

## Antifungal Activity of Silver Ions and Nanoparticles on Phytopathogenic Fungi

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### ABSTRACT

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Silver in ionic or nanoparticle forms has a high antimicrobial activity and is therefore widely used for various sterilization purposes including materials of medical devices and water sanitization. There have been relatively few studies on the applicability of silver to control plant diseases. Various forms of silver ions and nanoparticles were tested in the current study to examine the antifungal activity on two plant-pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea*. In vitro petri dish assays indicated that silver ions and nanoparticles had a significant effect on the colony formation of these two pathogens. Effective concentrations of the silver compounds inhibiting colony formation by 50% (EC<sub>50</sub>) were higher for *B. sorokiniana* than for *M. grisea*. The inhibitory effect on colony formation significantly diminished after silver cations were neutralized with chloride ions. Growth chamber inoculation assays further confirmed that both ionic and nanoparticle silver significantly reduced these two fungal diseases on perennial ryegrass (*Lolium perenne*). Particularly, silver ions and nanoparticles effectively reduced disease severity with an application at 3 h before spore inoculation, but their efficacy significantly diminished when applied at 24 h after inoculation. The in vitro and in planta evaluations of silver indicated that both silver ions and nanoparticles influence colony formation of spores and disease progress of plant-pathogenic fungi. In planta efficacy of silver ions and nanoparticles is much greater with preventative application, which may promote the direct contact of silver with spores and germ tubes, and inhibit their viability.

Silver is a prehistoric element. Even ancient Babylonians and Greeks possessed many silver mines (23). However, the use of silver for medicine or local antibacterial agents was not recognized until the nineteenth century (9,19,28). Since then, the antimicrobial property of silver has been investigated and employed more extensively than any other inorganic antibacterial agent (24). Silver is known to attack a broad range of biological processes in microorganisms including the alteration of cell membrane structure and functions (16,20,26). Silver also inhibits the expression of proteins associated with ATP production (30), although its specific antimicrobial mechanisms are still not completely understood.

Micromolar doses (1 to 10  $\mu$ M) of silver ions are sufficient to kill bacteria in water (14), while silver can be toxic at high doses to mammals (5,8) and freshwater and marine organisms (3,17), probably compromising the growth and shape of

animal cells by disrupting a variety of biological functions (5). Such micromolar concentrations of silver have no harmful effects on humans (2). Therefore, silver has been widely used for the development of many biological and pharmaceutical processes, products, and appliances such as coating materials for medical devices (22), orthopedic or dental graft materials (10,15), topical aids for wound repair (6), water sanitization (9,13,14), textile products (27), and even washing machines (12).

The use of nano-sized silver particles as antimicrobial agents has become more common as technological advances make their production more economical. One of the potential applications in which silver can be utilized is in management of plant diseases. Since silver displays multiple modes of inhibitory action to microorganisms (4), it may be used for controlling various plant pathogens in a relatively safer way compared to synthetic fungicides (21). Until now, limited research provided some evidence of the applicability of silver for controlling plant diseases (21). Primary requirements for the potential use of silver in plant disease control include more information about antifungal activity of various silver compounds to plant pathogens and the development of better application strategies to increase the efficacy of disease suppression. The objectives of this study were to determine the inhibitory property of silver ions and

nanoparticles on colony formation of plant-pathogenic fungi, and to evaluate the efficacy of the silver compounds for disease control on plants.

### MATERIALS AND METHODS

**Fungal isolates.** Two conidia-producing fungi that are economically important pathogens of grasses were used in the study: *Bipolaris sorokiniana* (Sacc.) Shoem, which causes seedling blight, root rot, crown rot, and leaf spot blotch on various gramineous species, and *Magnaporthe grisea* (Hebert) Barr, which causes blast on rice (*Oryza sativa* L.) and gray leaf spot on turfgrass. A single-spore isolate of each fungus was used to avoid possible compound effects caused by using a mixture of genetically different isolates. A single-spore isolate (LS-WI06) of *B. sorokiniana* was collected from infected plants of perennial ryegrass (*Lolium perenne* L.) in Verona, WI in 2006. One lab isolate (6082) of *M. grisea*, which was capable of pathogenicity on both rice and perennial ryegrass, was provided by S. Leong at the University of Wisconsin. For long-term storage, these isolates were stored as frozen conidia on filter paper at  $-20^{\circ}\text{C}$ .

**Silver preparation.** The silver preparations used in this study were AgNO<sub>3</sub>, AgCl, nanoparticle Ag (20 to 30 nm in diameter; Quantum Sphere Inc., Santa Ana, CA [indicated as Ag(p)]), and electrochemical Ag (indicated as Ag(e)). For preparing AgCl and Ag(p), AgCl salt or Ag(p) powder was weighed and suspended in sterile, deionized water using a sonicator (Branson, Branson Ultrasonic Corporation, Danbury, CT). Ag(e) solution was generated by electrolysis at 30 V with two silver electrodes (10 cm long, 2 mm diameter, and 3 cm between electrodes) in 500 ml of distilled water as previously described by Simonetti et al. (25). Silver ion concentrations of each silver preparation were measured at room temperature using an ion meter equipped with a silver ion-selective electrode (Weiss Research Inc., Houston, TX). Particularly, to further examine the antifungal effect of silver ions, each silver preparation was titrated with NaCl (500 mM) until silver ions were removed and undetected by the ion meter. To prepare different silver concentrations, subsamples of each silver preparation were diluted with sterile, deionized water. The range of silver concentrations used to test the sensitivity of each pathogen to each

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silver preparation was determined based on our preliminary trials and is listed in Table 1.

**Inhibition of colony formation by silver.** The antifungal activity of silver ions and nanoparticles was examined on the basis of colony formation by in vitro petri dish assays. Each fungal isolate was cultured on growth media that induced prolific conidia production. The *B. sorokiniana* isolate (LS-WI06) was grown on V8 agar medium, and the *M. grisea* isolate (6082) was grown on oatmeal agar medium. Conidia were collected from cultures that were incubated at 25°C for 10 days and diluted with sterile, deionized water to a concentration of 10<sup>6</sup> spores ml<sup>-1</sup>. Aliquots of the conidial suspension were mixed with serial concentrations of silver preparations to a final volume of 1 ml (Table 1), and were also mixed with sterile, deionized water as water controls.

A 10-μl subsample of the conidia and silver mixture stock was taken at 0, 1, 3, and 6 h after silver treatments and diluted 100-fold with sterile, deionized water. A 10-μl aliquot of the diluted spore suspension was spread on potato dextrose agar (PDA; Becton, Dickson and Company, Sparks, MD) medium. PDA was used because it was easier to examine colony formation than on V8 or oatmeal medium. Three PDA plates per each combination of exposure time and silver concentration were tested. The number of colonies forming on plates was counted after 2 and 4 days at 25°C for *B. sorokiniana* and *M. grisea*, respectively. The average number of colonies from silver-treated spore suspensions was compared with the number on the water control (percent colony formation) in order to calculate the concentration of each silver preparation that caused a 50% reduction in colony formation (EC<sub>50</sub>). This experiment was conducted twice. For each treatment combination of exposure time and silver concentration, a linear regression of the percent colony formation versus the log-transformed silver concentration was obtained to calculate the EC<sub>50</sub> value.

**In planta efficacy of silver.** The efficacy of silver ions and nanoparticles in reducing diseases on plants was examined with in planta inoculation experiments

conducted in a growth chamber. Perennial ryegrass was seeded on 25 cm<sup>2</sup> pots filled with potting mix (Metro-Mix, Sun Gro, Bellevue, WA), and 20 to 25 plants were grown per plot for 1 month before use in the experiment. These pots were arranged at random with four replicates per treatment. Silver compounds were diluted to two different concentrations with sterile, deionized water (Table 2) and mixed with 0.2% surfactant (Spreader Sticker, Gardens Alive Inc., Lawrenceburg, IN) before application.

To examine whether application timing would affect the efficacy of silver, the silver preparations were applied at two times: 3 h before or 24 h after conidia inoculation. For preinoculation treatments, silver preparations were sprayed on perennial ryegrass plants with an aspirator (Badger 350, Badger Air-Brush Co., Franklin Park, IL) and allowed to air-dry at room temperature for 3 h followed by inoculation with conidial suspensions (10<sup>5</sup> conidia ml<sup>-1</sup> + 0.2% Tween 20) of *B. sorokiniana* or *M. grisea*. The inoculated plants were incubated in a plastic container

with lid (Sterilite, Townsend, MA; 76 cm L × 45 cm W × 33 cm H) for 24 h at 25°C and 100% relative humidity, and then transferred to a growth chamber (25°C, 40 ± 10% relative humidity, and 12-h photoperiod). In the case of the postinoculation treatments, plants were inoculated with conidial suspensions of *B. sorokiniana* or *M. grisea* and then incubated in the plastic container (100% relative humidity; 25°C) for 24 h prior to silver treatments. The plants were treated with silver compounds as previously described and transferred to a growth chamber. Severity of leaf spot or gray leaf spot was measured as percent diseased areas of 10 randomly selected leaves per pot at 7 days after inoculation. The inoculation experiment was performed twice for each disease. Analysis of variance (ANOVA) was conducted to test the efficacy of silver preparations against the two diseases.

## RESULTS

**Inhibition of colony formation by silver.** Silver compounds showed various

**Table 2.** Disease severity (%) of leaf spot caused by *Bipolaris sorokiniana* and gray leaf spot caused by *Magnaporthe grisea* on perennial ryegrass treated with silver preparations and averaged across two experiments

Treatment [conc. ppm]	<i>B. sorokiniana</i>		<i>M. grisea</i>	
	Pre-inoc <sup>a</sup>	Post-inoc <sup>b</sup>	Pre-inoc	Post-inoc
Water	84.4	87.5	70.0	62.5
AgNO <sub>3</sub> [50]	0.6*	85.0	0.0*	52.5
AgNO <sub>3</sub> [25]	2.9*	88.8	0.3*	58.8
Ag(p) [200] <sup>d</sup>	6.9*	80.0	3.1*	53.8
Ag(p) [100]	26.9*	91.3	5.1*	60.0
Ag(e) [50] <sup>e</sup>	5.8*	62.5*	2.4*	53.1
Ag(e) [25]	15.0*	87.5	4.1*	57.5
AgNO <sub>3</sub> [50] + NaCl <sup>f</sup>	71.3*	90.0	39.4*	67.5
AgNO <sub>3</sub> [25] + NaCl	77.5	91.9	43.8*	75.0
Ag(p) [200] + NaCl	18.1*	75.0*	5.8*	55.0
Ag(p) [100] + NaCl	20.0*	83.8	8.9*	54.4
Ag(e) [50] + NaCl	8.8*	80.0	16.4*	56.3
Ag(e) [25] + NaCl	32.3*	82.5	51.3*	55.0
AgCl [200]	80.6	81.3	55.0*	61.3
AgCl [100]	82.5	91.9	76.9	60.0
LSD at P = 0.01	11.1	10.3	13.1	22.8

<sup>a</sup> Silver preparations were sprayed on plants 3 h before inoculation of spores (10<sup>5</sup> conidia ml<sup>-1</sup>).

<sup>b</sup> Silver preparations were sprayed on plants 24 h after inoculation with spores (10<sup>5</sup> conidia ml<sup>-1</sup>).

<sup>c</sup> Asterisks indicate significantly less disease compared with water control using Fisher's protected least significance differences at P = 0.01.

<sup>d</sup> Nanoparticle silver, 20 to 30 nm.

<sup>e</sup> Electrochemical silver generated by electrolysis.

<sup>f</sup> Neutralized by adding NaCl to [Ag<sup>+</sup>] = 0.

**Table 1.** Concentrations of silver preparations used for in vitro colony formation assay<sup>a</sup>

Treatment	pH	Conc. for <i>Bipolaris sorokiniana</i> (ppm)					Conc. for <i>Magnaporthe grisea</i> (ppm)				
AgNO <sub>3</sub>	5.8	100	10	5	1	0.5	5	1	0.5	0.25	0.1
Ag(p) <sup>b</sup>	7.8	500	250	125	100		200	100	50	25	
Ag(e) <sup>c</sup>	7.4	125	62.5	31.25	25		50	25	12.5	6.25	
AgCl	6.1	10,000					10,000				
AgNO <sub>3</sub> + NaCl <sup>d</sup>	6.2	100	10	5	1	0.5	5	1	0.5	0.25	0.1
Ag(p) + NaCl	8.3	500	250	125	100		200	100	50	25	
Ag(e) + NaCl	7.4	125	62.5	31.25	25		50	25	12.5	6.25	

<sup>a</sup> Concentrations used in this study were determined in pilot experiments.

<sup>b</sup> Nanoparticle silver, 20 to 30 nm.

<sup>c</sup> Electrochemical silver generated by electrolysis.

<sup>d</sup> Neutralization by adding NaCl to obtain [Ag<sup>+</sup>] = 0.

levels of inhibition on colony formation of *B. sorokiniana* (Fig. 1) and *M. grisea*. As concentrations of the silver compounds increased, colony formation decreased. Antifungal activity of silver to reduce the colony formation was apparent within 1 h. Extended exposure times of spores to AgNO<sub>3</sub> (Figs. 2A and 3A), Ag(p) (Figs. 2B and 3B), and Ag(e) (Figs. 2C and 3C) from 1 h up to 6 h did not significantly reduce colony formation ( $P > 0.05$ ).

Since the measurement of colony formation did not differ significantly between two repeated experiments ( $P > 0.05$ ), the data from the two independent experiments were averaged for each treatment and used to calculate EC<sub>50</sub> (Table 3). The range of EC<sub>50</sub> at 1 to 6 h after silver treatment on *B. sorokiniana* conidia was 1.2 to 2.2 ppm of AgNO<sub>3</sub>, 4.8 to 8.8 ppm of Ag(p), and 6.1 to 8.4 ppm of Ag(e). The EC<sub>50</sub> values for *M. grisea* were always numerically lower than those for *B. sorokiniana*, ranging 0.8 to 1.0 ppm of AgNO<sub>3</sub>, 3.9 to 4.7 ppm of Ag(p), and 5.6 to 6.8 ppm of Ag(e) at 1 to 6 h after silver treatment.

The antifungal activity of AgNO<sub>3</sub>, Ag(p), and Ag(e) against *B. sorokiniana* was significantly diminished by chloride ions ( $P < 0.01$ ). Silver cations of these silver preparations were neutralized with NaCl to produce AgCl, which caused an increase in the average colony formation of *B. sorokiniana* by 20 to 30% compared with corresponding nonneutralized silver preparations (Fig. 2). In comparison to *B. sorokiniana*, *M. grisea* was more sensitive to the neutralized silver preparations, showing much lower EC<sub>50</sub> values (Table 3). The antifungal activity of Ag(p) against *M. grisea* significantly diminished after neutralization by NaCl ( $P < 0.01$ ), but neutralized AgNO<sub>3</sub> and Ag(e) still showed similar antifungal activities as corresponding nonneutralized silver solutions (Table 3 and Fig. 3).

AgCl at a relatively high concentration (10,000 ppm) almost completely inhibited colony formation of both *B. sorokiniana* and *M. grisea*, and was also significantly influenced by chloride ions. An addition of NaCl to the AgCl preparation resulted in increased colony formation of *B. sorokiniana* up to 64.3% after 1 h exposure, 32.3% after 3 h exposure, and 10.8% after 6 h exposure. In the case of *M. grisea*, the AgCl preparation treated with NaCl increased colony formation up to 27.2% after 1 h exposure, 19.3% after 3 h exposure, and 16.1% after 6 h exposure.

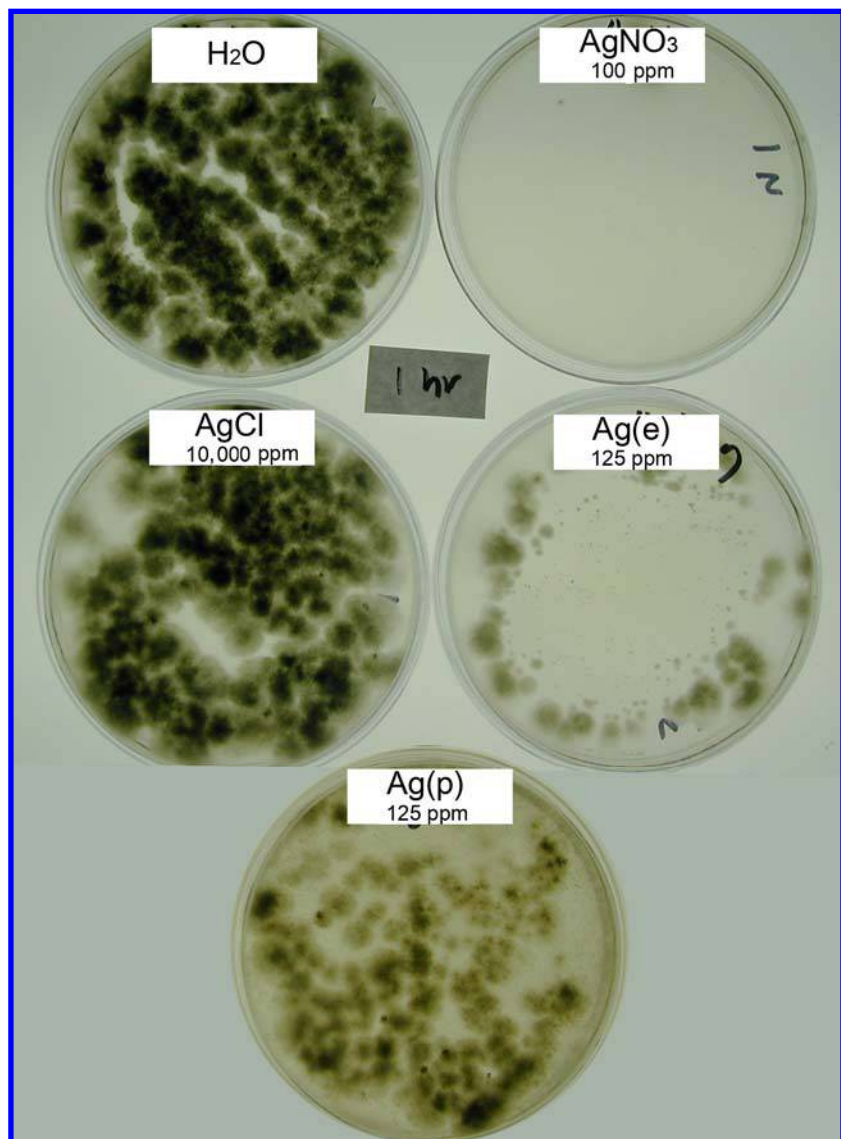
**Reduction of disease severity in plants by silver.** Silver ions and nanoparticles effectively reduced leaf spot (Fig. 4) and gray leaf spot on perennial ryegrass without noticeable phytotoxicity, but NaCl significantly decreased their antifungal efficacy. The efficiency of silver in reducing the diseases significantly differed between 3 h before and 24 h after conidia

inoculation ( $P < 0.01$ ). Most silver preparations applied at 3 h before spore inoculation on the plants significantly reduced both diseases compared with the water control ( $P < 0.01$ ). The preinoculation applications of AgNO<sub>3</sub> (25 and 50 ppm), Ag(p) (200 ppm), and Ag(e) (50 ppm) were found to be the most effective, allowing less than 7% foliar damage by *B. sorokiniana* and *M. grisea* under highly disease-conducive environmental conditions, which caused over 70% damage on water-treated control plants (Table 2). Some preinoculation treatments of neutralized AgNO<sub>3</sub>, Ag(p), and Ag(e) but not AgCl moderately reduced disease, but they still were less effective than nonneutralized silver preparations (Table 2). In contrast, delayed applications of silver preparations at 24 h after spore inoculation (postinoculation treatments) did not effectively reduce both diseases, causing >50% foliar damage (Table 2).

## DISCUSSION

Management of fungal diseases on food crops and ornamental plants is economically important. Recently, more efforts have been given to develop safe management methods that pose less danger to humans and animals, and have focused on overcoming deficiencies of synthetic fungicides. The current investigation showed that silver ions and nanoparticles with low toxicity and a broad spectrum of antimicrobial activity were also very effective for reducing plant diseases caused by spore-producing phytopathogenic fungi. However, the extrapolation of these findings to more general cases is limited due to the fact that this current study is based on in vitro petri dish and in planta growth chamber evaluations. Still, this research provides valuable preliminary efficacy data of silver compounds for control of plant diseases.

There have been numerous reports of the antimicrobial activity of silver. How-



**Fig. 1.** Colony formation of *Bipolaris sorokiniana* on potato dextrose agar medium, 1 h post-treatment of spores with water, AgNO<sub>3</sub>, AgCl, silver nanoparticles [Ag(p)] and electrochemical silver [Ag(e)]. Images were captured after culture plates had been incubated for 6 days at 25°C.

ever, antifungal properties of different forms of silver often coexisting in solution have not been investigated fully. Furthermore, there have been few studies conducted for testing antifungal activity of silver for plant-pathogenic fungi. Results presented in this study confirm that silver ions and nanoparticles have significant

inhibitory effects on colony formation from conidia of *B. sorokiniana* and *M. grisea*. The sensitivities of the two fungal species to silver compounds were different. Conidia of *B. sorokiniana*, with bigger size (median length more than 60  $\mu\text{m}$ ) and darker color, showed higher tolerance to silver than those of *M. grisea* (17 to 28

$\mu\text{m}$ ). This is consistent with previous reports that stated antimicrobial activity of silver was different depending on microbial species (7,25,27). The colony formation from conidia of both plant-pathogenic fungi was influenced in the range of  $\text{EC}_{50}$  values of 0.9 to 2.2 ppm of  $\text{AgNO}_3$ , 3.9 to 8.8 ppm of  $\text{Ag(p)}$ , and 5.6 to 8.4 ppm of  $\text{Ag(e)}$ . This

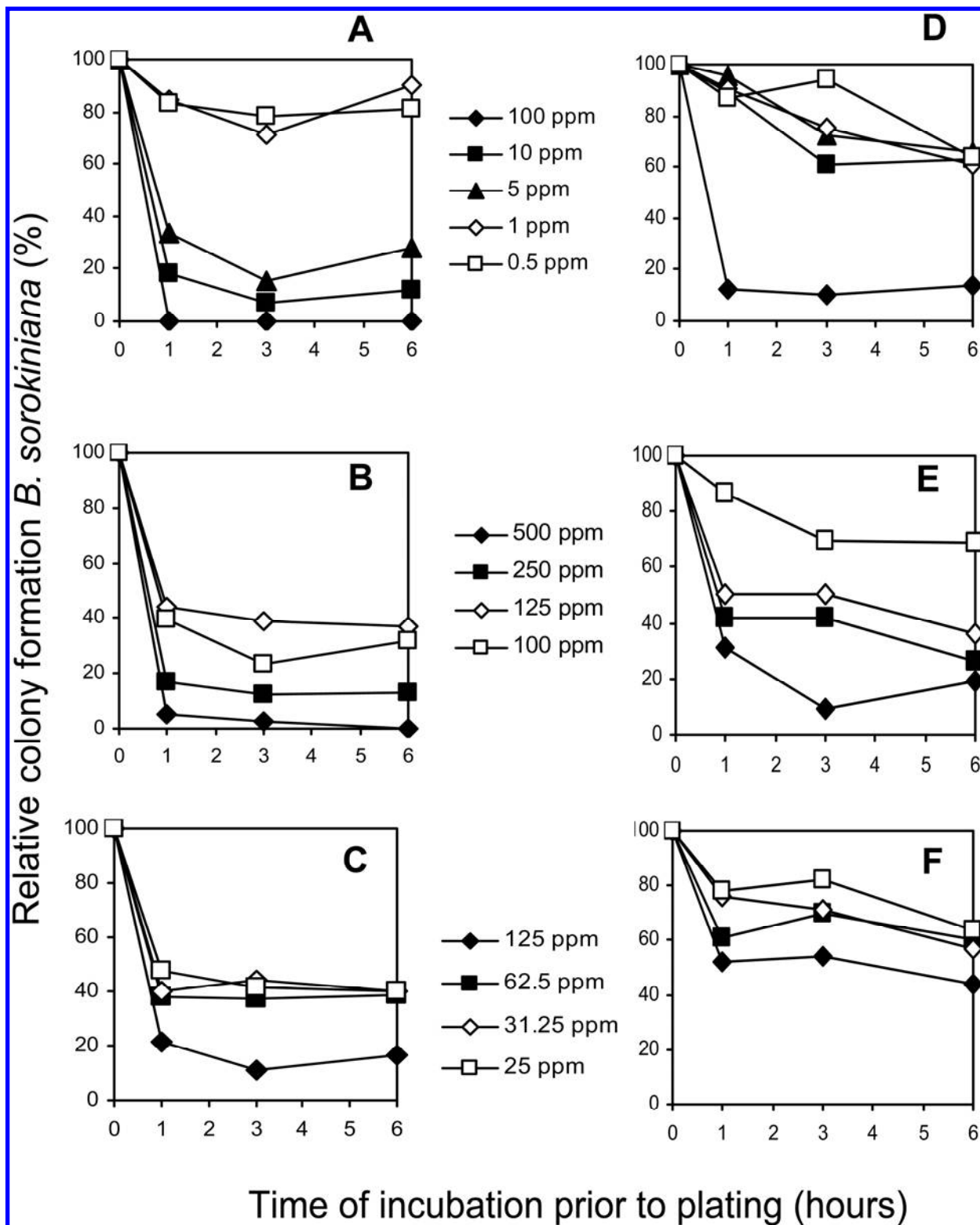


Fig. 2. Colony formation of *Bipolaris sorokiniana* affected by A,  $\text{AgNO}_3$ , B, silver nanoparticles [Ag(p)], C, electrochemical silver [Ag(e)], D, neutralized  $\text{AgNO}_3$ , E, neutralized Ag(p), and F, neutralized Ag(e) after silver cations were removed by NaCl.

range of silver doses is 1 to 8 times higher than the maximal concentration of silver (1.142 ppm) allowed in drinking water for human consumption for short-term exposure (1 to 10 days) by the U.S. EPA (1).

This study was the first to demonstrate that ionic forms of silver have strong antifungal activity against *B. sorokiniana* and *M. grisea*, while their antibacterial effects have been previously well described (18,20,25). The silver ionic forms of  $\text{AgNO}_3$  caused a significant reduction of colony formation within 1 h at low concen-

trations ( $\text{EC}_{50}$  for *B. sorokiniana* = 2.2 ppm and  $\text{EC}_{50}$  for *M. grisea* = 0.9 ppm). Another piece of direct evidence of the importance of silver ions for antifungal activity is that silver compounds are significantly influenced by NaCl. After silver cations of  $\text{AgNO}_3$  reacted with chloride ions and formed the insoluble precipitate  $\text{AgCl}$ ,  $\text{AgNO}_3$  showed a dramatic reduction in antifungal activity and yielded higher  $\text{EC}_{50}$  values than nonneutralized  $\text{AgNO}_3$  (Table 3).  $\text{AgCl}$  has also been known to have antimicrobial activity (25)

but was much less effective than silver ions for inhibiting colony formation and suppressing disease development by *B. sorokiniana* and *M. grisea*.

Silver nanoparticles showed significant antifungal activity in both in vitro petri dish assays and in planta inoculation experiments. Silver nanoparticles may directly attach to and penetrate the cell membrane to kill spores, although penetration of silver nanoparticles into microbial cell membranes is not completely understood (18). There was a significant reduc-

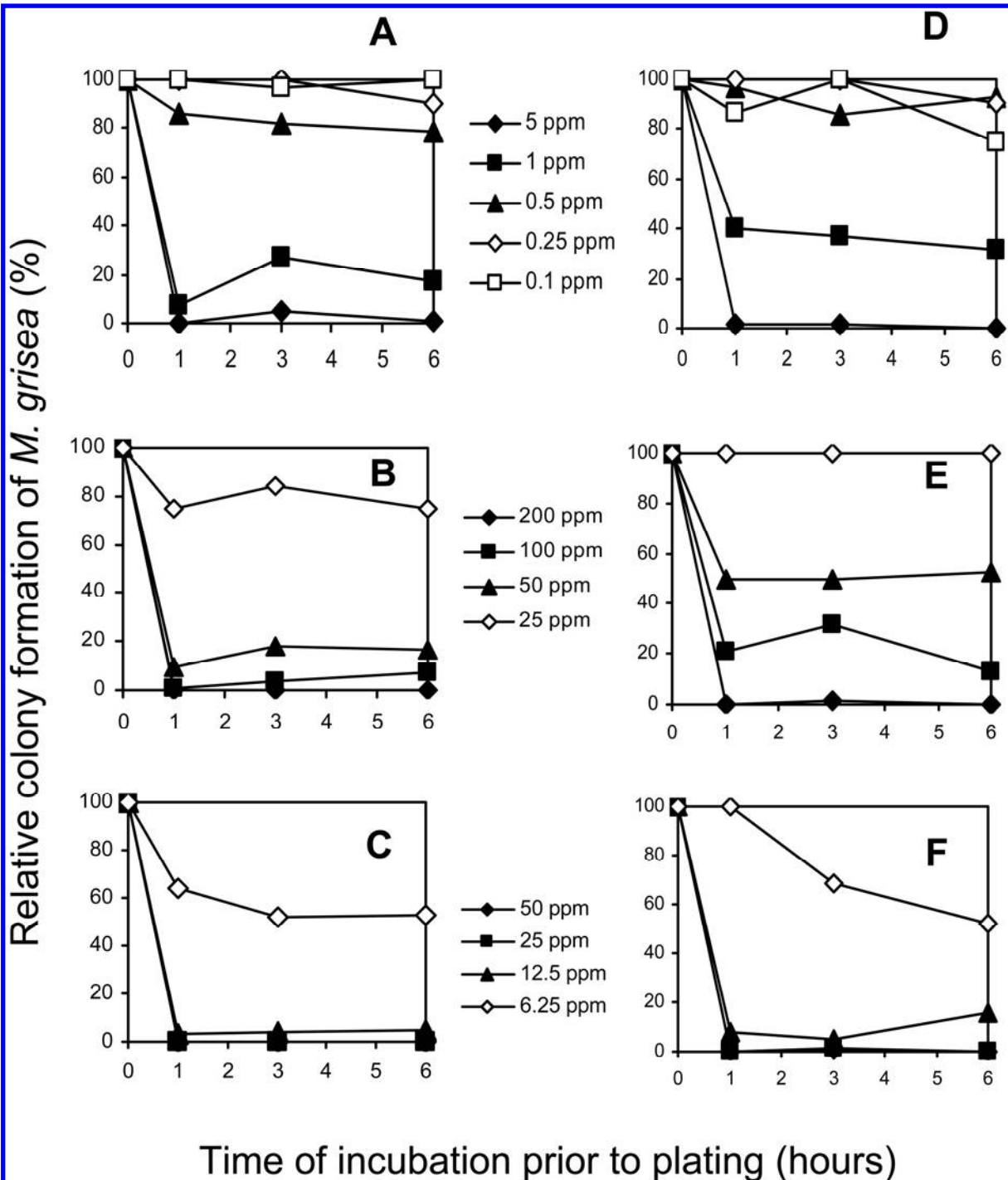


Fig. 3. Colony formation of *Magnaporthe grisea* affected by A,  $\text{AgNO}_3$ , B, silver nanoparticles [Ag(p)], C, electrochemical silver [Ag(e)], D, neutralized  $\text{AgNO}_3$ , E, neutralized Ag(p), and F, neutralized Ag(e) after silver cations were removed by NaCl.

tion of antifungal activity of Ag(p) after neutralization of silver cations with NaCl (Figs. 2B and E, and 3B and E). The reduction of antifungal activity results from limiting the concentration of silver cations in solution by producing AgCl, a less effective form of silver. Similarly, the antifungal activity of Ag(e) generated by electrolysis was significantly reduced after neutralization by NaCl, indicating that its primary antifungal property is attributable to silver ions (Figs. 2C and F, and 3C and F). In addition to ions, nano-sized particles in Ag(e) are likely to be involved in affecting spores, which might account for the remaining antifungal property of Ag(e).

The preventative application of the silver preparations more effectively reduced disease severity on plants than the postinoculation application. A mechanism of this antifungal activity is suggested by the germination and infection process in these fungi. Both *B. sorokiniana* and *M. grisea* cause foliar diseases and reproduce as asexual conidia. Disease infection is initiated by the attachment of spores to the

plant surface and formation of germ tubes (29). Under favorable conditions of high humidity (~100% relative humidity) and warm temperature (25°C), conidia germinate, and the resulting germ tubes penetrate plant surfaces within 24 h (11). Antifungal efficiency of silver was reduced at 24 h after inoculation, suggesting that direct contact of silver with spores or germ tubes is critical in inhibiting disease development.

In summary, antifungal activity of ionic or nanoparticle silver has a great potential for use in controlling spore-producing fungal plant pathogens. Silver may be less toxic to humans and animals than synthetic fungicides. Multiple modes of action targeting a broad range of biological pathways of microbes provide an important benefit for avoiding the development of resistance, which has been increasingly important in terms of current issues for the chemical management of many plant fungal diseases. Since the efficacy of silver is greatly influenced by application timing, preventative applications of silver ions and

nanoparticles work better before spores penetrate and colonize within the plant tissue. Our follow-up research focuses on extended applicability of silver for control of *B. sorokiniana* and *M. grisea* in the field, and evaluation of the efficacy of silver on different types of pathogens such as soilborne sterile fungi that rarely produce spores. Further research should focus on the development of silver compounds, compositions, and carriers than have improved stability against chloride ions. At the same time, the environmental tracking of silver when applied in the field is important to assess the impact on environmental and human health. This information is imperative for future registration and labeling of the silver nanoparticles as fungicides for crop protection.

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**Table 3.** Effective concentration of silver preparations to cause a 50% reduction in colony formation (EC<sub>50</sub>) for *Bipolaris sorokiniana* and *Magnaporthe grisea*

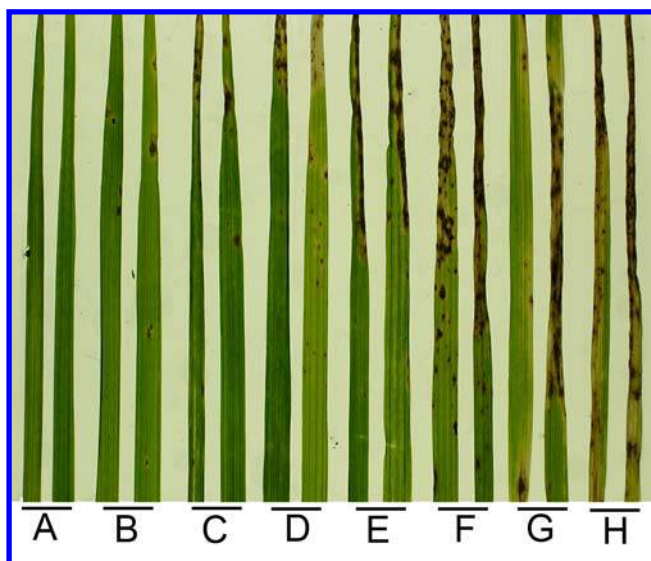
Treatment	<i>B. sorokiniana</i> (ppm)			<i>M. grisea</i> (ppm)		
	1 HAI <sup>a</sup>	3 HAI	6 HAI	1 HAI	3 HAI	6 HAI
AgNO <sub>3</sub>	2.2	1.2	2.0	0.9	1.0	0.8
Ag(p) <sup>b</sup>	8.8	4.8	5.4	3.9	4.7	4.0
Ag(e) <sup>c</sup>	8.4	6.1	6.1	6.8	5.7	5.6
AgNO <sub>3</sub> + NaCl <sup>d</sup>	92.9	13.6	6.9	1.2	1.1	1.1
Ag(p) + NaCl	216.8	160.5	121.4	60.7	64.4	59.2
Ag(e) + NaCl	134.9	182.7	92.3	10.3	7.4	6.1

<sup>a</sup> Hour after incubation prior to plating.

<sup>b</sup> Nanoparticle silver, 20 to 30 nm.

<sup>c</sup> Electrochemical silver generated by electrolysis.

<sup>d</sup> Neutralization by adding NaCl to obtain [Ag<sup>+</sup>] = 0.



**Fig. 4.** Disease symptoms on leaves of perennial ryegrass 7 days postinoculation with *Bipolaris sorokiniana*. Three hours before conidia inoculation, the plants were treated with **A**, electrochemical silver [Ag(e), 50 ppm], **B**, silver nanoparticles [Ag(p), 200 ppm], **C**, AgNO<sub>3</sub> (50 ppm), **D**, neutralized Ag(e) (50 ppm + NaCl), **E**, neutralized Ag(p) (200 ppm + NaCl), **F**, neutralized AgNO<sub>3</sub> (50 ppm + NaCl) after silver cations were removed by NaCl, **G**, AgCl (200 ppm), and **H**, water.

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