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Phosphorous Acid for Late Blight Suppression in Potato Leaves

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ABSTRACT

Late blight is the most devastating disease of potatoes, resulting in global constraints in potato production. In this study, field trials were conducted in Prince Edward Island, Canada for three years to evaluate the efficacy of a phosphorous acid-based fungicide for the suppression of foliar late blight. Leaves from each treatment were detached from plants in early August of each year and were manually inoculated with a sporangial suspension of a local US-8 (A2) strain of *P. infestans*. Analysis of disease severity showed that the treatments had a significant effect on disease development and the treatment effect interacted with incubation time and cultivar type. In all three years, the combined treatment of phosphorous acid with chlorothalonil provided the best disease suppression, followed by treatment with chlorothalonil alone. Phosphorous acid alone provided increased protection to the leaves, but the effect was not as marked as the chlorothalonil alone or combination treatments. When late blight was detected in the field in 2008 and 2009, disease severity in the field was also evaluated. The results of field ratings matched the findings of the detached leaf experiments. The outcome demonstrated the value of detached leaf study as a reasonable replacement for field trials with late blight. Phosphorous acid-based fungicides appear to provide benefit in a foliar program for late blight control, particularly when combined with the regular application of a protectant fungicide. Phosphorous acid also has an excellent environmental profile, so that its usage will reduce the environmental impact of pesticides applied to the potato crop.

Keywords: detached leaves, disease development, fungicide, reduced environmental impact

INTRODUCTION

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a devastating disease of potatoes that occurs worldwide and causes significant crop losses annually (Hijmans *et al*. 2000). Control of the disease during the past 50 years has relied primarily on the use of fungicides in both developed and developing countries. Late blight control is challenging since the pathogen overwinters as mycelium in seed tubers, in tubers in cull piles, and in un-harvested diseased tubers which survive the winter and become sources of inoculum. Since new strains of *P. infestans* began to dominate populations of the pathogen in Canada in the mid 1990s (Peters *et al.* 1998, 1999, 2001), disease control has become even more difficult. These new strains were often of the A2 mating type, very aggressive, appeared early in the season, and were commonly resistant to metalaxyl, a systemic fungicide which was effective against the old A1 strain (US 1) of the pathogen in Canada (Deahl *et al*. 1995; Peters *et al.* 1998, 2001). At present, protectant fungicides are most commonly employed for late blight control. These include chlorothalonil (Bravo®), or mancozeb (Dithane® or Manzate®). On average, 10 applications of protectants per growing season are applied to the potato crop in Prince Edward Island (PEI), Canada. In late blight epidemic years, the number of fungicide application could double.

It is believed that the economic and environmental limits of systematic, frequent spraying schedules have been reached and there is a progressive shift towards composite strategies based on pesticide applications with genetic means of control (Lizarraga 2002; Andrivon *et al*. 2006). In spite of the efforts of the worldwide late blight research communities, the mechanisms for its control are still not completely understood. Recent reports have shown that potato foliage treated by some phosphorous acid (PA)-based fungicides can reduce foliar late blight (Lobato *et al.* 2008; Mayton *et al.* 2008) as well as late blight and pink rot in tubers (Cooke and Little 2002; Johnson *et al.* 2004; Al-Mughrabi and Peters 2006; Peters *et al*. 2006; Mayton *et al.* 2008; Peters *et al.* 2008). As well, postharvest application of PA to tubers entering storage is effective at killing pathogen spores on the tuber surface (Miller *et al.* 2006; Peters *et al.* 2008). To further test the efficacy of PA for control of late blight in field-grown potatoes, a three-year trial (2007, 2008, and 2009) was conducted on Prince Edward Island using the two processing varieties 'Russet Burbank' and 'Shepody'. Both varieties are the most common for French fry processing in Canada and United States. They are also the most cultivated varieties in Eastern Canada where the research was conducted. Since it is not permissible to inoculate field trials with late blight pathogen to collect data, we chose to use inoculation of detached leaf procedure as a technique for rating the relative efficacy of fungicides. Even though no late blight occurred in the field in 2007, research plots in both 2008 and 2009 were become infected by naturally-occurring inoculum of the pathogen, and so the impact of treatments on natural disease epidemics was also assessed.

MATERIALS AND METHODS

Field experiments

Two French fry processing varieties 'Russet Burbank' and 'She-

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pody' were grown at the Cavendish Farms' research field located in New Annan, PEI, Canada, in 2007, 2008 and 2009. A two year crop rotation was practiced, potatoes followed by barley underseeded with clover. The field experimental design was a split-plot with the fungicide treatment as the whole plot factor and cultivar as the subplot factor. Each treatment was replicated four times. The plots were comprised of plot islands each having two 20-foot (6 meters) rows of each of 'Shepody' and 'Russet Burbank'. The two outside rows were guard rows and the two centre rows were used for assessments of the blight in foliage and as harvest rows for yield data and assessment of tuber rot.

The fungicide Bravo® (chlorothalonil) was obtained from Syngenta Crop Protection Canada Inc. (Guelph, Ontario). The commercial product of PA used in the experiments was Confine™, provided by The Agronomy Company of Canada (Thorndale, ON). This PA formulation is a mixture of mono- and di-potassium salts. The four treatments were: 1) plots sprayed with water as control; 2) PA (Confine™) applied alone at the rate of 5.8 L product/250 L water/ha; 3) chlorothalonil (Bravo®) applied alone at the rate of 2 L product/250L/ha, and 4) PA + chlorothalonil, both at the same rates as individual applications. Fungicide applications were made with a tractor mounted commercial sprayer. Fungicide application took place once a week. In 2007, 10 fungicide applications were made; 8 PA and PA + chlorothalonil applications were made, with water and chlorothalonil applied during weeks 2 and 3. In 2008 and 2009, a total of 11 fungicide applications were made. PA and PA + chlorothalonil applications were made every second spray alternating with water and chlorothalonil, respectively, resulting in 5 applications of each PA and PA + chlorothalonil in 2008 and 6 in 2009.

In 2007, the treatments took place from July 3 to September 10; in 2008, the treatments took place between July 11 and September 16; in 2009, the treatments took place from July 2 to September 11. No late blight was observed in the 2007 field; whereas late blight was found in the fields in both 2008 (started in early August) and 2009 (started in late July). Late blight severity was scored in the field in 2008 from August 20 to September 25 and in 2009 from July 28 to September 3 based on the percentage of defoliation. The score for percent defoliation was based on the total number of plants per plot, the number of infected plants and the severity of the infections (James 1971a).

Inoculum of *Phytophthora infestans*

A local PEI isolate of *P. infestans* (A2 mating type; US-8 genotype) was used in all studies. This is the same strain that is colonized in the field on the Island. The strain was maintained on excised potato leaves (cv. 'Green Mountain') in a humid chamber at 15°C and transferred weekly to maintain isolate virulence. Inoculum was prepared by swirling infected leaves inoculated 7 days previously in 250 mL of distilled water to dislodge sporangia. The resulting suspension was examined microscopically to determine sporangial concentration (with the aid of a hemacytometer). The inoculum was then diluted to 10-20,000 sporangia/mL and filtered through cheesecloth prior to inoculation.

Inoculation of detached leaves with *Phytophthora infestans*

Asymptomatic leaves from each treatment were detached in early August of each year (August 10, 2007; August 19, 2008; August 4, 2009). Four stems from four plants in each treatment were randomly taken. New leaves from the top were discarded; the first fully expanded leaf from the top (named as P) was taken and frozen in liquid nitrogen for protein analysis. The top second (I-1) and the third (I-2) fully expanded leaves were selected and individually infected. Each leaf was placed in a clear plastic bag, then taken to a nearby shed to prevent infection of the field. Each leaf was inoculated by spraying the adaxial surface with 1 mL of the sporangial suspension (prepared as above). Immediately after spraying, each leaf was sealed in the plastic bag with wet paper towel and then stored in styrofoam boxes with ice packs until being transferred to a growth chamber. Eight leaves per treatment/replicate were collected, for a total of 256 leaf samples in each year. As well, 16 leaves (8 randomly picked from 'Russet Burbank' and 8 randomly picked from 'Shepody' of water treated plots) were sprayed with water as a negative control for the *in vitro* infection experiment. The inoculated leaves were incubated in a growth chamber for 7 days at 15°C with 12 hr photoperiod. The inoculated leaves were evaluated daily for disease severity based on estimating percentage of diseased leaf area (James 1971b). Since no significant disease symptoms occurred prior to day 4, the data used for analysis included only day 4 to day 7 in each year.

Statistical methods

In each year of study, the response variable (disease severity scores), was measured repeatedly on Day 4, 5, 6 and 7 after inoculation, and the data were analyzed as repeated measures. The three factors of interest (Treatment, Cultivar and Day) were considered as fixed, and Block was considered as random. The data in the three years were analyzed separately using the Mixed Procedure of SAS (SAS Institute 2003). The most appropriate covariance structure was determined to be AR(1) using Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (BIC). The validity of constant variance and normal distribution assumptions on the error terms were verified by examining the residuals as described in Montgomery (2009). Multiple means comparison was completed for significant ($P < 0.05$) effects. In all years, since either Treatment × Day or Treatment × Cultivar × Day interaction effects were significant, the Least Squares means of all 16 or 32 treatment combinations were compared using the lsmeans statement of Proc Mixed with pdiff option to produce p-values for all pairwise differences, and then letter groupings generated using 1% level of significance to protect Type I experimentwise error rate from over inflation.

RESULTS AND DISCUSSION

This three-year field study was aimed at investigating the efficacy of a PA-based fungicide on controlling late blight in potato foliage. The commercial product Confine™ was used in all experiments. Treatments including a water control, the protectant fungicide Bravo® alone and the combination of Confine™ with Bravo® were included for comparison. **Table 1** summarizes the Treatment, Cultivar and Day effects and their interactions from each trial season.

In 2007, a three-way interaction of Treatment × Cultivar × Day was detected (**Table 1**). Further analysis of the interaction effects is shown in **Fig. 1**. On day 4, no disease symptoms were observed in all treatments in both 'Russet Burbank' and 'Shepody'. Disease symptoms became apparent in the control (water sprayed) samples from day 5. PA-treated samples showed significantly reduced disease symptoms from day 5 to day 7 compared with the control. This was observed in both cultivars. However, significant differences in disease expression between the cultivars were observed (**Fig. 1**). In this case, 'Russet Burbank' responded to PA better than in 'Shepody'. Even better disease suppression was observed in chlorothalonil-treated and PA + chlorothalonil treated samples. For both cultivars, the chlorothalonil + PA treatment was more effective than chlorothalonil alone (**Fig. 1**). In both treatments, 'Russet Burbank' responded better than 'Shepody'.

In 2008, the cultivar difference was shown to be signi-

Table 1 Effects of Treatment, Cultivar and Day on the severity of infection (%) in 2007, 2008 and 2009. Significant effects that need multiple means comparison are shown in bold face.

Source of Variation	2007	2008	2009
Treatment	0.001	0.001	0.001
Cultivar	0.001	**0.001**	0.175
Treatment × Cultivar	0.002	0.835	0.002
Day	0.001	0.001	0.001
Treatment × Day	0.001	**0.001**	0.001
Cultivar × Day	0.001	0.183	0.643
Treatment × Cultivar × Day	**0.002**	0.211	**0.013**

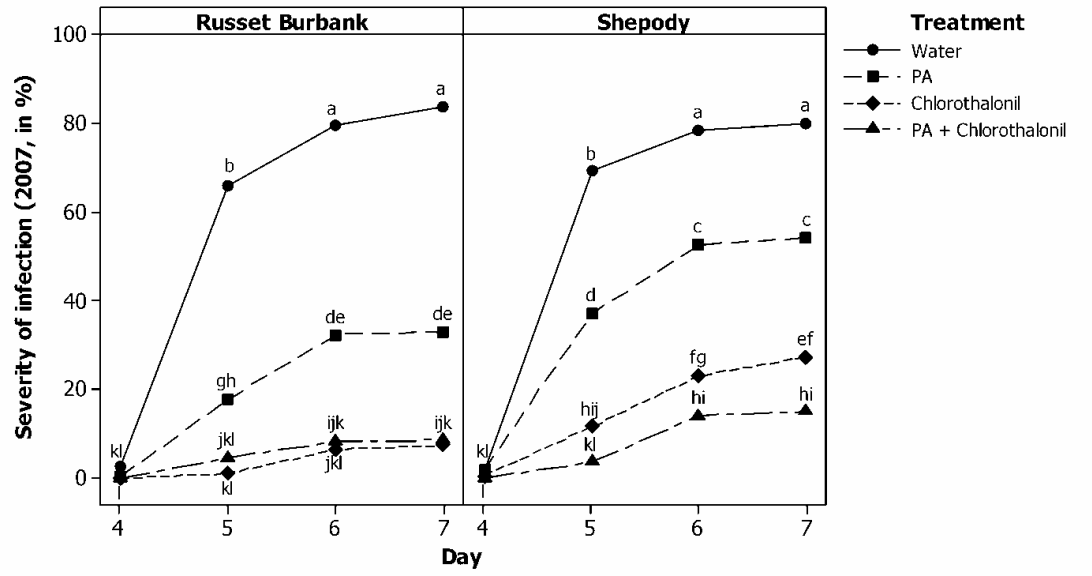

Fig. 1 Interaction plot of disease severity (%) versus Day for the four treatments and the two cultivars in 2007. Means sharing the same letter are not significantly different at the 1% level of significance.

ficant (**Table 1**). Disease symptoms in 'Shepody' were more severe (on average 22.6%) than that of 'Russet Burbank' (on average 14.9%). **Fig. 2** demonstrates the interaction effects of Treatment × Day in 2008 samples. Since the Cultivar × Treatment interaction effects were not significant, the means were averaged across the two cultivars. As shown in **Fig. 2**, on day 4, disease had developed in the control samples and the PA-treated samples, even though disease severity in PA-treated samples was significantly less than in the control. No disease was observed in the chlorothalonil and PA + chlorothalonil-treated samples at this time point. From day 5 to day 7, the disease was rapidly expressed in both control and PA-treated samples, however, the PA-treated samples still showed significant delay in disease development compared to the control on day 5. This difference in disease expression was lost after day 6 (**Fig. 2**). Both chlorothalonil and PA + chlorothalonil treatments provided outstanding disease suppression in this trial. No significant differences were observed between these two treatments. Even though no late blight was observed in the field at the time of sampling detached leaves, late blight was observed in the field later in August this year.

In 2009, a three-way interaction of Treatment × Cultivar × Day was detected (**Table 1**). On day 4 following the leaf inoculation, disease was seen in all three treatments of control, PA, and chlorothalonil alone, but not in the PA + chlorothalonil combined treatment. The disease rapidly developed on the control samples after day 5 and reached the highest level in day 7 in both cultivars (**Fig. 3**). PA treatment significantly delayed disease development in 'Russet Burbank'; however, this was not seen in 'Shepody'. Chlorothalonil suppressed disease in both cultivars, but was less effective in 'Shepody' as no significant differences were detected after day 6 when compared to the control samples. Nevertheless, the treatment of PA + chlorothalonil provided exceptional disease suppression in both cultivars (**Fig. 3**). The disease severity in this treatment on day 7 was only 10.8% for 'Russet Burbank' and 13.0% for 'Shepody'. We have consistently observed that the onset of late blight development is much earlier on 'Shepody' than in 'Russet Burbank'.

As indicated earlier, late blight was identified in our field trials in both 2008 and 2009, in which late blight was prevalent in commercial fields across PEI. Because the field data is only used to compare the detached leaf data done in a laboratory setting, we wanted to show only patterns that our detached leaf data matches the observations from the samples in these field plots in 2008 and 2009. The complete analyses of the field data will be presented in a separate

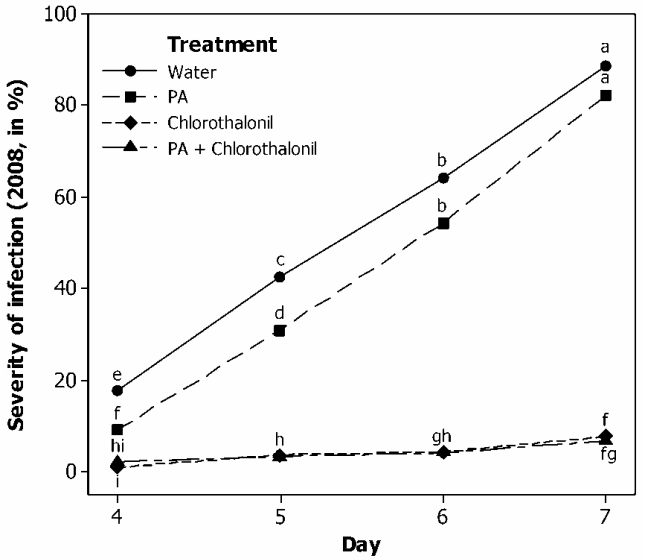

Fig. 2 Interaction plot of disease severity (%) versus Day for the four treatments in 2008. Means sharing the same letter are not significantly different at the 1% level of significance.

manuscript. Disease severity in the research plots due to infection of plants by naturally-occurring inoculum (ascertained to be of the US-8, A2 genotype) was rated in both years as shown in **Fig. 4**. In general, disease symptoms appeared in PA-treated plants at least one week after being confirmed in the control plots. The onset of late blight symptoms was even later in the plots treated with chlorothalonil, but this protection broke down as the season progressed and disease pressure increased. In general, the patterns of disease development in the field were very similar to those observed in the detached leaf experiments, which serves to validate the latter approach for treatment comparison. Chlorothalonil alone was less effective in suppressing late blight in 2009 than in the other two years, likely due to the very early onset of disease in 2009 July 22 in 2009 vs mid August in 2008, and the inability of the protectant program to keep up with the field infection rate. Nevertheless, the combined treatment of PA + chlorothalonil was consistently the best for disease suppression in all years of the study.

Our studies indicate that PA-based products can provide significant disease control benefits when incorporated into a

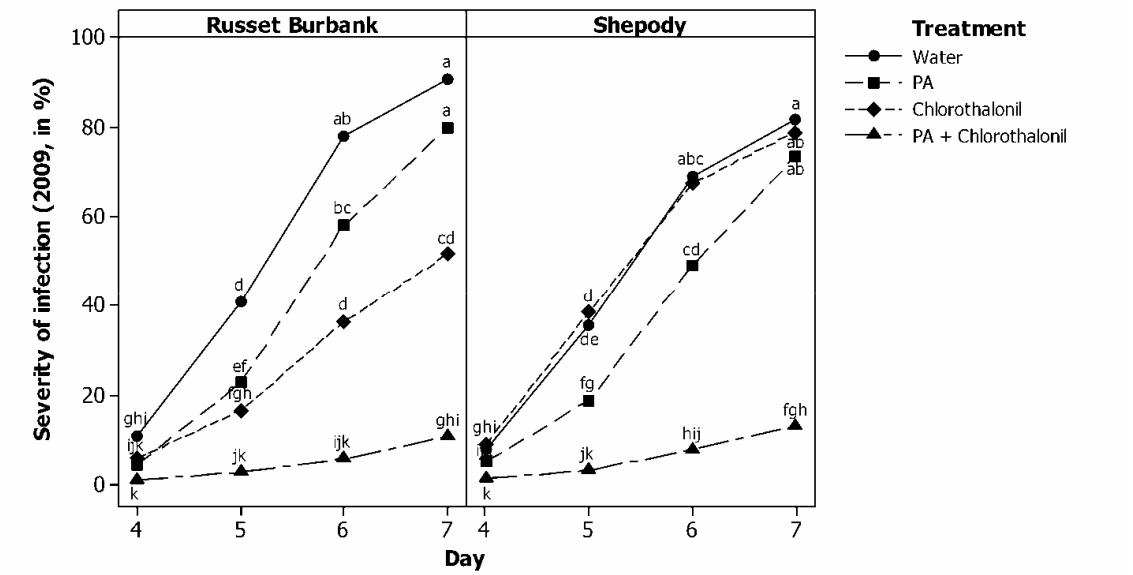

Fig. 3 Interaction plot of disease severity (%) versus Day for the four treatments and the two cultivars in 2009. Means sharing the same letter are not significantly different at the 1% level of significance.

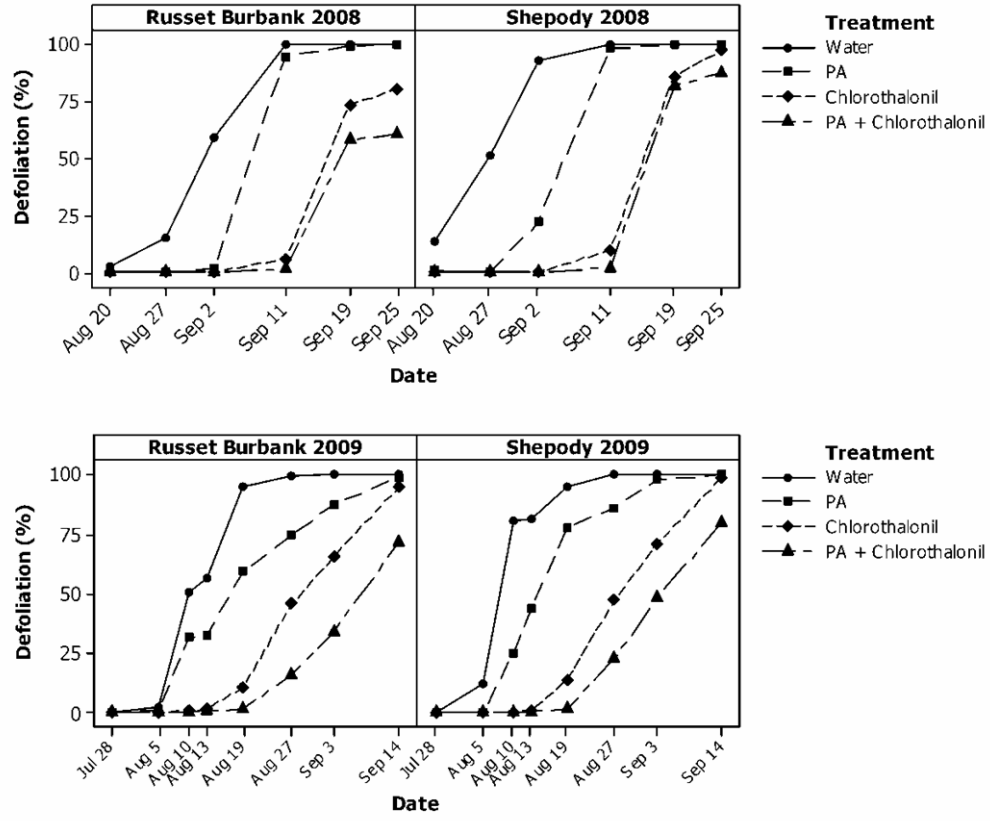

Fig. 4 Disease severity in the fields of 2008 and 2009 seasons. Ratings took place from August 20 to September 19 in 2008, and July 28 to September 14 in 2009, based on the defoliation percentage.

late blight management program. Previous studies have documented the dual nature of activity of PA against oomycete pathogens (Thao and Yamakawa 2009). While a direct fungitoxic activity has been reported (Grant *et al.* 1990; Guest and Bompeix 1990), stimulation of host defence responses has also been recorded (Guest and Bompeix 1990). PA, when applied alone, delayed disease epidemic development in our studies. This could be an important benefit for growers during those times when application of protectant products in a timely fashion is difficult due to adverse weather conditions. The combination of Confine™ with Bravo® was a particularly effective approach likely due to the synergies captured by combining a truly systemic (Confine™) with a protectant (Bravo®) product. In addition to achieving foliar disease suppression, the suppression of tuber rot achieved by incorporating a PA-based product into an oomycete management program must also be underscored (Cooke and Little 2002; Peters *et al.* 2008).

The commercial product Confine™ (mixture of mono- and di-potassium salts of phosphorous acid) is the only PA-based fungicide currently registered in Canada. It is registered for postharvest treatment of potato tubers (Registration No. 29100 Pest Control Product Act). An emergency registration for foliar application of Confine™ for late blight control was granted in 2009; however, it was not approved until too late in the season for meaningful application. Growers in Canada will likely have full access to this product in the near future. An added benefit of the PA class

of compounds is their relatively low toxicity. In the USA, an exemption from residue data requirements has been granted by the EPA (US Environmental Protection Agency) and there are a number of PA based products registered in USA that are not registered in Canada to date. Since PA-based products are recognized as environmentally friendly, their incorporation into disease management programs may significantly reduce the usage of other fungicides. Studies are underway by our research team to ascertain whether the concentration or frequency of application of toxic protectant products can be reduced when PA-based products are used as part of a late blight management program.

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