Effect of Formulated Yeast in Suppressing the Liberation of *Botrytis cinerea* Conidia

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ABSTRACT

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Eight yeast isolates that bound directly to *Botrytis cinerea* germings were assessed for the ability to suppress spore liberation of conidia from *B. cinerea*. After the yeast cell suspension from each isolate was mixed with cellulose and dried, the product was milled into a fine powder. This yeast-cellulose formulation was applied as a dry powder to sporulating *B. cinerea* colonies on kiwifruit (*Actinidia deliciosa*) leaf disks, where the particles from the formulation attached to conidiophores and conidia. Some of these formulations significantly suppressed the liberation of conidia from treated colonies. Suppression of conidial liberation could provide another management tool for the biological control of sporulating *B. cinerea* with applications during late epidemic development. Using α -cellulose prepared with *Candida pulcherrima* in the conditions imposed in the present study, there was an approximately 50% reduction in the number of conidia released with the treatment of the *B. cinerea* lesions. The suppression of disease through a reduction in the population of liberated conidia is discussed.

Additional keywords: adhesion, attachment, biocontrol, cellulose, Sigmacell

Spore transport is an important process in the development of disease epidemics (1). Dispersal of the pathogen *Botrytis cinerea* (Pers.:Fr.), can be achieved by various mechanisms (19), including airborne conidia (13,20), insect-vectored conidia (14,15), or splash-dispersed conidia (19). The contribution made by airborne conidia to epidemic development varies with the pathosystem under consideration (12,20).

Reduction in the number of airborne conidia by suppressing *B. cinerea* sporulation has been shown to be a successful approach for management of this pathogen (27,29). Kohl et al. (23) manually removed necrotic leaves from a planting of onions, which reduced the spore load above the crop and significantly retarded epidemic development. A similar study in kiwifruit confirmed that removal of necrotic tissue to suppress spore populations resulted in a significant reduction in postharvest disease (12). A number of fungi and yeasts have been reported to reduce the sporulation of *B. cinerea* (11,27) and some have significantly retarded epidemic development in the field or under glasshouse environments (21,22,24). The use of plastic filters to

exclude specific wavelengths of sunlight suppressed sporulation in vitro (10,13) but results were inconsistent in the glasshouse (10). Reductions of 35 to 75% in aerial spore populations were reported but these levels did not reduce disease incidence and it was concluded that the spore population within the glasshouse was not a limiting factor in epidemic development (10).

Many of the reports of suppression of sporulation and retarded epidemic development require application of the biocontrol agent (BCA) to be made early in pathogen lesion development. Although there have been some recent reports in which a BCA was applied 5 days after *B. cinerea* inoculation (28), these applications appear to be prior to pathogen sporulation. Timely antagonist application still is required in order to maximize the beneficial effects of the antagonist. Although certain phases of the pathogen, (for example, conidial germination) have been targeted for biocontrol, *B. cinerea* sporulation is a phase of the pathogen life cycle that remains relatively new for biocontrol opportunities.

B. cinerea produces conidia on determinate, highly branched conidiophores that project from the host surface (18). Spore release is regarded as passive (1,2,18), although it has been proposed that a twisting motion in the conidiophore, induced by changes in relative humidity, assisted spore liberation both within a single conidiophore and by physically moving neighboring conidiophores (19). These reproductive structures exposed to the environment

could present opportunities for colonization by products that could interfere with normal spore dispersal processes. The basis of such products may be found in a group of yeasts that were selected for the ability to bind to *B. cinerea* germings (5,8) and to suppress *B. cinerea* infection in vivo (8) in postharvest storage (7) and in glasshouses (4). This adhesion behavior could be harnessed and the yeasts formulated into a product with enhanced binding to *B. cinerea*, thereby trapping conidia within the lesion.

In this article, the effect on conidial release and dispersal of yeasts formulated into such a product, then applied to sporulating *B. cinerea* colonies growing on kiwifruit leaf disks, is presented. A series of three experiments are described, each designed to specifically address three questions. Does the application of the yeast reduce conidial release? Is the reduction of release likely to be epidemiologically significant by comparing treatment effects with no spore release? What is the fate of those spores that are released from a treated colony?

MATERIALS AND METHODS

Inoculum preparation. *Botrytis cinerea* (Pers.:Fr.) isolate BC20 (4,7,8) was stored in 15% glycerol at -20°C and was used in all experiments in this study. Stock cultures were grown on potato dextrose agar (PDA; Difco Laboratories, Detroit) for 10 days at 18°C , and exposed to a 12-h photoperiod from near-UV lights (Sankyo FL30 BLB, 30W) and fluorescent lights (NEC 30SSBR-HG) located 40 cm above the petri dishes.

Dried leaf disks (20 mm in diameter) from kiwifruit (*Actinidia deliciosa* cv. Hayward) were sterilized by gamma irradiation, then rehydrated in high humidity chambers for 16 h at 18°C . The adaxial surface of each leaf disk was inoculated with dry *B. cinerea* conidia by placing the disks directly onto a sporulating stock culture. The disks were then lifted from the culture that was used as inoculum and incubated in the high-humidity chambers for 10 days. Following incubation, sporulating leaf disks were visually evaluated and those with a similar density of conidiophores were selected. Each disk was cut in half and pairs of half-disks were randomly selected to form each experimental unit which, unless otherwise stated, defined the population of conidia from

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which liberation effects were studied following the application of the yeast–cellulose treatments.

The yeast isolates used in this study were isolated and described previously (8). Cells of *Candida sake* (Saito & Ota) van Uden & Buckley (isolates, 561, 532, and 662dia), *C. pulcherrima* (Lindner) Windisch (isolate 662dib), *Trichosporon pullulans* (Lindner) Diddens & Lodder (isolate 622b), and *Galactomyces geotrichum* (E. E. Butler & L. J. Peterson) Redhead & Malloch group A (isolates 552c and 572c) were stored in 15% glycerol at -20°C until required. The isolates were cultured in 100 ml of nutrient yeast dextrose broth (NYDB; nutrient broth at 8 g liter $^{-1}$ [Difco Laboratories], yeast extract at 5 g liter $^{-1}$ [Difco Laboratories], and D-glucose at 10 g liter $^{-1}$ [BDH Laboratory Supplies, Poole UK]) and the pH was adjusted to 4.5 with 5 M HCl. This broth was inoculated with 15 μl of the yeast in glycerol, then incubated at 18°C in 250-ml baffled conical flasks shaken at 150 rpm for 72 h.

The spent culture broth was removed by centrifuging the yeast culture at $766 \times g$ (Jouan M-22 centrifuge) and the yeast cells were resuspended in 30 ml of sterile distilled water (SDW).

The efficacy of various cellulose materials as the carrier of the yeast isolates was a second experimental factor examined in this study. α -Cellulose (Sigma C8002; Sigma-Aldrich, St. Louis), composed of cellulose particles ranging from in size from 80 to 200 μm , or Sigmacell (Sigma S3504, Sigma-Aldrich), composed of 20- μm cellulose particles, was added to the yeast at a rate of 0.1 g ml $^{-1}$ of suspension.

The cellulose–yeast suspension was thoroughly shaken by hand until a homogenous slurry was formed. The slurry was then centrifuged at $766 \times g$ to remove the liquid. The cellulose–yeast paste was spread thinly over the base of a sterile glass petri dish (90 mm in diameter), then placed under a low vacuum at 25°C for 16 h to dry. Each cellulose–yeast preparation then was powdered using a coffee grinder (Breville Model CG2) set at maximum speed for 30 s. Control preparations of cellulose were made by adding SDW to the α -cellulose or to Sigmacell and processed as described above.

Application of formulated yeast to sporulating *B. cinerea*. Plastic transfer pipettes (3-ml volume; Corning Samco Corp., San Fernando, CA) were modified by removing most of the barrel and retaining the bulb plus a 1-cm-long portion of the barrel. Yeast powder was added into the bulb, then the bulb-barrel was connected to a settling chamber (6). Briefly, the settling tower was composed of an octagonal glass cylinder 60 cm high and 32 cm wide. The bulb-barrel unit was connected directly to the inlet port (5 mm in diameter) which directed discharged inoculum into the settling chamber. Yeast powder was discharged into the settling chamber by puncturing a small hole into the side of the bulb and injecting a short (<1 s) pulse of compressed CO_2 (400 kPa gauge pressure) into the bulb (6). The cellulose–yeast powder then was allowed to settle during a 4-min period onto the two randomly selected half-disks of sporulating *B. cinerea*.

Measuring the inhibition of spore release. Standard, reproducible wind speed

conditions for spore liberation were created using a Tubeaxial Fan (Innovative Industrial Co, Taiwan), with a fan diameter of 73 mm, mounted in a square tube that was 80 mm wide, 80 mm high, and 100 mm long (Fig. 1). A detailed wind speed profile was measured (Fig. 1) using a Thermoanemometer (Anlor Instrument Co., Shoreview, MN). The apparatus also provided a standardized position for the placement of each pair of treated, sporulating, leaf half-disks (Fig. 1) and a standard exposure time of 30 s.

Unless otherwise stated, treatment effects of yeast application to sporulating *B. cinerea* colonies were determined by measuring the number of conidia that remained in the sporulating *B. cinerea* colony following exposure to the wind. The number of conidia that remained was quantified by first collecting conidia from the *B. cinerea* colony using a Cyclone surface sampler (Burkard Manufacturing, Rickmansworth, UK). The sampler deposited the conidia into an Eppendorf tube. The conidia were suspended in 1 ml of mineral oil (Shell NZ Ltd., Auckland, New Zealand), then enumerated by measuring the absorbance of conidia at 400 nm using a spectrophotometer (Jasco V530). Conidial concentration was calculated from absorbance values using the following equation derived from fitting a linear regression (SAS 6.12) to data obtained from a dilution series of conidia suspended in mineral oil (*unpublished data*): $\text{Abs}_{400} = 1.2 \times 10^{-7} \times \text{no. conidia} - 0.05$; $P_{(\text{slope})} = 0.0001$, $P_{(\text{intercept})} = 0.12$, $P_{(\text{model})} = 0.0001$, $R^2 = 0.97$.

Experiment 1: Reduction of conidial release following yeast application. Each

Fig. 1. The wind speed profile (meters per second) from the apparatus used to provide standard conditions for the liberation of conidia from *Botrytis cinerea* colonies grown on kiwifruit leaf disks. Dimensions of the apparatus and the position of the sporulating leaf samples also are indicated.

yeast isolate was formulated in α -cellulose, then applied at 0.2-, 0.3-, or 0.4-g dry powder doses to two half-disks of sporulating *B. cinerea*. Four replicate applications were made to the sporulating *B. cinerea* colonies, in which the half-disks were positioned on the settling chamber floor according to a standard design. An α -cellulose-only control also was applied at the same three doses in four replicate applications. The experiment was carried out according to a randomized block experimental design. Following the yeast-cellulose application, all leaf half-disks were incubated for 1 h at 4°C, to induce water condensation on the surface of the pathogen and encourage the adhesion of the yeast to sporulating *B. cinerea*. The treated disks were transferred to 18°C at ambient relative humidity for 16 h. The effect of each isolate formulated with α -cellulose on spore liberation was determined as described above.

The effect of yeast isolate formulated in Sigmacell on suppression of spore liberation also was examined in a repeat of the above experiment.

In both experiments, spore concentration was transformed by a $\log_{10}$ function to best fulfill the assumptions for analysis of variance (ANOVA) and analyzed using the SAS system (ver. 6.12). The effect of each yeast isolate on the number of conidia that remained in the treated *B. cinerea* colony was compared with the number of conidia that remained in *B. cinerea* colonies treated with cellulose-only using linear contrasts.

Experiment 2: Optimization of the suppression of conidial liberation. A factorial experiment was carried out to examine the following factors: yeast isolate, cellulose type, the concentration of the preparation applied, and two-way and three-way interactions among these factors.

Yeast isolates 561, 662dib, and 552c formulated in both α -cellulose and Sigmacell products were applied to sporulating leaf segments as described above at 0.2, 0.3, and 0.4 g. Controls composed of pairs of half-disks of sporulating *B. cinerea*

treated with either α -cellulose or Sigmacell and sporulating *B. cinerea* that was untreated and either exposed or not exposed to wind using the apparatus illustrated in Figure 1. Four replicate treatments and controls were prepared and exposed (or not exposed) to the wind. The conidia that remained in the *B. cinerea* colony were harvested using the Cyclone surface sampler. The conidia were suspended in 1 ml of mineral oil and the absorbance measurements, taken at 400 nm, were converted to spore concentration and $\log_{10}$ transformed for analysis by ANOVA, as described for experiment 1. In the present experiment, each yeast treatment, the cellulose-only controls, and the untreated control exposed to the wind were compared with the no-wind, untreated control using linear contrasts. These comparisons were chosen because the objective of the suppression of conidial release was to retain to as close as possible the original population of conidia in each colony. In the present experiment, a significant biological effect of suppressing spore liberation was conferred when the treatment mean was not significantly ($P > 0.05$) from the no-wind untreated control.

Experiment 3: The fate of those spores that were released from a treated *B. cinerea* colony. To monitor the fate of spores liberated from treated and untreated sporulating *B. cinerea* colonies, the spore cloud from sporulating leaf disks positioned in the apparatus illustrated in Figure 1 was discharged into a wind tunnel 5 m long, 50 cm high, and 70 cm wide. The apparatus was positioned at the head of and on the longitudinal axis of the wind tunnel. At 0.5-m intervals along the wind tunnel, nine sampling drawers were used to locate and retrieve passive spore traps. Inoculum sources of differing sizes (2, 1, 0.5, and 0.25 sporulating disks) were placed at the head of the wind tunnel and the fan was turned on for 30 s to launch the pathogen conidia. The fan was turned off and this was followed by a settling time of 5 min. The wind speed at the far end of the wind

tunnel during these experiments was below the detection range of the therm-anemometer. However, experiments carried out during the construction of the wind tunnel (*unpublished data*), confirmed that this experimental system was sufficient to liberate most of the conidia from an untreated sporulating *B. cinerea* lesion and deposit liberated conidia within the 5-m length of the wind tunnel.

Dispersal from leaf disks treated with 0.4 g of the yeast 662dib (*C. pullulans*) formulated in α -cellulose was compared with untreated leaf disks. Conidia released from treatments using 1 to 2 disks were trapped onto three agar-coated microscope slides at each sampling site. For the 0.5- and 0.25-disk treatments, three 90-mm-diameter petri dishes with PDA amended with Triton N101 at 5,000 ppm were used at each sampling site. These plates were incubated at 18°C until discrete *B. cinerea* colonies were visible, then counted.

There were no spore density gradients detected along the wind tunnel; therefore, the mean spore density at each sampling site was combined to calculate an overall mean density throughout the tunnel. The means from four replicates were compared using ANOVA and Fisher's least significant difference.

RESULTS

The design of, and wind speed profile of, the apparatus to create standard conditions for spore liberation is illustrated in Figure 1. A wind speed of 4.4 m s⁻¹ was measured where leaf disks were placed; this velocity was sufficient to remove >90% of conidia from an untreated sporulating *B. cinerea* lesion (D. W. M. Cook, *unpublished data*).

Effect of yeast isolates on suppression of conidial liberation. Application of 0.2 g of *C. sake* (662dia) or *G. geotrichum* (552c) resulted in a significant increase in the number of conidia retained compared with an α -cellulose-only control (Table 1). At the 0.3-g dose, yeast isolates 561, 532, 552c, and 572c conferred significant in-

Table 1. Effect of yeast isolate applied to sporulating *Botrytis cinerea* colonies on the suppression of conidial liberation^y

Treatment, isolate	Species	Spore concentration (spores $\times 10^6$ ml ⁻¹) ^z					
		α -cellulose			Sigmacell		
		0.2 g	0.3 g	0.4 g	0.2 g	0.3 g	0.4 g
Cellulose	...	4.1	4.3	2.9	2.4	4.8	6.9
532	<i>Candida sake</i>	6.0	6.8**	10.9***	2.2	7.8	6.0
561	<i>C. sake</i>	6.9	8.5**	11.6***	5.1	8.1	13.0*
662dia	<i>C. sake</i>	9.0*	4.7	10.2***	1.8	9.8	12.9*
662dib	<i>C. pulcherrima</i>	5.4	5.7	13.4***	7.0	1.9	15.1**
622b	<i>Trichosporon pullulans</i>	4.4	5.5	9.3***	1.9	1.2	3.5
552c	<i>Galactomyces geotrichum</i>	10.6**	8.9***	14.8***	4.4	2.6	5.3
572c	<i>G. geotrichum</i>	5.9	8.4*	10.5***	5.3	7.7	4.8

^y Liberation = the quantity of *Botrytis cinerea* conidia that remained in a sporulating lesion on kiwifruit leaves after exposure to a 30-s pulse of wind at 4.4 m s⁻¹. Means are from four replicates; *, **, and *** = $P < 0.05$, 0.001, and 0.0001, respectively, and were calculated for the comparison between the accompanying treatment and the cellulose-only control according to linear contrasts.

^z Spore concentration was calculated from absorbance data, $\log_{10}$ transformed, then presented in this table as back-transformed data. The yeast/cellulose product was applied to sporulating *B. cinerea* colonies in either α -cellulose or Sigmacell at 0.2-, 0.3-, or 0.4-g doses of the formulated yeast per application to the settling chamber.

creases in the number of conidia retained. At the 0.4-g dose, all of the formulated yeast isolates conferred significant increases in the number of conidia retained compared with the α -cellulose control (Table 1).

Overall, Sigmacell-based formulations were less effective in retaining conidia on sporulating *B. cinerea* colonies (Table 1). Only at the highest dose of 0.4 g, isolates of *C. sake* (561 and 662dia) and *C. pulcherrima* (662dib) conferred a significant increase in the number of conidia compared with the Sigmacell-only control.

Optimization for the suppression of spore release. The effect of various combinations of the cellulose–yeast formulation and concentration on suppression of liberation of *B. cinerea* conidia is shown in Table 2. Following exposure to wind, a significant quantity of conidia were removed from untreated sporulating colonies, from the Sigmacell or the α -cellulose controls, and from colonies treated with the yeasts compared with the untreated no-wind control (Table 2); specifically, isolates 561 and 552c formulated with Sigmacell and applied at the 0.2-g dose, or the yeast 561 formulated in Sigmacell and applied at 0.3 g. For the remaining yeast treatments, there was no significant difference between a treatment mean and the no-wind control, suggesting that these treatment combinations were all highly effective at suppressing conidial liberation.

The main effects in this factorial experiment (isolate, dose, and cellulose type) were all highly significant. The interaction between cellulose and isolate was statistically significant and the remaining two-way and three-way interactions were not significant (Table 2).

Fate of liberated conidia. Regardless of the initial size of the *B. cinerea* colony placed at the head of the wind tunnel, there was a significant ($P < 0.05$) suppression of conidial liberation by isolate 662dib (*C. pulcherrima*) formulated in α -cellulose (Table 3). For each of the quantities of sporulating *B. cinerea*, approximately half of the number of conidia were liberated from treated lesions compared with untreated controls (Table 3). There were no significant differences ($P > 0.05$) in the number of conidia trapped at the various distances along the tunnel, which resulted in an even distribution of conidia throughout the length of the wind tunnel.

DISCUSSION

Conventional strategies for the biological control of *B. cinerea* require BCA application before or shortly after pathogen inoculum arrival. In contrast, BCA application to an already sporulating pathogen presents some unique challenges and potential opportunities for the suppression of subsequent epidemic development. In the present study, yeasts that attached to conidia and to conidiophores of *B. cinerea* and applied to sporulating pathogen colonies grown on kiwifruit leaf disks were able to significantly suppress spore liberation. The application of these yeasts to suppress conidial liberation could be used to reduce airborne inoculum and reduce epidemic development, which has been previously demonstrated following the removal of infected and sporulating plant tissue (23). Spore release has been studied and modeled for its contribution to epidemic development (1,20), but studies of BCA application on spore release are lacking. Therefore, the mechanisms for the

suppression of disease following BCA applications to sporulating pathogens are poorly understood.

Selection of the yeast isolate and the cellulose component in the formulation was shown here to be important for suppression of spore liberation. The α -cellulose, which was highly effective in suppressing spore liberation at a dose of 0.4 g regardless of yeast isolate, was composed of large particles (80 to 200 μ m). In contrast, yeasts formulated with the smaller Sigmacell particles (20 μ m) conferred significant suppression of spore release at the 0.4-g dose for only three of the seven yeast isolates tested. The mechanism by which this suppression is achieved was not specifi-

Table 3. Total *Botrytis cinerea* conidial liberation from treated and untreated sporulating colonies of various sizes^y

Inoculum dose	Treatment, isolate	Mean spore density mm ^{-2z}
2 Disks	Untreated	5.62 a
	662dib	2.77 b
1 Disk	Untreated	3.28 a
	662dib	1.94 b
½ Disk	Untreated	0.69 a
	662dib	0.37 b
¼ Disk	Untreated	0.38 a
	662dib	0.17 b

^y Colonies were treated with the yeast *Candida pulcherrima* (isolate 662dib) formulated in α -cellulose.

^z Spore liberation was calculated from conidial densities measured at each sampling point along a 5-m wind tunnel, then combined to calculate a single value for each treatment ($n = 4$). The same letters following mean spore density are not significantly different at $P > 0.05$ according to Fisher's least significant difference analysis.

Table 2. Optimization of the suppression of *Botrytis cinerea* conidial liberation from colonies grown on kiwifruit leaf disks^y

Spore concentration (spores × 10 ⁶ ml ⁻¹) ^w									
Factor	Treatment	Control ^y	0.2 g ^x		0.3 g		0.4 g		P value
			α	Sigma	α	Sigma	α	Sigma	
Wind exposure									
No-wind	Untreated	8.2	...	...	...	...	...	...	...
Plus wind	Untreated	0.5***	...	...	...	...	...	...	...
Plus wind	Cellulose	...	1.9***	3.4**	2.6**	0.9**	3.2**	1.7**	...
Plus wind	561	...	9.4	3.4*	12.2	3.6*	8.3	9.6	...
Plus wind	662dib	...	5.9	4.7	5.4	5.0	6.3	5.6	...
Plus wind	552c	...	5.9	3.2**	17.6	5.8	8.8	5.8	...
ANOVA ^z									
Cellulose	...	...	...	...	...	...	...	...	0.0001
Isolate	...	...	...	...	...	...	...	...	0.0001
Dose	...	...	...	...	...	...	...	...	0.0002
Cellulose × dose	...	...	...	...	...	...	...	...	0.007
Isolate × dose	...	...	...	...	...	...	...	...	0.26
Cellulose × isolate	...	...	...	...	...	...	...	...	0.11
Cellulose × isolate × dose	...	...	...	...	...	...	...	...	0.27

^y Interaction effects of selected yeast isolates, formulation and concentration. Liberation = the quantity of *Botrytis cinerea* conidia that remained in a sporulating lesion after exposure to a 30-s pulse of wind at 4.4 m s⁻¹. Means are from four replicates. Treatments with an associated asterisk were statistically significantly different (*, **, and *** = $P < 0.05$, 0.001, and 0.0001, respectively) from the no-wind untreated control according to linear contrasts.

^w Spore concentration was calculated from absorbance data, log₁₀ transformed, then presented in this table as back-transformed data.

^x Controls included a no-wind untreated control, or plus wind, untreated control.

^z Yeast isolates *Candida sake* (isolate 561), *Candida pulcherrima* (isolate 662dib), or *Galactomyces geotrichum* (Isolate 552c) formulated with either α -cellulose or Sigmacell were applied at 0.2-, 0.3-, or 0.4-g doses to sporulating *B. cinerea* colonies.

^z Analysis of variance.

cally examined in this study; however, during the experiments, a number of observations were made with the use of light microscopy. Particles from the α -cellulose appeared to become lodged on top of the conidiophores while, in contrast, particles from Sigmacell passed through the dense canopy of conidiophores (*unpublished data*). In addition, the conidiophores could vibrate under the influence of the wind to a greater degree in untreated controls compared with colonies treated with the α -cellulose. The α -cellulose resting on the conidiophores may have formed a rigid scaffold that may have resulted in considerably less conidiophore movement (*unpublished data*). Mechanical movement in the form of leaf shaking (17), the impact of water droplets (19), or from already released spores (9) and air turbulence (1) aid spore liberation. Conidia that are terminally borne (18) are subject to rapid release compared with those borne on lower conidiophores (17). It is possible that the α -cellulose-based yeast formulation residing on the conidiophores could come into direct contact with many of the terminally borne conidia and interrupt their release. Nevertheless, these proposed mechanisms of the suppression of conidial liberation would require further detailed study.

The impact of suppressing spore liberation on subsequent development of *B. cinerea* epidemics will be influenced by a number of epidemic factors. In instances where inoculum is not the limiting factor in perpetuating the epidemic, reductions in airborne populations may not be effective (10). Several antagonistic fungi have significantly retarded epidemic development as a result of suppressing pathogen sporulation and reducing airborne inoculum (21,22,24,29). In those studies, BCA application was made prior to sporulation and the suppressive effect was the result of prolonged and cumulative interaction with the BCA. In contrast, suppression of liberation represents a strategy with a short interaction time that would not benefit from long cumulative suppression. Wind tunnel experiments in the present study showed that approximately the same proportion of conidia are prevented from being liberated from treated colonies regardless of the initial quantity of sporulating tissue. Therefore, successful suppression of an epidemic will, among other factors, be influenced by the absolute amount of sporulating tissue at the time of BCA application. Infection studies have shown that a single *B. cinerea* conidium could induce a lesion in gerbera flowers (26) and cold-stored grape berries (3). In contrast, Coertze and Holz (3) found that single conidia did not induce lesions in fresh grape berries. Therefore, the effect of suppressing spore liberation on subsequent disease development will depend on dose thresholds for infection.

There are inconsistent reports as to the nature of *B. cinerea* conidia upon release.

Salinas et al. (26) and Coertze and Holz (3) stated that inocula were released as single spores. In contrast, Harrison and Lowe (17) stated that conidia tend to be released as clumps. In the present study, conidia were observed in the wind tunnel traps as single conidia unless they were attached to particles from the yeast powder. When inocula are released as clumps of conidia, they settle from the air currents at a higher rate (16), provided the atmosphere is not turbulent (1). Aylor (1) also proposed that clumping of conidia reduced the efficiency of pathogen dispersal during an epidemic. *B. cinerea* spores attached to particles from yeast powder are analogous to clumped *Botrytis* spores and potentially subject to enhanced rates of settling and dispersal inefficiencies. Although clumping was found periodically in the wind tunnel experiments, there was no evidence in the data to suggest there was a change in the dispersal patterns.

Suppression of conidial liberation as described in the present study may be unique in two aspects. BCA application to sporulating *B. cinerea* is a strategy seeking biocontrol opportunities during a phase in the pathogen life cycle that was previously unexplored. Suppression of spore liberation from an already sporulating lesion may provide opportunities for disease control late in an epidemic in a treatment designed to restrict further epidemic development. However, studies are required to investigate whether there would be ongoing suppression of the epidemic. Plaut and Berger (25) found there was no significant difference in the final disease severity from epidemics started from three levels of initial severity. Higher infection rates were measured in the epidemic that started with the lower initial severity. It remains unknown if patterns like those observed by Plaut and Berger (25) would be repeated following the treatment of plants with a liberation suppressant.

Second, provided that attachment competency can be maintained, there is no need to apply a living BCA to suppress conidial liberation. The BCA formulation described in the present study also could be applied as an inert product, achieving suppression of spore liberation primarily through a proposed adhesion mechanism. Whereas pre-infection and post-infection strategies require a living microorganism to be applied to the plant surface, this is not necessary in suppressing spore liberation in the manner described in the current study. Not only must the maintenance of attachment competency be determined, but careful consideration of the formulation also will be required in order to minimize any reduction in the photosynthetic rate, because the leaves will be coated in the preparation.

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