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Mycoactive Acetate Esters from Apple Fruit Stimulate Adhesion and Germination of Conidia of the Gray Mold Fungus

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Ethyl acetate, 2-methylbutyl acetate, butyl acetate (BA), and hexyl acetate were detected by solid-phase microextraction and gas−liquid chromatography inside slices of Golden Delicious apple and in water droplets on the skin of slices incubated in sealed glass jars. Conidial adhesion and germination of the gray mold fungus, *Botrytis cinerea*, was assessed on apple slices after exposure or no exposure to the esters in the headspaces of glass jars. Attached conidia were dislodged by sonication and remaining conidia on apple slices were counted by microscopy. Adhesion generally increased as BA increased to 7.2 μg mL^{-1}, but declined with greater concentrations. BA at 0−3.6 μg mL^{-1} for 24 h stimulated adhesion 2-fold greater compared to that at 4 h. Adhesion stimulated by BA increased as a function of time (0−24 h), showing linear trends ($r^2 = 0.99$; $p = 0.01$) during 0−12 h. The four esters were similar in their ability to stimulate adhesion. Germination of conidia exposed to BA increased linearly ($r^2 = 0.95$−0.98; $p = 0.01$) during 4−12 h. Conidial adhesion stimulated by BA preceded conidial germination by 2 h. The four esters stimulated conidial germination to similar levels. Results indicated that acetate esters formed in apple fruit are mycoactive, influencing life-cycle events of *B. cinerea* important to its survival on the fruit. The similar responses of three *B. cinerea* isolates to four acetate esters suggests a common stimulation mechanism may operate in *B. cinerea.*

KEYWORDS: Acetate esters; apple; *Botrytis cinerea*; fruit volatiles; fungal spore adhesion; fungal spore germination; bioactive compounds

INTRODUCTION

Plants produce numerous flavor and aroma compounds that are bioactive to fungi (*1*). These and other mycoactive compounds may influence one or more events in the life cycle of fungal pathogens on fruit (*1*−*7*). *Botrytis cinerea* Pers.:Fr., a fungal pathogen of numerous plant species (*8*−*9*), causes a decay of apple fruit called gray mold, which can cause serious economic loss in postharvest storage (*10*−*12*). On infected apples *B. cinerea* sporulates, producing abundant conidia that can be dispersed by various means to healthy apples to incite new infections (*8*). It is generally accepted that before infection can occur, conidia of *B. cinerea* must first adhere to host surfaces (*9*), reducing dislodgement by rain or wind, and perhaps signaling metabolic events required for germination. To infect, a conidium must germinate, producing the hyphae that will grow and infect the apple. As *B. cinerea* is a necrotrophic fungus (*13*), survival is furthered by sporulation and dispersal of conidia which complete the life cycle. Clearly, conidial adhesion and germination of *B. cinerea* are life-cycle events important to its survival on apple fruit.

Apples form hundreds of flavor and aroma compounds (*14*−*17*). Many of these are released to the air as the fruit develops on the tree and further matures in storage and on the grocer's shelf. Acetate esters are produced in maturing apples on the tree and later in storage (*15*−*17*). Concentrations of acetate esters wax and wane as the fruits develop on trees and are maintained in storage. Although 18 acetate esters have been isolated from apple (*14*), the main acetate esters routinely found in apple include butyl acetate (BA), ethyl acetate (EA), 2-methylbutyl acetate (2MBA), and hexyl acetate (HA) (*15*−*17*). Brown (*18*) first showed that EA from apple fruit stimulated germination of *B. cinerea* conidia. Ethyl acetate, BA, and HA were later shown to stimulate conidial germination of *B. cinerea* on polycarbonate membranes inside glass jars containing these acetate esters in the headspace (*6*). Also, Golden Delicious apples in sealed jars exposed to BA had more decay caused by *B. cinerea* than nonexposed apples (*4*, *6*).

Adhesion of *B. cinerea* conidia to polycarbonate membranes was increased in the presence of BA, and germination was highly correlated with conidial adhesion to the membranes when conidia on the membranes were exposed to BA (*6*). Whether or not conidia of *B. cinerea* on the skin of apple fruit will respond to acetate esters in a similar way to their responses on membranes is not known. Therefore, the objective of this research was to assess the effect of acetate esters found in apple on the adhesion and germination of conidia of *B. cinerea* on the skin of apple fruit. The response of conidia to 2MBA also was investigated.

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MATERIALS AND METHODS

Source of Apples and Preparation for Experiments. Large (ca. 8-cm diam and 260−280 g) apples (*Malus* × *domestica* Borkh.) cv. Golden Delicious were purchased from a local supermarket. They were used on the day of purchase or stored at 2 °C for no longer than one week before use. Apples were free of obvious bruises, with a soluble solids content of 11.0−12.2% and a fruit firmness of 42.5−51.0 N. Apples were removed from cold storage and preincubated at the temperature of the planned experiment for 16−24 h. Just prior to an experiment, apples were washed with a paper towel in warm tap water and wiped dry with paper towels. Slices of apples (2.5−3.0 cm diam × 0.4−0.6 cm thick; 2.00−2.25 g) for experiments were cut with a sharp knife, and each slice was handled so that the skin was not contaminated by sugars in the juice released during cutting. About 6−8 slices were typically cut from an apple, and these, plus slices from other apples, were randomly placed cut-side down on moist paper towels inside a plastic tray. Apple slices were used in experiments within 30 min of preparation.

Detection of Acetate Esters inside Apple Slices or in Water Drops on Apple Slices Exposed to Acetate Esters. Using paper-clip wire, a 1-mm diam hole was pushed through the middle of a freshly cut apple slice. The slice was held at one end, cut-side down, in a clamp fastened to a metal stand. Acetate esters in apple juice inside an apple slice were adsorbed on a 65-μm PDS/DB, 2-cm StableFlex, solid-phase microextractor (SPME; Supleco, Inc., Bellefonte, PA). The extractor needle was pushed through the hole, and the adsorptive fiber was extended and then gently pulled back into the hole. The extractor was then clamped to the stand, and 100 μL of sterile distilled water (sdH_2O) was added to the top of hole. After 4 h the fiber was withdrawn, and the acetate esters were thermally desorbed at 275 °C in the injector of a gas chromatograph fitted with a DB-23 capillary column (30 m, 0.25 mm i.d.; J & W Scientific, Folsom, CA). Helium at 55 mL min^{-1} was the carrier gas. The oven was programmed at 45 °C for 3 min, followed by a 10 °C min^{-1} increase to 155 °C, and then held at 155 °C for 1 min (*19*). The flame ionization detector was at 300 °C. Quantification of esters was based on comparisons of retention times and integrated peak areas of acetate ester standards adsorped by SPME from dilutions in water. Acetate esters in two apple slices were quantified per experiment and three experiments were conducted.

In another experiment, an apple slice bearing a 50-μL sdH_2O droplet was sealed inside a jar, and the jar airspace was injected with 4.1 μL of butyl acetate (99.5% pure; Fluka Chemika, Buchs, Switzerland), 2-methylbutyl acetate (99.9%; Sigma-Aldrich Chemical, St. Louis, MO), or hexyl acetate (99.5%; Fluka Chemika), or 4.0 μL of ethyl acetate (99.9%; Sigma-Aldrich) to give 7.2 μg mL^{-1} of an ester. There were six jars for each ester, and six jars for the control which received no injections. After 4 h at 23 °C, the jars were opened, and the droplets were sucked into a pipet tip and combined in a 1-mL conical-well, glass vial with a silicone rubber septum. The SPME fiber was incubated in the composited water for 30 min prior to GC injection (*19*).

Fungi and Their Culture. Sources for isolates F-J-4, WMA1, and WMG2 of *B. cinerea* were previously reported (*4*, *6*). Maintenance of *B. cinerea* strains and the production of conidia on potato-dextrose agar (PDA; Difco, Inc., Detroit, MI) have been reported elsewhere (*12*). Conidia were dislodged by tapping the top cover of an inverted PDA culture on a bench, and the dislodged conidia were suspended in sdH_2O in a centrifuge tube and vigorously agitated. Suspensions of 1−1.4 × 10^5 conidia mL^{-1}, as determined in a hemocytometer, were used in all experiments.

General Method for Adhesion Experiments. A sonication method (*20*) with modifications was used to quantify conidial adhesion. Briefly, 50 μL of a conidial suspension was pipetted on the center of each of five apple slices resting cut-side down on a plastic Petri dish. These were dried at 50 °C for 30 min, and the centers of the slices were stained with a thin film of crystal violet in ethanol/water (1:1, v/v). These slices were used to determine the initial density of applied conidia. Conidia (50 μL) also were deposited on a slice on moist filter paper inside a glass jar (500 mL; Kerr, Los Angeles, CA). Each jar was sealed with a lid fitted with a small rubber septum. Jars were injected with acetate esters, but controls were not. There were five replicates for each treatment or control. Jars were randomized on a bench and incubated at 23 °C for various durations. The jars were unsealed, and an apple slice was carefully lifted out of a jar without disturbing the droplet. A small piece of apple skin bearing the droplet was excised with an X-acto knife (X-acto, Statesville, NC) and placed in a 15-mL plastic centrifuge tube containing 8 mL of ice-cold sdH_2O. Samples and controls were immediately sonicated by inserting the probe of a model 550 sonic dismembrator (Fisher Scientific, Houston, TX) into a tube and sonicating at 150 W for 10 s. Apple pieces were removed with a forcep, stained with crystal violet, and dried for 30 min at 50 °C. Stained apple pieces were observed with epi-illumination microscopy at 400×, and only blue, ovoid, intact conidia with and without germ tubes were counted on the apple skin. The mean number of counted conidia in 10 randomly selected microscopic fields was determined for each replicate. The average initial density of applied conidia was used as a basis for calculating the % adhesion of conidia exposed or not exposed to acetate esters.

Effect of Acetate Ester Concentration and Exposure Duration on Adhesion. The effect of low concentrations of BA in the headspace for 24 h, and the effect of greater concentrations for 4 h, on conidial adhesion of isolate F-J-4 were assessed. In the 24-h experiments jars containing conidia on apple slices were injected with 1 μL of BA or 10 μL of dsH_2O with various concentrations of dissolved BA to give headspaces of 1.8, 0.9, 0.09, and 0 μg mL^{-1}. In the 4-h experiments, jars were injected with 0−8.2 μL of BA to give headspaces of 0−14.4 μg mL^{-1}. For each duration there were five replicates per treatment per experiment.

The adhesion responses of isolates F-J-4, WMA1, and WMG2 of *B. cinerea* to apple skin were each assessed in jars injected with 4.1 μL (7.2 μg mL^{-1}) of BA. Adhesion of each isolate was assessed at 0, 2, 4, 8, 12, and 24 h after BA injection. Zero hour measurements were made by opening a jar lid immediately after injection, placing the excised apple piece in water, sonicating it, and processing the apple skin for conidium counting. There were five jars for each sampling time.

Effect of BA on Adhesion of Viable Compared to Nonviable Conidia. Conidia of isolate F-J-4 were poisoned with sodium azide, washed by centrifugation with sdH_2O, and used in experiments to assess the importance of viable conidia in adhesion. Poisoned conidia on PDA for 24 h did not germinate and were considered nonviable. Nonviable conida were compared to viable conidia for their adhesion on apple slices in jars that were exposed for 4 h to 0 or 7.2 μg mL^{-1} of BA. There were five replicates per treatment or control.

Effect of Other Acetate Esters on Adhesion. Isolates of *B. cinerea* on apple slices were exposed to 7.2 μg mL^{-1} of BA, 2MBA, EA, or HA. Jars without esters were the control. After 4 h incubation at 23 °C, adhesion was measured. There were five jars for each ester and the control, and one separate experiment was done for each isolate of *B. cinerea*.

Effect of Acetate Esters on Germination of Conidia. In all germination experiments, conidia of isolate F-J-4 on apple slices were exposed to acetate esters at a headspace concentration of 7.2 μg mL^{-1} in glass jars, as described in the adhesion experiments. After exposure, the apple slices were removed from the jars, and 5 μL of rose bengal stain was added to each droplet on the slices. Slices were heated at 50 °C for 45 min, and the red stained areas were washed with ca. 0.5−1 mL of water. The wash did not dislodge heat-fixed conidia from an apple slice. Slices were heated at 50 °C for 15 min to dry the stained area. The area was excised as a thin section with a razor blade. The cut side was blotted dry on tissue paper and fixed to a smear of rubber cement on a glass microscope slide. Conidial germination in 300 observations per section was counted at 400×. A conidium was considered germinated when the germ tube length was greater than the width of the conidium.

Germination of conidia on apple slices that were exposed or not exposed to BA was assessed after 4, 8, 12, and 24 h. There were three replicates per treatment or control.

The effect of a 4-h exposure to BA, 2MBA, EA, or HA on germination of conidia was also assessed in jars containing apple slices. Jars without acetate esters were the controls. There were five jars per treatment or control.

Table 1. Retention Times and Concentrations of Acetate Esters Detected in Apple Slices or in Water Droplets on Apple Slices for 4 h in Sealed Jars Not Injected or Injected with 7.2 μg of an Acetate Ester

ester	retention time[b] (minutes)	concentration (μg mL^{-1})[a]		
			in water drop on apple slices in jars	
		in apple slices	not injected	injected
ethyl acetate	1.10 ± 0.06	0.09 ± 0.02	0.07 ± 0.02	223.3 ± 41.5
butyl acetate	2.45 ± 0.09	1.98 ± 0.56	0.77 ± 0.29	135.0 ± 33.0
2-methylbutyl acetate	4.17 ± 0.10	0.45 ± 0.09	0.37 ± 0.26	218.0 ± 21.9
hexyl acetate	5.93 ± 0.12	0.53 ± 0.07	0.90 ± 0.39	112.2 ± 34.4

[a] Values are the means of five replicates ± one standard deviation. [b] Means of retention times for each acetate ester in a composite standard analyzed four times.

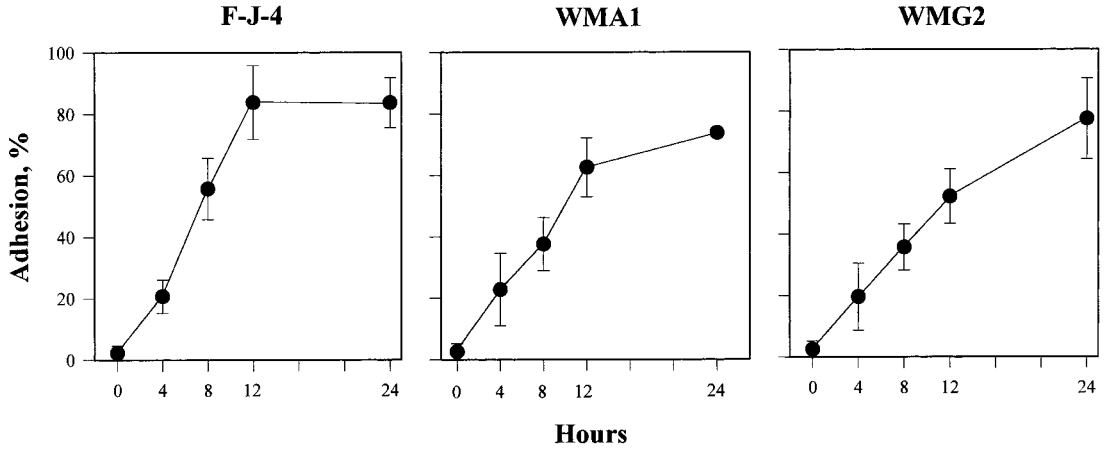

Figure 1. Percentage conidial adhesion of isolates F-J-4, WMA1, or WMG2 of *B. cinerea* incubated for 24 h at 23 °C on the skin of Golden Delicious apple slices inside 500-mL glass jars with 7.2 μg mL^{-1} of butyl acetate in the headspace. Bars represent means of five replicates ± one standard deviation.

Data Analysis. All experiments were repeated once, unless otherwise noted. Results from repeated experiments were reproducible. Percentage adhesion and germination data were subjected to an arcsine square-root transformation prior to analysis of variance (AOV). Means were separated using the Student−Newman−Keul's Test ($p \leq 0.05$). AOV and mean separation was determined using SigmaStat (ver. 2; Jandel Corp., San Rafael, CA) Values given in the text or figures are means ± one standard deviation (SD). The relationships of conidial adhesion or germination to time were analyzed by regression ($p \leq 0.01$) using SigmaPlot (ver. 4.0; Jandel).

RESULTS

Detection of Acetate Esters. EA, BA, 2MBA, and HA were found in slices of Golden Delicious apples that were incubated in the open air (**Table 1**). These esters were sufficiently separated by capillary GLC to identify them by retention time. Spiking selected samples with an acetate ester standard produced an enhanced detector response at the expected retention time for that ester, verifying their identification. The BA concentration in apple slices was ca. 4-fold greater than that of 2MBA or HA and 22-fold greater than EA.

These esters accumulated in 50-μL water droplets on apple slices that were incubated for 4 h in sealed jars that were not injected with acetate esters. Except for EA which was at the lowest level, concentrations of the three other esters did not differ and were ca. 5−13-fold greater than EA (**Table 1**). In sealed jars injected with 7.2 μg mL^{-1} of an acetate ester, water droplets on apple slices absorbed twice as much EA or 2MBA than BA or HA.

Adhesion Experiments. Conidia adhered to the skin of apple slices not exposed to BA, reaching 35.8% adhesion after 24 h (**Table 2**). Nonviable conidia adhered less than 1% to apple slices whether exposed to BA or not. Butyl acetate at 0.09 μg mL^{-1} did not stimulate greater adhesion, even after 24 h exposure. Adhesion at ≥0.9 μg mL^{-1} was greater than that for nonexposed conidia for both time durations. A 4 h exposure to 0.9−7.2 μg mL^{-1} BA increased adhesion, but greater concentrations decreased adhesion.

Table 2. Effect of Butyl Acetate Concentration and Exposure Duration on Adhesion of *Botrytis cinerea* Conidia to Apple Slices in Sealed Jars[a]

concentration (μg mL^{-1})	% adhesion after duration of	
	4 h	24 h
0.0	14.7 ± 3.2	35.8 ± 6.3
0.09	22.1 ± 7.1	53.2 ± 13.6
0.9	26.1 ± 7.4	59.6 ± 7.4
1.8	32.8 ± 3.9	88.2 ± 11.6
3.6	46.6 ± 4.3	90.8 ± 9.8
7.2	53.2 ± 5.5	nd
10.8	34.4 ± 5.0	nd
14.4	19.2 ± 6.4	nd

[a] Values are the means of five replicates ± one standard deviation at $p = 0.01$; nd, not determined.

Conidial adhesion of all isolates exposed to 7.2 μg mL^{-1} BA generally increased as a function of exposure duration (**Figure 1**). Significant adhesion occurred by 4 h. During 0−12 h, all isolates exhibited linear increases in adhesion. Regression analysis of adhesion (%*A*) to hours of exposure (*H*) showed a high degree of fit for all isolates ($r^2 = 0.99$; $p = 0.01$), and yielded the following models: $\%A = -8.29 + 6.99H$ for F-J-4, $\%A = -2.69 + 4.87H$ for WMA1, and $\%A = -1.41 + 4.13H$ for WMG2. The isolates did not differ in adhesion at 24 h, with 83.8 ± 12.0% adhesion for F-J-4, 77.5 ± 13.2% for WMG2, and 73.8 ± 13.0% for WMA1. During 0−12 h, however, the adhesion rate model of F-J-4 showed a greater slope (6.99) than

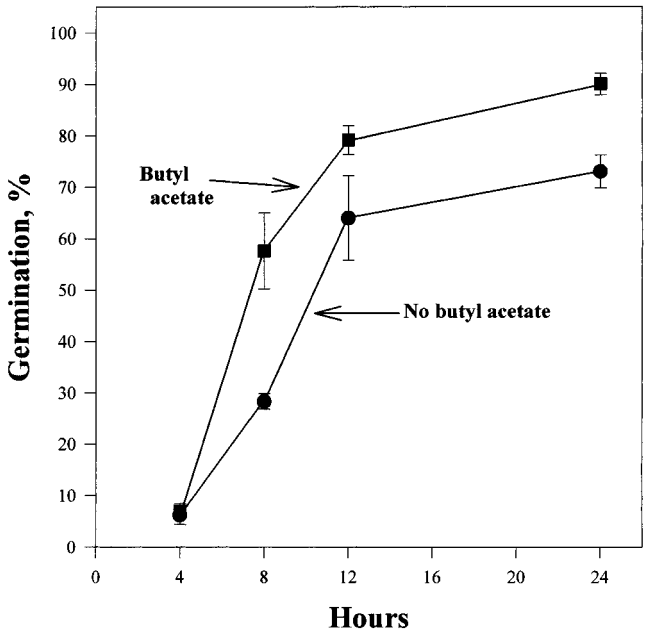

Figure 2. Percentage conidial germination of isolate F-J-4 of *B. cinerea* incubated for 24 h at 23 °C on the skin of Golden Delicious apple slices inside 500-mL glass jars with no butyl acetate or 7.2 μg mL^{-1} of butyl acetate in the headspace. Bars represent means of five replicates ± one standard deviation.

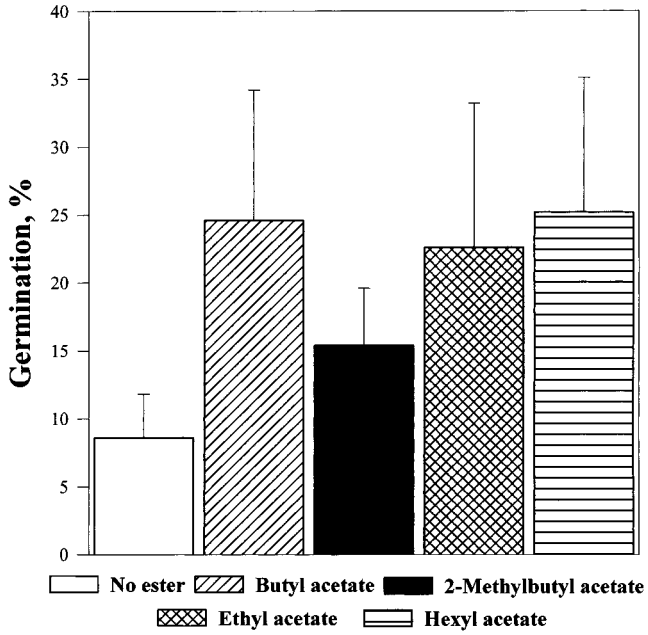

Figure 3. Percentage conidial germination of isolate F-J-4 of *B. cinerea* incubated for 6 h at 23 °C on the skin of Golden Delicious apple slices inside 500-mL glass jars with no acetate ester or 7.2 μg mL^{-1} of butyl acetate, 2-methylbutyl acetate, ethyl acetate, or hexyl acetate in the headspace. Bars represent means of five replicates ± one standard deviation.

that for WMA1 (4.87) or WMG2 (4.13), both of which were quite similar in adhesion rate. By 8 h, for instance, WMA1 and WMG2 showed 37.6 ± 8.6% and 35.7 ± 7.5% adhesion, whereas F-J-4 exhibited 55.7 ± 10.0% adhesion.

All acetate esters at 7.2 μg mL^{-1} stimulated conidial adhesion of all isolates to apple slices after 4 h of exposure. Adhesion of nonexposed conida of F-J-4, WMA1 and WMG2 was 28.6%, 19.0%, and 22.0%, respectively, whereas adhesion of exposed conidia of F-J-4, WMA1, and WMG2 was increased 1.5−2.4-fold, 2.4−3.0-fold, and 1.9−2.6-fold, respectively. However, the isolates of *B. cinerea* exhibited no differences in their responses to any acetate ester.

Germination Experiments. A 4-h exposure to BA did not increase conidial germination compared to that of nonexposed conidia (**Figure 2**). Thereafter, conidial germination was greater when exposed to BA. Typically, 6 h of exposure was required before appreciable germination occurred in exposed conidia, and by 8 h significant differences between exposed and nonexposed conidia were found. After 24 h germination was 90.0 ± 3.2% for exposed conidia and 73.0 ± 3.5% for nonexposed conidia. Regression analysis showed a linear relationship ($p = 0.01$) between germination (%G) and hours of exposure (H) from 0 to 12 h that yielded the following models: $\%G = -24.20 + 9.01H$ ($r^2 = 0.95$) for exposed conidia and $\%G = -24.97 + 7.22H$ ($r^2 = 0.98$) for nonexposed conidia.

Germination of conidia increased after a 6-h exposure to 7.2 μg mL^{-1} of each acetate ester (**Figure 3**). Esters did not differ in their mycoactive effect. Germination of exposed conidia was 1.8−2.9-fold greater than that of nonexposed conidia (8.6%).

DISCUSSION

Findings presented herein show that adhesion and germination of conidia, two life-cycle events important to the survival of *B. cinerea* on the surface of apple fruit, were stimulated by acetate esters. This is the first report of this acetate ester effect on fungal spores on apple fruit, although the effect has been reported for conidia on polycarbonate membranes (*4*, *6*). This is also the first report of the stimulation of conidial adhesion and germination by 2MBA.

Apple slices in this study were randomly taken from a pool of similar-sized slices that were cut from a few whole fruit to minimize the expected physiological variation in store-bought apples. Conidial adhesion and germination always occurred on apple slices used for controls, and conidia exhibited no abnormal behavior when on apple slices. In most of the experiments conidia of isolate F-J-4 was the model organism because it is a aggressive gray mold pathogen of apples (*10*−*12*) and its conidia respond to acetate ester stimulation (*4*, *6*). Butyl acetate was routinely used because it is frequently found in higher concentrations in apple tissue, as shown by the ca. 4−40-fold greater concentration than other acetate esters in apple slices that is reported herein. Most adhesion experiments were for 4 h because this duration was sufficient to observe statistically significant responses.

The responses of other *B. cinerea* isolates to other acetate esters in other experiments were comparable with those found for isolate F-J-4 and BA. Therefore, the conidial adhesion and germination responses found in this study were most likely characteristic of all the *B. cinerea* isolates studied. It should be noted that adhesion and germination of WMG2, which was isolated from geraniums with gray mold, was affected by acetate esters. Therefore, isolates of *B. cinerea* from other habitats should be examined for their responses to acetate esters to see how common acetate ester stimulation is for this species.

Although conidia of some isolates of *B. cinerea* show good germination levels in sdH_2O, most isolates exhibit little germination. These nutrient-dependent isolates typically require sugars or some other carbon energy source for germination (*21*−*24*). Conidia of the *B. cinerea* isolates used in this study were nutrient-dependent (*12*, *21*). The spores of other nutrient-dependent fungi, such as *Penicillium* and *Aspergillus* spp. (*5*, *24*) should be examined for acetate ester responses.

The similar responses of three *B. cinerea* isolates to four acetate esters suggest a common stimulation mechanism may operate in *B. cinerea*. How acetate esters stimulate conidium germination is not known; however, Filonow (*6*) reported that butyl acetate did not appear to be an energy-yielding substrate for *B. cinerea* conidia. Butyl acetate caused the conidia of *B. cinerea* to become leaky, so a putative adhesion hypothesis (*6*) suggests that conidia of *B. cinerea* leak an adhesive (*25*, *26*), the leakage of which is increased by acetate esters. Other fungal spores have been reported to secrete adhesives (*27*, *28*).

Nutrient-dependent spores, such as those in some species of *Botrytis*, *Penicillium*, etc. are generally considered to have an exogenous dormancy (*24*) that is different from the endogenous, i.e., constitutive, dormancy of many spores, such as those in *Phycomyces* and *Neurospora* spp. (*29*). Germination in these spores has to be activated by chemical or environmental stimuli. As acetate is an activator of germination for *P. blakesleeanus* sporaniospores (*30*, *31*), it would be interesting to see if acetate esters activate these spores.

It is generally accepted that adhesion of pathogenic fungal spores to plant host surfaces is a prerequisite for pathogenesis, because adhesion would reduce dislodgement of spores due to weather, and may aid the fungus in host recognition and as an anchor for spore germination. Spores of several plant pathogenic fungi typically exhibit adhesion prior to germination on host surfaces (*32*, *33*). Findings reported herein indicated that acetate esters stimulated conidial adhesion of *B. cinerea* to apple skin prior to germination. Additional studies are needed to clarify whether acetate esters or other volatile compounds from plants can accelerate adhesion of fungal spores, leading to stimulated germination. Initial adhesion of *B. cinerea* conidia to surfaces also has been shown to be a weak interaction prior to germination, and after emergence of germ tubes, adhesion becomes much stronger (*25*, *34*).

In the present study EA, BA, 2MBA, and HA were found in slices of Golden Delicious apples or in water drops on the skin of apple slices inside sealed jars. Others have identified these esters in apple or apple headspaces (*4*, *15*, *17*, *19*). The effect of these esters on conidial adhesion was a function of concentration and time of exposure. A BA headspace of 0.09 μg mL^{-1} was not stimulatory to conidial adhesion even after 24 h of exposure. However, concentrations greater than this were stimulatory up to a point (3.6−7.2 μg mL^{-1}), then adhesion declined with increasing BA concentration, most likely due to toxicity of greater concentrations (*4*). In the present study killed conidia did not adhere strongly to apple slices, regardless of BA exposure, indicating that adhesion is controlled primarily by fungal metabolism. A 24-h exposure to BA produced ca. twice the stimulation effect that 4 h did, suggesting that an accumulated dose of acetate ester is needed for conidia to be physiologically stimulated. How much of a dose is required by *B. cinerea* conidia has yet to be determined.

The effect of acetate esters on *B. cinerea* conidia was investigated on the skin of apple fruit; however, *B. cinerea* primarily colonizes wounds in apple fruit, and further study should address the effect of acetate esters on conidial adhesion and germination in wounds. In this regard, Filonow (*6*) reported greater *B. cinerea* decay in aged wounds in apple fruit exposed to butyl acetate than in aged wounds that were not exposed. It is reasonable to speculate that some pathogenic fungi that rely primarily on sugars and other readily available nutrients to stimulate spore germination in fresh wounds, may have an alternative, nonnutritive mechanism to stimulate germination when nutrients are scarce. Presumably, aged or healed wounds in apple fruit have less readily available nutrients than fresh wounds, but there appears to be no published comparison.

The present findings were obtained from experiments done on apple slices in sealed jars which had headspaces augmented with acetate esters to expedite mycoactivity and simplify interpretation of cause and effect. The findings on the skin of apple fruit confirmed those from polycarbonate membranes (*4*, *6*), showing that acetate esters formed in apples can stimulate adhesion and germination of conidia, and thus mediate the life-cycle of *B. cinerea* on the fruit. It would now be worthwhile to look for this phenomenon in apple trees, and during postharvest storage, etc., and investigate variables, such as fruit phenology and weather, that may influence the magnitude of the phenomenon. In addition, only 4 of the 18 acetate esters isolated from apple (*14*) have been investigated for their effect on fungal adhesion and germination. It is possible that other flavor and aroma chemicals formed in apple fruit may be more mycoactive than the acetate esters reported here.

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