1) and thus did not meet our goal threshold, this treatment did provide a statistically beneficial shift in scab reduction compared to control.

Figure 1. Scab percent composition of treatments, treatment key and scab category rating key.



Scab Categories Key

Scab-Free: No visual scab up to ≤ 15% surface scab.

Surface Scab: ≥ 15% surface scab or one pit

Pitted Scab: ≥ 2 pitted scab lesions

Figure 1
(continued)

Treatments	inoculum	Carrier	Milled with Fir Bark?	Strain	Inculum wet or dry	Inoc. Mode
1	Kinkel	Vermic + OMB	No	93	wet	in furrow
2	Kinkel	Vermic + OMB	No	Mix I	wet	infurrow
3	Kinkel	Vermic + OMB	No	Mix II	wet	infurrow
4	Kinkel	Vermic + OMB	Yes	93	dry*	seed piece
5	Kinkel	Vermic + OMB	Yes	Mix	dry	seed piece
6	Kowalski	NI DP	Yes	93	dry	seed piece
7	Kowalski	NI DP	Yes	Mix I	dry	seed piece
8*	Kowalski	NI DP	Yes	Mix II	dry	seed piece
9	Kowalski	NI DP	Yes	Mix II	dry	seed piece
C=control		none	No	None	n/a	None

*NI DP is proprietary scale up of a dry powder carrier from Natural Industries, Houston TX
*Mix I is equal concentrations of Strains 15, and 93
*Mix II is equal concentrations of Strains 15, 93, and 2-12
*Milling to be done by TFX Bioscience
* Water added to seed to help coat resulted in wet breakdown of seed and poor stand

Data Analysis

Analysis of Variance (ANOVA) shows significance for reductions in pitted scab ($p<0.023$) and for increases in scab-free tubers ($p<0.004$) (Fig. 2). Treatment 1 gave both a significant decrease in pitted scab and increase in scab-free tubers when compared to control. In addition, treatments prepared in wet vermiculite (Treatments 1, 2 and 3) all resulted in a significant decrease in pitted scab (Fig. 2). Treatment 5, which was a dry-powder whey protein formulation coated on to seed at time of planting, resulted in a significant decrease in pitted scab an in a significant increase in scab-free tubers (Fig. 2).

Figure 2. ANOVA test and pair wise comparisons of treatment effects on pitted scab and scab-free categories. Pair wise comparison by Fisher's pair wise comparisons (see Fig. 1 for scab category ranking).

		Pairwise comparisons*						
TRT	Pitted Scab	1	2	3	4	5	6	7
7	6.5625							
1	7.6875							
2	8.25							
3	9.1875							
5	9.625							
9	10.25							
4	10.58333							
6	11.25							
chk	13.188							
p-value	0.023							

		Pairwise comparisons*		
TRT	Scab free	1	3	5
1	11.825			
3	10.625			
5	8.6875			
7	8.3125			
9	8.125			
2	7.3125			
4	6.083333			
Chk	5			
6	4.916667			
p-value	0.004			

*Pairwise comparison significantly different
*Pairwise comparison not significantly different

The greatest statistical significance resulted in the comparison of vermiculite carriers to control, irrespective of strain (Fig. 3). We therefore conclude that the suppressive strain technology is directionally valid but not optimized.

Figure 3. ANOVA test and pair wise comparisons of treatment effects on pitted scab and scab-free categories. Pair wise comparison by Fisher's pair wise comparisons (see Fig. 1 for scab category ranking).

		Pairwise comparisons*			
TRT	Scab-free	1	3	2	
1	11.825				
3	10.625				
2	7.313				
Chk	5				
p-value	0.0001				

*Pairwise comparison significantly different
*Pairwise comparison not significantly different

Conclusions. We conclude that although control is less than what would be commercially viable for Heartland Farms, the 2005 test resulted in high scab pressure from which we were able to show statistical benefits of suppressive strains vs. control. The best control was achieved through strains prepared in wet vermiculite, but to a lesser magnitude, control was also achieved through the preparation of dry inoculum used to coat potato seedpieces (e.g., Treatment 5).

Our test was statistically underpowered to make conclusions about the optimal strain or strain combinations or optimal dry inoculum preparation method (dry vermiculite vs. whey powder as prepared by Natural Industries).

Description of Information or Educational Materials

Even though treatments in this trial had significantly less scab than did controls, we were unable to demonstrate to our grower-cooperator the commercial viability of a biocontrol product. We conclude that communications and public relations with the grower-cooperator are as important as the science. We have demonstrated that our product does control scab under high scab pressure situations. It would be our recommendation to other developers of biocontrol product to do large scale testing as soon as feasible. For our test with our grower-cooperator, we most likely would have been able to demonstrate statistical and meaningful significance on a broader scale (strip trials across an entire field) as scab intensity would not have been as high as in our selected small-scale trial. We did not have an appropriate carrier to be able to formulate our strains into a format suitable for large-scale trials. It was learned through our testing and grower interactions that a priority should be placed on the development of a carrier prior to field testing. Once a carrier is developed, specific strain (s) to formulate in the inoculant may be optimized per varying geographical locations.

Future Research and Product Development

TFX Bioscience has made it a priority to find a business partner to cooperate in the development of highly-concentrated biocontrol strains in a user-friendly carrier. We are in the process of building a relationship with such a partner and have licensed from them, a process to manufacture the ideal aforementioned inoculum.

Future plans include testing the shelf life of our licensed product and field testing our biocontrol strains alone and in mixtures of other biocontrol agents with the potential of reducing scab and other soil-borne disease of potato such as *Rhizoctonia.*

Recommendations to Enhancing Benefits of the Project

Recommendations to enhance the benefits of this project are two fold:

1. Business partner with technical, marketing, and sales success. We will leverage our partner's knowledge of their proprietary base of biocontrol products for potato diseases to identify and test with growers in the Pacific

Northwest. It is anticipated that we will be able to formulate a seed piece inoculant that will control several potato diseases.

2. Broad-geography testing. We have tested our product with one grower-cooperator in 2004 and 2005. We recognize the importance of building relationships with other growers both within and outside Wisconsin. We envision that a suitable forum such as the Wisconsin Vegetable and Potato Growers Association (WVPGA) will stimulate grower interest and result in additional grower-cooperators.