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Phosphites Effect on Late Blight Control and Physiological Parameters in Commercial Potato (*Solanum tuberosum* L.) in Argentina

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ABSTRACT

Phosphite (Phi) has been recommended to enhance plant resistance against *Phytophthora* spp. The purpose of this study was to evaluate the effect of calcium phosphite (PhiCa) and potassium phosphite (PhiK) under field conditions, taking into account physiological parameters, performance and protection against late blight in the foliage of two potato cultivars, and comparing these results to controlled *in vitro* experiments. Experimental design was a split plot in randomized complete blocks with four replicates. The main plot comprised two potato cultivars ('Kennebec' and 'Spunta') and the sub-plots three late blight control treatments: control, PhiCa and PhiK. The interaction between cultivar and treatment on the area under disease progress curve (AUDPC) was determined. In 'Kennebec', the PhiK treatment showed a significantly lower AUDPC than control and PhiCa treatments. No differences were detected between treatments on 'Spunta'. In the detached-leaf tests, there was a significant treatment effect ($P < 0.01$). In both cultivars, PhiK application resulted in a significantly lower damage growth rate (DGR) compared to the control and PhiCa treatments, although there was less difference in 'Spunta'. Treatments tested had no significant effect on yield. However, PhiK application tended to increase yields by 20.5 and 14.8% in relation to control and PhiCa treatments, respectively. Under experiment conditions, Phi decreased disease severity and increased yields, with differences between cultivars and chemical compounds.

Keywords: chemical control, phosphite, *Phytophthora infestans*, *Solanum tuberosum* L.
Abbreviations: AUDPC, area under disease progress curve; **DGR**, damage growth rate; **PhiCa**, calcium phosphite; **PhiK**, potassium phosphite

INTRODUCTION

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is the most devastating potato disease and one of the major limiting factors in the production of this crop (Hijmans *et al.* 2000). In temperate areas, the pathogen generally persists from year to year in infected tubers, increases in foliage throughout the growing season, and washes into the soil to infect new tubers. Therefore, yield losses are attributed to both premature death of the foliage and diseased tubers (Lambert *et al*. 2005).

To control the pathogen and the disease it causes, the usual strategy is fungicide application, which increases production costs and damages the environment (Huarte and Capezio 2003). Additionally, partially-resistant cultivars can be used to reduce the input of fungicides (Kirk *et al.* 2005).

Potato genetic resistance against late blight is classified into two different types: specific resistance which is generally monogenic and is only effective against a subset of strains of the pathogen and quantitative resistance which is polygenic and thought to be effective against all strains of the pathogen. Potato cultivars containing specific resistance proved ineffective in the field because of new virulent races of the pathogen. In contrast, cultivars that possess quantitative resistance proved durable (Turkensteen 1993). Argentina currently produces well over 2 million tonnes (t) of potatoes per annum on just over 100 000 ha (the 80% is represented by the cultivars 'Spunta' and 'Kennebec') with an average yield of 29.5 t/ha) (Mantecon 2009). These cultivars do not have quantitative resistance to late blight. Thus, there is enormous interest in finding effective alternatives to protect potato crop against late blight (Mizubuti *et al.* 2007).

Phosphites (Phi), derived from phosphorous acid, are fungitoxic chemicals that can be combined with different elements such as calcium, copper, manganese, magnesium, potassium or zinc. These compounds are classified by the US Environmental Protection Agency (US-EPA) as biopesticides, specifically biochemical pesticides (see: http://www.epa.gov/pesticides/biopesticides/). Biochemical pesticides classified in the US are composed of substances that occur naturally in the environment (Mayton *et al.* 2008). Thus, they have a low environmental impact (Guest and Grant 1991). Kovach *et al.* (1992) developed a method to quantify the damage caused by common pesticides used in agriculture. They determined that Phi has an environmental impact quotient (EIQ) of 7.33 whilst mancozeb, the most commonly used fungicide in potato crops, has a quotient of 14.6.

Lobato *et al.* (2010) have assessed the *in vitro* antimicrobial activity of potassium phosphite (PhiK) and calcium phosphite (PhiCa) against different potato pathogens such as *P. infestans*, *Fusarium solani*, *Rhizoctonia solani* and *Pectobacterium carotovorum* (syn. *Erwinia caratovora*) founding that Phi are fungistatic rather than fungitoxic. Besides their fungistatic or fungicidal activity (Fenn and Coffey 1984; Cohen and Coffey 1986; Guest and Grant 1991; Wilkinson *et al.* 2001; Mills *et al.* 2004; Lobato *et al.* 2008), Phi stimulate the activation of defence mechanisms in plants against diseases (Smillie *et al.* 1989; Saindrenan and Guest 1994; Guest and Grant 1991; Daniel and Guest 2006; Andreu *et al*. 2006; Lobato *et al.* 2008; Lobato *et al.* 2011) and promote growth (Guest and Grant 1991; Thao

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and Yamakawa 2009). For this reason, the horticultural industry widely uses Phi for oomycetes control (Pilbeam 2003). Phi have been reported to effectively control many *Phytophthora* species infecting a range of crops (Pegg *et al.* 1985; Guest *et al.* 1994; Cooke and Little 1996; Forster *et al.* 1998; Jackson *et al.* 2000; Cooke and Little 2001; Johnson *et al*. 2004; Thao *et al.* 2008; Garbelotto *et al.* 2009).

In potato, under greenhouse conditions (Lobato *et al.* 2008), Phi applied to potato seed tubers, immediately after cutting, increased resistance against *P. infestans*, *F. solani* and *R. solani*. Protection was high against *P. infestans*, intermediate against *F. solani* and low against *R. solani* for all cultivars tested. The foliar applications of PhiCa or PhiK controlled *P. infestans* in all cultivars tested, although protection degree was cultivar-specific, dose and time of application dependent. On the other hand, tubers issued from plants treated in foliage showed a smaller colony diameter of *P. infestans* and a reduction of the lesion area caused by *F. solani* and *E. carotovora* when artificially infected; suggesting that this compound induced a systemic defence response. PhiK had a stronger effect than PhiCa (Lobato *et al.* 2011).

Similarly, Miller *et al.* (2006) showed significant reductions in late blight incidence on potato tubers when applying these compounds as post-harvest treatments. In contrast, under field conditions, Cooke and Little (2001) did not record significant reductions in leaf infection caused by *P. infestans* in potato. However, they considered that the response to Phi is influenced by application time, the cultivar used, the location and disease incidence and severity. In Argentina, there is no research on the efficacy of foliar applications of Phi under field conditions on potato crops.

The objective of this study was to evaluate the effect of PhiCa and PhiK under field conditions, taking into account physiological parameters, performance and protection against late blight in the foliage of two potato cultivars, and comparing these results with controlled *in vitro* experiments.

MATERIALS AND METHODS

Field experiments

1. Chemicals

PhiK (30% P_2O_5, 20% K_2O, dose 3 l/ha) and PhiCa (25% P_2O_5, 8% Ca, dose 3 l/ha) were formulated by PFG International SA (Lerida, Spain) and provided by Agro-EMCODI SA (Buenos Aires, Argentina).

2. Experimental design and plant material

Field experiments were conducted in the 2006/2007 and 2007/2008 growing seasons in Balcarce, Buenos Aires, Argentina (37° 45′ S; 58° 18′ W), over a complex of Typical Argiudoll and Petrocalcic Paleudoll soils. Experimental design was a split plot in randomized complete blocks with four replicates. The main plot was comprised of two potato cultivars: 'Kennebec' (Chippewa × Katahdin) × (Earlaine × 3895-13), moderately susceptible to late blight) and 'Spunta' (Béa × USDA 96-56, highly susceptible to late blight) and the sub-plots consisting of three late blight control treatments: control sprayed with water, PhiK and PhiCa.

3. Crop management

Potato cultivars were planted on 10/30/2006 and 11/21/2007; each plot had four rows 5 m long and 85 cm apart. Tubers were planted every 20 cm along the rows (100 tubers per plot). PhiK and PhiCa (3 l/ha) were weekly applied using water at a rate of 250 l/ha since the beginning of tuberization with a backpack sprayer with constant pressure (2.75 bar), brand name HD 20, equipped with ceramic disctype cone nozzles 0.40 m. Weed control, insect control, and fertilization were conducted according to standard recommended practices for the region and spray irrigation was applied to avoid water deficiencies.

4. Field evaluations

Field experiments were carried out under natural conditions for late blight development. Artificial inoculation was not used. In each plot, readings were made based on the leaf area percentage affected (Henfling 1987) by *P. infestans* starting from symptoms manifestation (74, 77, 86 and 99 days after planting (DAP)). With these infection percentage values, the area under disease progress curve (AUDPC) was estimated as established by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n-1} ((Xi + 1 + Xi)/\ 2)\ (ti + 1 - ti).$$

where Xi = the affected tissue rate in observation i, t = time, measured in days, in observation i and n = number of observations.

An additional variable, relative AUDPC (rAUDPC) was estimated by dividing AUDPC by the disease evaluation period duration. During the 2007/2008 season, radiation interception was determined using a radiation sensor bar Li-Cor 1914 SB (LICOR USA) at 45, 58 and 73 DAP, five measurements (one reading over canopy and four readings below the last green leaves strata) were determined for each plot. The interception rate was established according to the formula (1-A/A0) (100), where A is the radiation value reaching the soil and A0 the value registered above the crop. Potato plants, from the two middle rows, were harvested at maturity and potato tuber yield per plot in Kg was converted to t/ha.

In vitro experiments

1. Biological material

For the detached-leaf test (Goth and Keane 1997), a strain of *P. infestans* (race 2, 3, 6, 7, 8, 9; mating type A2 (Andreu *et al.* 2010), grown on rye-agar medium (Agar bacteriological, Agar Nº1, LP0011, OXOID Ltd, Basingstoke, England) and on potato tuber slices was used. Slices were incubated in closed plastic boxes with moist paper to maintain approximately 90% relative humidity and were maintained in the darkness at 18°C. After 7 days, the mycelium that developed on the slices was washed with distilled water, then filtered through 20 µm Nalgene filters and placed for 2 h at 4°C to stimulate the release of zoospores. The sporangial suspension was observed under light microscope for quantification before use as inoculum. The concentration of sporangia was adjusted to 40 000 sporangia/ml using a hematocytometer.

2. Evaluation of in vitro resistance

From each plot, two leaves per plant of the upper portion of the foliage of 10 plants, were cut, 45 DAP corresponding to maximum expression of resistance (Juan Landeo, International Potato Center, Peru), and placed in 15 cm Petri dishes on 2% water-agar. In the laboratory, the detached leaves were artificially inoculated with *P. infestans* sporangial suspension by placing a 50 µl droplet in the centre part of the abaxial side of each leaf. The Petri dishes were then placed in a growth chamber at 18-20°C and 80% relative humidity during the rest of the experiment. The damage growth rate (DGR) was estimated as established by Juárez Soto (2001). Each leaf was photographed as soon as the first necrotic damages (incubation period) were observed, 148 h after inoculation (HAI) when the damage area (DA) was estimated using Sigma Scan Pro software (Jandel Corporation, San Rafael, CA). With these values, DGR was estimated according to the formula:

$$DGR = \frac{\sqrt{\frac{DA}{\pi}}}{\frac{148 - IP}{24}}$$

where DGR = damage growth rate (mm/day), DA = damage area (mm^2), 148 = time through which DA was evaluated (HAI), IP = incubation period (HAI), 24 = hours to days conversion factor and $\pi = 3.14$.

Data analysis

Analysis of variance and simple regression analysis were made using GLM and REG procedures respectively, included in the software Statistical Analysis System (SAS, SAS Institute Inc. 1985). When significant treatment differences were found, the least significant difference (LSD) test was used.

RESULTS

Effect of calcium and potassium phosphite under field conditions

During the 2006/2007 season, the interaction between cultivar and treatment on AUDPC was determined (**Table 1**). In fact, on the 'Kennebec' cultivar, the PhiK treatment showed a significantly lower AUDPC than control and PhiCa treatments (396.4, 505.0 and 499.6%/days, respectively) ($P < 0.05$). However, no differences were detected between treatments on the 'Spunta' cultivar (417.6, 437.0 and 511.6%/days for PhiK, control and PhiCa treatments, respectively) (**Table 1**).

No significant cultivar × treatment interaction and no significant differences among treatments were observed in yield data (**Table 1**). However, PhiK application tended to increase yields by 20.5 and 14.8% in relation to control and PhiCa treatments, respectively. In contrast, there was a significant effect between cultivars in relation to yields (**Table 1**). Indeed, in 'Spunta', the yield was 16.8% higher than in 'Kennebec' cultivar (42.4 and 36.3 t/ha, respectively). In 2006/2007 season, an increase in the AUDPC value produced a decrease in yield in this experiment, establishing a linear, inverse and significant ($P < 0.05$) relationship between these two variables. However, AUDPC only accounted for 26% of the yield variation (**Fig. 1**).

In the 2007/2008 season, tubers were sown 22 days later than in the previous season (11/21/07 and 10/30/06, respectively). Thus, adequate crop growth and development for late blight infection fell behind until February, when high temperatures (**Fig. 2**) hindered disease expression. By the end of the crop cycle, abundant and prolonged rainfall (360 mm in 15 days) (**Fig. 2**) speeded up the disease development, creating then difficulties to correctly record late blight progress in this last stage. This situation disguised the differences between treatments. Hence, AUDPC could not be correctly estimated, neither could total yields, because of the excessive precipitation by the end of the crop cycle (**Fig. 2**) which caused tubers to rot in th e field.

In the 2007/2008 season, there were no significant differences ($P > 0.10$) in radiation intercepted between treatments (**Fig. 3**). However, in 'Kennebec' in the first samples, PhiK treatment intercepted more radiation than control. At 45 DAP, the average radiation intercepted was 65.9 and 69.4% for control and PhiK treatments, respectively (**Fig. 3**). On the other hand, the PhiCa treatment did not show differences with control on any sampling date. However, on 'Spunta', no significant differences between treatments were observed (**Fig. 3**).

Effect of calcium and potassium phosphite on the *in vitro* resistance

In the detached-leaf tests, it took 3-4 days to all combinations to sporulate (latent period), with high infecting efficiency (80-90%) and a lesion area of 25 to 70% of the leaflet. No significant cultivar × treatment interaction on DGR and no significant differences among cultivars were observed. Contrarily, significant differences among treatments were observed. In both cultivars, PhiK application resulted in a significantly lower DGR in relation to control and PhiCa treatments, although with less difference in 'Spunta' (**Table 1**).

Table 1 Total tuber yield, the area under the disease progress curve (AUDPC), and the relative area under the disease progress curve (rAUDPC) during the 2006/2007 growing season and average damage growth rate (DGR) of the detached-leaf test during the 2006/2007 and 2007/2008 growing seasons, for two cultivars: 'Spunta' (S) and 'Kennebec' (K) and three treatments: calcium phosphite (PhiCa), potassium phosphite (PhiK) and control (C).

Cultivar	Treatment	AUDPC %/days	Yield t/ha	rAUDPC	DGR mm/day
K	C	505.0	30.13	0.20	2.55
	PhiK	396.4	43.10	0.16	1.23
	PhiCa	499.6	35.73	0.20	2.55
S	C	437.0	42.48	0.17	2.40
	PhiK	417.6	44.35	0.17	1.43
	PhiCa	511.6	40.47	0.20	2.58
Means	K	467.0 a	36.32 a	0.19 a	2.11 a
Cultivars	S	454.4 a	42.43 b	0.18 a	2.13 a
Means	C	471.0 b	36.30 a	0.19 b	2.48 b
Treatments	PhiK	407.0 a	43.73 a	0.16 a	1.33 a
	PhiCa	505.6 b	38.10 a	0.20 b	2.56 b
		ANOVA (analysis of variance)			
Treatments		*	ns	*	**
Cultivars		ns	†	ns	ns
Cultivars × Treatments		†	ns	†	ns
CV (%)		22.2	17.9	22.2	34.0

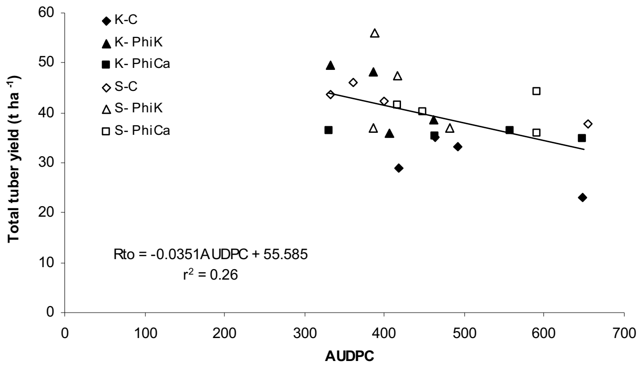

Fig. 1 Relation between total tuber yield and the area under the disease progress curve (AUDPC) during the 2006/2007 growing season for two cultivars: 'Spunta' (S) and 'Kennebec' (K) and three treatments: calcium phosphite (PhiCa), potassium phosphite (PhiK) and control (C).

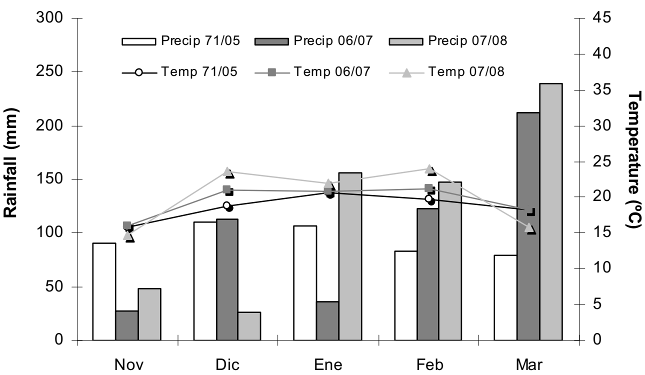

Fig. 2 Average monthly precipitation (mm) and temperature (ºC) during the 2006/2007 and the 2007/2008 growing seasons and history average for the period 1971/2005.

DISCUSSION

Resistant potato cultivars require less fungicide (Fry 1978). 'Spunta' and 'Kennebec' cultivars do not have quantitative resistance to late blight and have low specific resistance. Consequently, in Argentina, commercial potato production relies heavily on fungicide application. In the present work,

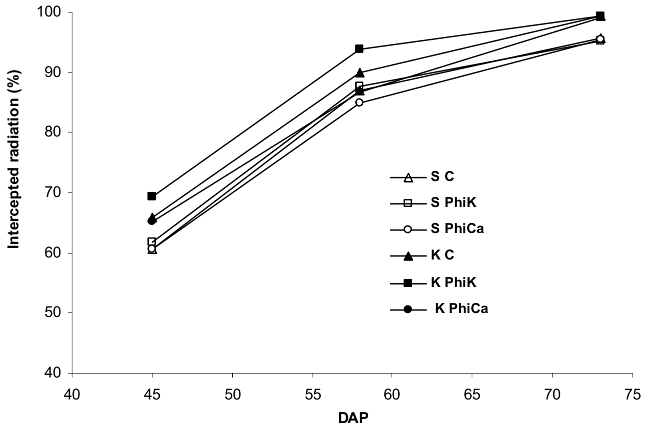

Fig. 3 Intercepted radiation as a function of time (expressed as days after planting (DAP) in the 2007/2008 growing season) for two cultivars: 'Spunta' (S) and 'Kennebec' (K) and three treatments: calcium phosphate (PhiCa), potassium phosphite (PhiK) and control (C).

the action of Phi in the defence response of potato plants to *P. infestans* and physiological parameters was studied. These compounds have a low environmental impact (Guest and Grant 1991). Therefore, they could be used to reduce fungicide input while achieving an acceptable control of late blight.

The results obtained indicated that Phi induced protection in foliage on 'Kennebec' (**Table 1**) which could be explained by the antifungal activity of Phi (Guest and Bompeix 1990; Lobato *et al*. 2010). Sporulation and germination of *Phytophthora* species have also been shown to be suppressed by these biopesticides (Garbelotto *et al.* 2009) because these compounds caused hyphal distortions and lysis of cell walls and had an adverse effect on hyphal growth (King *et al.* 2010). In addition, these biopesticides can activate the defence mechanisms (Smillie *et al*. 1989; Saindrenan and Guest 1994; Guest and Grant 1991; Daniel and Guest 2006; Andreu *et al*. 2006; Lobato *et al*. 2008; Lobato *et al.* 2011). In this regard, Lobato *et al.* (2008) determined that Phi application increases the expression of β-1,3-glucanases which have an anti-fungal effect and might increase plant resistance by inductor production. No differences were detected between treatments in 'Spunta' (**Table 1**). These results were consistent with previous studies which indicated that the effects of these compounds vary depending on potato cultivar and Phi type (Andreu *et al*. 2006; Lobato *et al.* 2008). The differences between cultivars may be explained by factors such as the skin ability and epidermal cells resistance to penetration, and chemical activity in leaves that inhibits spore germination and penetration (Henfling 1987). In addition, differences were detected between PhiK and PhiCa treatments, suggesting that the protective effect of Phi against pathogens can be affected by the cationic form of Phi used (Lobato *et al.* 2008) and by the amount of active ingredients of each compound (Taipe *et al.* 2008).

Mayton *et al.* (2008) point out that Phi could reduce foliar late blight symptoms. However, they also state that no Phi application was as effective as conventional treatments. Furthermore, these authors showed that the combined application of Phi and fungicides did not differ significantly from conventional treatment. Thus, Phi could be useful to control late blight in foliage within an integrated management scheme (Mayton *et al.* 2008), reducing, thus, the environmental impact compared to conventional treatments (Kovach *et al.* 1992).

PhiK application tended to increase yields even though no significant differences were observed among treatments (**Table 1**). Although lower AUDPC was found, no significant tuber yield increments due to PhiK application were found because of the low infection levels during the evaluation period (expressed in rAUDPC). These results agree with those reported by Inglis *et al.* (1997). Hence, late blight had little influence in potato crop yield determination in this experiment (**Fig. 1**). In addition, significant differences between cultivars were observed based on yield data. In fact, in 'Spunta', the yield was 16.8% more than 'Kennebec' (**Table 1**). Caldiz (2006) points out that although 'Spunta' is a high yielding cultivar, quality is low and its susceptibility to potato blight is high. Thus, in 'Spunta', the yield was less negatively associated with AUDPC than in 'Kennebec' ($r^2 = 0.21$ and $r^2 = 0.38$, respectively).

The greatest growth in early stages resulted from an earlier emergence of plants. Lobato *et al.* (2008) reported that Phi application to seed tubers resulted in earlier emergence of plants. By the time that control plants reached approximately 80% emergence, Phi-treated seed tubers reached at least 99% emergence for PhiK treatment. Growth is a direct function of the radiation intercepted by the crop (Echeverría 2005). In the 2007/2008 season, no significant differences ($P > 0.10$) in radiation intercepted were found between treatments at any sampling date (**Fig. 2**). However, in the first 'Kennebec' samples, PhiK treatment intercepted more radiation than control (**Fig. 2**). Based on these results, the emergency would have been greater in 'Kennebec' due to PhiK application. Contrarily, Thao *et al.* (2009) did not detect any beneficial effect of Phi on plant growth and suggested that in practical agricultural production, the application of Phi might have some positive effects on plant growth as a result of the fungicidal properties of this compound.

In the detached-leaf tests, the PhiK treatment showed a significantly lower DGR in relation to control (**Table 1**). This may be explained by the suppression of spore production and germination of *Phytophthora* sp. (Cohen and Coffey 1986), as well as mycelium growth inhibition and the reduction or alteration of phosphorylation reactions on the pathogen (Guest and Bompeix 1990). In addition, no significant differences among PhiCa and control treatments were observed (**Table 1**), coinciding with the results of AUDPC recorded under field conditions (**Table 1**).

In the 2006/2007 season, the correlation between DGR and AUDPC was low (data not shown). This can occur because in the field genotype behaviour towards disease may vary depending on environmental conditions (Turkensteen 1993). Furthermore, detached-leaf tests can show considerable differences in some cultivars between experiments and disease severity may also vary within an experiment. Thus, this type of test requires a large number of repetitions in order to verify results (Dorrance and Inglis 1997).

CONCLUSIONS

In the present study, Phi application decreased late blight severity and increased yields, with differences between cultivars and treatments. These results suggest the need to continue analyzing Phi application for disease control in potato crops with different Phi rates, Phi types, disease pressure and cultivars, in order to evaluate the usefulness of this compound as a potential control method and to reduce the use of conventional fungicides which have a detrimental impact on the environment.

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