Title: Use of Plant Activators to Control Common Diseases of Tomato

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Abstract:

Plant activators induce defense responses in plants, which can prevent or slow pathogen infection and some of these products have been reported to enhance yield. This project tested several activators labeled for use in NY to determine whether induction of defense response pathways could be followed in the field and whether these product could be utilized in a manner that if there is any yield improvement or efficacy against common tomato diseases. While we did not see a yield enhancement using products that are suggested to increase yield, we did see encouraging results for disease control. We found that one class of activators (the SAR-inducing type) controlled bacterial speck of tomato without negatively affecting yield. The ISR-inducing activator failed to provide protection from bacterial diseases. Also, this compound produced inconsistent growth enhancement over several field seasons and it is not clear whether this type of compound (ISR-inducing) provides yield enhancement. Based on these findings, we believe that the SAR-inducing compound could be a valuable addition to an integrated management program of bacterial disease of tomato.

Background and Justification:

Activation of plant defense responses against diseases and insect pests can provide a powerful management tool. Induced resistance has been studied in the laboratory for decades, though this information has not translated to consistent control of pests in the field. Plant defense-activating compounds are known as plant activators, plant defense activators or systemic acquired resistance (SAR) inducers and are frequently termed biopesticides. Many of these compounds claim additional benefits by increasing plant health and yield, and are expected to be environmentally friendly, having no direct effect upon the pathogen. Plant activators could be a viable component of an integrated pest management (IPM) program via delaying initial fungicide and/or insecticide applications or they could be alternated with chemical control. Organic production systems could benefit as some activators are certified for organic use. While several such compounds are commercially available, their use by NY growers is quite limited. Unfortunately, this is most likely due to the inability of plant activators to consistently control many pests in the field.

Control of bacterial speck and spot of tomato is one of the few examples where a plant activator is currently recommended to growers in the Northeast, but the compound is not widely used. Tomato bacterial disease control is a high priority for the vegetable commodity group, and a comprehensive study of plant activators is necessary to determine the efficacy and utility of these products in NY.

This study examined one example of each of the two types of plant activators. The first activator class consists of living microbes that colonize plant roots and activate a resistance mechanism known as induced systemic resistance (ISR). Activation of ISR is thought involve priming, where induced plants respond more quickly and to a higher level when a pathogen is present. Priming and ISR are thought to involve the ethylene and jasmonic acid plant defense response pathways. Frequently, these products are plant growth-promoting rhizobacteria (PGPR), which claim to reduce disease and increase yield. PGPR are living bacteria (many are *Bacillus* spp.) which can be incorporated into the growing medium just prior to planting seed. The exact mechanism by which PGPR act is unknown, though the yield-enhancement is thought to be a response to the 'growth-promoting' aspect of the organism. Bacterial colonization of plant roots aids in mineral and nutrient uptake while pest control is apparently due to activation of ISR.

A specific plant defense pathway known as systemic acquired resistance (SAR) is induced by the second type of activator. Acibenzolar-*S*-methyl (ASM) is the SAR activator included in this study, which is a salicylic acid analog that is applied to the foliage. ASM is commonly used in New Jersey and other states to control bacterial diseases of tomato, but has not been used in the Northeast. In contrast to ISR, there have been several reports of yield decreases following application of an SAR-inducing compound. This is thought to occur from the systemic activation of SAR, which diverts resources away from other areas of the plant, such as growth. While both ISR and SAR induce the plant's natural defense mechanisms, they are not the same and it is unknown which mechanism will have greater efficacy against tomato pathogens in NY. In addition, it is unknown if usage of the two products together could act synergistically to enhance both yield and disease control.

Previous studies have shown that plant activators have the greatest efficacy against bacterial pathogens, therefore this study focused on bacterial speck disease of tomato, caused by *Pseudomonas syringae* pv. *tomato*. While plant activator induction of tomato defense response pathways has been extensively studied in the greenhouse and growth chamber, this is the first study to examine gene activation in the field. In this study, marker genes for SAR (salicylic acid pathway) and ISR (ethylene and jasmonic acid pathways) were utilized to follow defense response pathway activation in the field. We also determined the effect of plant activators and a traditional control compound (copper) on *P. syringae* pv. *tomato* populations on the leaf surface. Quantification of bacterial populations could provide valuable insight into how these activators control disease. This information is crucial to determine the optimal application regime which will enable the highest level of disease control.

Objectives:

1. To determine the impact different activators have on disease control and yield
2. To evaluate the success of the project based on disease control and yield data which will lay the foundation for new IPM control strategies that incorporate activators. As this is a continuing project, the data for three seasons will be compiled.

Procedures:

1. All field trials were conducted at the Gates Road farm in Geneva, NY. Tomato seedlings, cultivar Sunchief, were grown in the greenhouse and transplanted into the field 5 weeks after sowing. Each plot consisted of 10 tomato plants spaced 45 cm apart. Treatments were arranged in a randomized complete block design with three replications.
2. Treatments were applied using labeled rates of commercially available products. Treatments included: Actigard (acibenzolar-*S*-methyl, produced by Syngenta, 0.75 oz/A), BioYield (plant growth-promoting rhizobacteria produced by Bayer, 2 lb/yd^3), Actigard + BioYield, the conventional pesticides Cuprofix 40 (copper sulfate, 2.5 lb/A) and Cuprofix MZ (copper sulfate + mancozeb, 5 lb/A). BioYield is a soil amendment and was added to seedling mix, as described by the product label. Foliar applications of all other treatments began two weeks prior to inoculation so that the plant had an opportunity to "activate" prior to the presence of the pest. The second application of the products occurred 12 hours prior to inoculation with the pathogen. To insure the presence of bacterial speck in our trial, plants were spray inoculated with *P. syringae* pv. *tomato* ($1x10^8$ bacteria/ml) 5 weeks after planting. Disease severity was assessed twice by counting the total number of lesions on 20 leaflets randomly picked from each plot.

3. Tomato tissue was harvested and flash frozen in liquid nitrogen for bacterial population assessment and tomato defense gene studies 12 hours prior to and 12, 36, 60, and 86 hours after inoculation with *P. syrinage* pv. *tomato*. Two leaves were collected from individual field plots for each of three replicates and stored at -80°C.

4. DNA was extracted from field tomato leaf samples using the Qiagen DNeasy extraction kit (Qiagen Inc., Madison, WI). To determine the amount of *P. syringae* pv. *tomato* present in samples, real-time quantitative PCR(qRT-PCR) was performed utilizing the SYBR green reporter dye system. Standard curves, made from serial dilutions of pathogen DNA, were used to calculate the amount of *P. syringae* pv. *tomato* in each sample.

5. Total tomato RNA was extracted from leaf tissue using the SV Total RNA Isolation System (Promega Corporation, Madison, WI) and DNase treated with Turbo DNA-*free* (Ambion Inc., Austin, TX). Two-step qRT-PCR was performed using Taqman fluorogenic probes for three marker genes for tomato defense response pathways and one housekeeping gene. Gene transcript levels for each sample were derived from tomato genomic DNA standard curves. Transcript levels were then normalized to the tomato housekeeping gene to control for plant-to-plant variation.

6. Yield (tomato fruit number and weight) were assessed at the end of the season.

7. The data collected have been evaluated to determine the utility of plant activators in fresh market tomato production in NY. Information on the efficacy of plant activators to control common tomato diseases is being presented at educator and grower meetings throughout the state. Results of the project have also been presented at the Fruit and Vegetable Expo and American Phytopathological Society National Meetings, and a written report of the results will be given to Extension Educators for use in newsletters.

Results and discussion:
This year, we were able to quantify the pathogen growing on tomatoes in the field over time for the first time (Figure 1). It is intriguing that growth on the untreated control follows the typical logarithmic growth pattern of *P. syringae* pv. *tomato*, while growth is modified by all of the other treatments.

Activation of SAR was followed using the acidic *PR-1* marker gene (Figure 2, A & D). Both Actigard treatments induced the salicylic acid defense response pathway, but a steady increase in activation from 12 – 60 hours after inoculation was only observed in inoculated plants. Interestingly, at symptom development, acidic *PR-1* activation dropped to baseline levels or below in Actigard-treated plants in both inoculated and non-inoculated plots. As plants became infected, untreated control and BioYield-treated plots showed increased SAR activation, though this response was not enough to prevent disease.

Basic *PR-1* was utilized to follow induction of the ethylene defense response pathway (Figure 2, B & E). As expected, Actigard application induced this pathway in both inoculated and non-inoculated plants. Trends in activation were similar to SAR induction, with the response being slightly greater in inoculated plants. No priming or ISR induction was observed in either of the BioYield treatments.

Examination of the jasmonic acid wound-induced pathway was performed using the marker gene *Pin2* (Figure 2, C & F). The hail storm immediately prior to pathogen inoculation activated this pathway at relatively low levels in both inoculated and non-inoculated plants at the 12 hour time point. It is possible that levels had been much higher following the storm and had dropped by this sampling time. As observed in greenhouse studies, activation in untreated controls and PGPR-treated plants increased as plants became infected (times 60 hours and symptom). This response was not observed in non-inoculated plants. *Pin2* expression was upregulated to very high levels in both Actigard + BioYield and Cuprofix 40-treated plants. This response could be due to an interaction between the wounding from the hail storm and the treatments. No priming effect was observed in BioYield-treated plots as the response was not faster or to a greater degree than the other treatments.

The Actigard treatments reduced the number of leaf lesions and the early Actigard treatment (starting one week after transplanting) had the fewest infected fruit and the highest yield. Yield for both BioYield and the control treatments were reduced compared to the other treatments, but this difference was not significant.

The Actigard results are encouraging for growers. In contrast to previous studies, we did not find any decrease in yield relative to the untreated control. Actigard treatments provided the best control of bacterial speck numerically, although they were not statistically different from the copper treatments. This provides evidence that Actigard can be used to compliment a copper spray program. Unfortunately, the PGPR product did not provide protection from bacterial speck nor did induce ISR or provide any growth enhancing effects.

In summary, we found that one plant activator, Actigard, does have efficacy against bacterial pathogens of tomato. This activator turns on SAR in tomato plants in the field without detrimentally affecting yield. Thus, it offers growers an additional component in an integrated management program. Many growers have asked about the efficacy of activators and yield-enhancement products, and wonder if they truly increase yield. We have found that this is dependent upon the year, as we saw an increase in yield in 2004, but not in 2005 or 2006.

Figure 1. Quantification of *Pseudomonas syringae* pv. *tomato* DNA from field tomato samples averaged over three replicated plots.

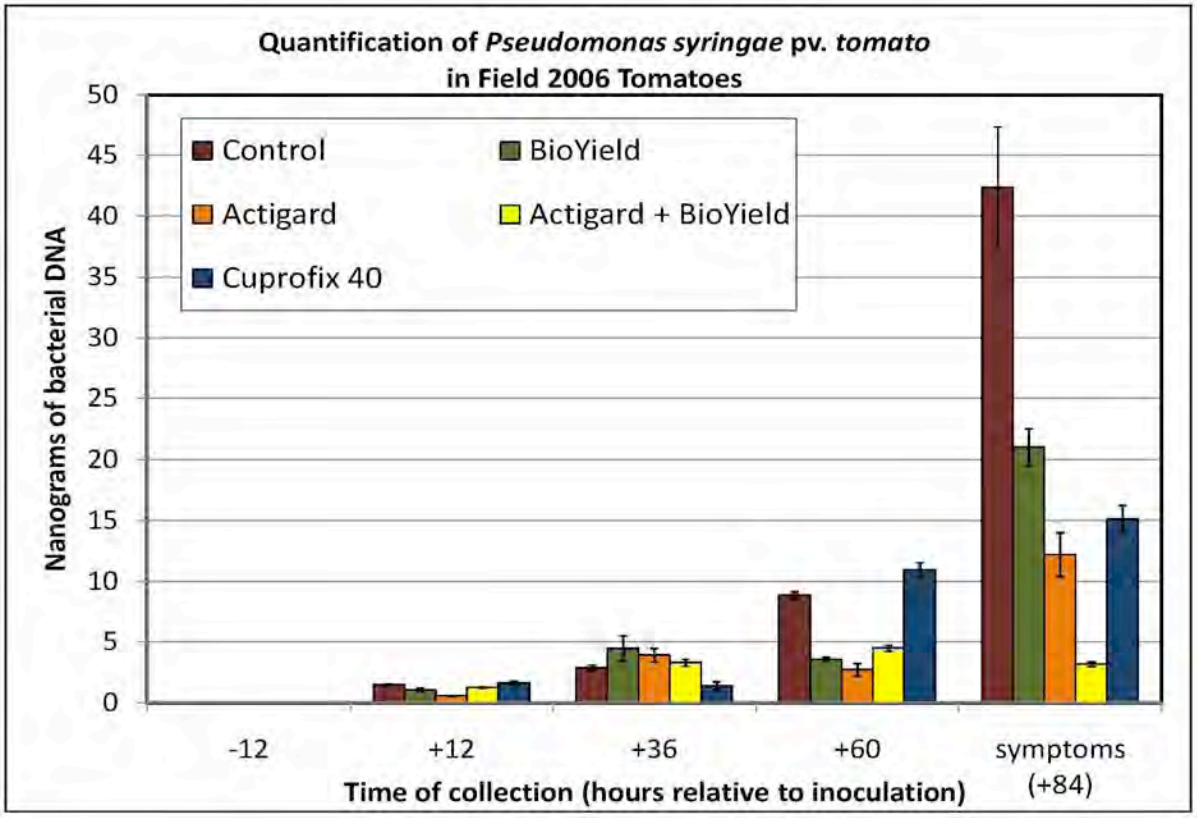

Figure 2. Expression patterns of three tomato defense response pathway marker genes in field-grown tomatoes.