Effectiveness of Seed Treatments in Reducing TMV Infection

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Abstract
Tobacco mosaic virus (TMV), named for the plant it was first discovered in, can infect hundreds of different species. It has no known invertebrate vectors, but TMV is spread mechanically through contact with other plants, clothes, tools, contaminated soil and seedborne. While TMV is known to be exceptionally stable, it has been reported that treatment of tools with powdered milk can significantly reduce the infection rate of the virus. While there is a seed treatment available for conventional agriculture, there is no seed treatment available for organic production. Organic growers, who grow heirloom tomatoes experience the highest rates of TMV infection. Since powdered milk has been reported as an effective means of treatment in tools, the purpose of this research is to determine whether it can be an effective seed treatment. To test this, tomato seeds were treated with powdered milk, soy milk, almond milk and water. A grow out test was then conducted in a greenhouse and plants were tested for infection using Polymerase Chain Reaction and antibody based ELISA testing. In the first grow out trial treatment little effect was seen, with infection rate for the powdered milk, almond milk, soy milk and control treatments were 60%, 34%, 32% and 47%, respectively. The seed treatment may have been ineffective because we did not add a surfactant and additional research is in progress to test this hypothesis.

Introduction
Tobacco Mosaic Virus (TMV) is a remarkably stable RNA virus that has been known to remain viable in vitro for as many as eight years and even longer in cold temperatures. TMV infects over 350 species of plants, most notably those in the Solanaceous family such as tomatoes, peppers, and tobacco for which the virus was named. TMV can easily be spread by contaminated tools, clothing, soil and seeds. Heirloom tomato varieties are becoming increasingly popular, especially in organic produce production, but are not resistant to TMV. This leads to increased yield losses due to stunted plant growth and symptomatic fruit (Fig. 1 and 2). Yield losses can range from 20% to 100% in tomatoes. These are obvious economic incentives for developing organic seed treatments. Powdered milk has been found to be a very effective means of TMV inoculation in tools, so it is the primary focus of the following experiments.

Materials and Methods
For the grow out test the Big Rainbow heirloom tomato variety was selected with 100% TMV positive seed. Seeds were treated for 15 minutes in either: 20% powdered milk solution, almond milk or soy milk solution. Seeds were planted in 72 cell trays with miracle grow potting mix and moved to a greenhouse. After three months leaves were collected from each plant and split in half. One half was ground in general extract buffer and tested for TMV infection using antibody based ELISA testing. Positive results were verified by extracting the RNA from the other half of positive leaves using reverse transcription PCR. Samples which were 750 base pairs were cut from the gel and sequenced. Germination trials were conducted to ensure that the treatment type did not affect seed germination rate. Seeds were treated as detailed above, then were placed in petri dishes on moist filter paper. Seeds were placed in an incubator and checked daily for germination.

Results/Discussion
In the grow out trial little effect was seen. The infection rate for powdered milk, almond milk, soy milk and control treatments were 61%, 34%, 32% and 47%, respectively. These treatments may have been ineffective because of the surface tension between the hairs on the seed and the water. If these hairs are creating enough surface tension, then parts of the seed will not come in contact with the treatment. To test this hypothesis further research is currently underway using surfactants in the treatments to break the surface tension. Interestingly, the number of germinated tomato plants for the soy treatment was 65% lower than that of other treatment types. Germination trials were conducted to determine whether this was a result of the treatment type, and it was shown that there is no relationship between germination and treatment type.

References