Influence of Jet+Wash or Jet+Surf surfactant on the mortality of fungi in peracetic acid.

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Summary: **Jet+Wash (400:1 dilution) inhibited the germination of conidia of *Alternaria alternata, and*** it markedly increased the potency of 30 ppm peracetic acid to inactivate conidia of *P. digitatum.* **Jet+Wash increased the rate of conidial mortality in PAA about ten-fold.** When **Jet+Wash** was present during the PAA spore mortality assay, the LT_{50} (time to kill 50% of the conidia) was 55 seconds, while if the PAA was in water alone the LT_{50} was about 9 minutes. In a second experiment, **Jet+Surf** surfactant (500:1 dilution) also enhanced PAA activity. **Jet+Surf surfactant increased the rate of PAA conidial mortality in PAA about three-fold.** When it was present during the PAA assay, the LT_{50} was about 4 minutes, while if the PAA was in water alone the LT_{50} was about 12 minutes.

Objectives: Determine the following: **1)** if a 400:1 dilution of **Jet+Wash** alone inhibits the germination of three fungi (*Penicillium digitatum*, *Botrytis cinerea,* and *Alternaria alternata*); **2)** evaluate the effect of the addition of a 1:500 dilution of **Jet+Wash** on the rate of mortality of the *P. digitatum* conidia in 30 ppm (=mg/L) peracetic acid (PAA); and **3)** evaluate the effect of the addition of a 1:500 dilution of **Jet+Surf** on the rate of mortality of the *P. digitatum* conidia in 30 ppm PAA.

Methods.

Fungi*. Penicillium digitatum* was isolated from an infected orange, ***Botrytis cinerea*** from an infected grape, and ***Alternaria alternata*** was isolated from 'Atwood' navel orange leaves. The isolates were identified as using the descriptions of these species in the book Fungi and Food Spoilage, Edition 3, by John I. Pitt and Ailsa D. Hocking. *P. digitatum* conidia were cultured by infecting an ethanol surface-sterilized navel orange and incubating the fruit at 45°F for 10 days. *P. digitatum* conidia were collected with a metal spatula from a sporulating lesion *B. cinerea* and *A. alternata* produced abundant conidia on V8 juice agar at room temperature after 10 days. For the sporocide assay, the surface of a 10-day old colonies grown on V8 agar media were flooded with sterile water, rubbed with a flame-sterilized glass rod, and the solution was poured through two layers of sterile gauze. A hemocytometer was used to adjust the conidial concentration used in the sporocide assay to about 5.5×10^5 conidia per ml.

Mortality of conidia in Jet+Wash alone. Conidia were immersed in **Jet+Wash** for one hour, then an aliquot was plated on potato dextrose agar and germination evaluated. The assay was conducted as follows: 1) 0.4 ml of diluted **Jet+Wash** solution was placed in a 1.5 ml capacity polycarbonate centrifuge tube. The dilutions of the **Jet+Wash** (final concentration in the tube after thiosulfate and conidial solution additions) were water alone, 1:400, 1:1800, 1:1600, and 1:3200 and were from the label of the product; 2) at Time = 0 seconds, a 0.05 ml volume of a dense conidial suspension (containing 2.75×10^4 conidia) was added, the tube was capped

and inverted several times; 3) at Time = one hour of exposure, 0.05 ml of the thiosulfate solution was added, the tube was capped and inverted several times. The thiosulfate was added to determine if it contributed any toxicity and it would be present in later peracetic acid assays; 4) the centrifuge tube was spun at 2000 x g in a micro-centrifuge; 5) the pellet of conidia was removed in a 0.1 ml volume and deposited on the surface of potato dextrose agar; 6) the agar surface dried under sterile air for several hours, the plate lid was closed, and after a total of 48 hours at 20-22^{o}C the conidia were examined with a compound microscope at 200x on the surface of the agar; and 7) about 100 conidia were examined per plate, and the number germinated was divided by the number examined to determine the percentage of survivors. If few or no germinated conidia were observed, the plate was examined after an additional 24 hours to determine if germination was stopped or just delayed by the treatment.

Peracetic acid solution. Peracetic acid solution was provided by Jet Harvest Solutions, Jet-AG® (Lot # 8255092201, Jet Harvest Solutions, Longwood Florida 32791). A volume of 500 ml of a stock PAA assay solution containing 33.7 ppm PAA (the higher than 30 ppm concentration was used because of dilution back to 30 ppm when the spore solution was added to initiate the assay) was prepared from (4.9% PAA) as follows: (33.7 ppm) (500 ml) = (49,000 ppm) (x ml), where x solves to 0.344 ml of the PAA formulation. The pH was 4.5 to 5, as determined by pH indicating paper (Hydrion, range 1-11). To confirm the PAA concentration, the assay solution was further diluted to be a calculated 5 ppm to be within the concentration

range of the DPD reaction employed to quantify PAA using a Hach DR900 colorimeter. The dilution was as follows: (5 ppm) (50 ml) = (33.75 ppm) (x ml), where x solves to 7.41 ml. A 45 second reaction time was used in this assay (program 88, following Application Note: "Determination of Peracetic Acid (PAA) and Hydrogen Peroxide (H_2O_2) in Water") This solution was analyzed 5 times and indicated a mean concentration of 5.0 ppm was present in the diluted solution in water alone or a 400:1 dilution of **Jet+Wash**, which indicated 37.5 ppm was present in the sporicidal assay solution. This is the concentration needed before the assay solution was diluted by the addition of the conidial solution to a final concentration of 33 ppm, slightly higher than the 30-ppm desired.

Figure 1. Jet+Wash container labels.

Surfactant. Jet+Wash was received two weeks before use. The label indicated it should be used at a dilution of 400:1. It was present to a final concentration of 400:1 during the PAA assays. **Jet+Surf** was received 3 months before use. The label

indicated it should be used at a dilution of 500:1. It was present to a final concentration of 500:1 during the PAA assays. The label indicated it should be used at 24 oz/100 gallons, or in metric units 709.7 ml surfactant in 378.5 L of water, or approximately 1 ml in 500 ml. To 250 ml of the PAA assay solution that contained 112.5 ppm PAA, 0.469 ml of **Jet+Surf** was added. The pH of the resulting diluted solutions was 4.5 to 5.0, and like the pH of 4.5 of the PAA solution prepared in water alone.

Sporocide assay. The sporocide assay used exposures (from 2.5 to 30 minutes) of the conidia to the PAA solution in water or surfactant followed termination of the exposure by the addition of a thiosulfate solution (pH 5.5) to oxidize the PAA and hydrogen peroxide to water and acidic acid. The concentration of PAA during the assay was unchanged. A concentration of 85.4 mM thiosulfate (25 g/L) in a volume of 0.1 ml was sufficient to eliminate the PAA and hydrogen peroxide and terminate the exposure. This was confirmed empirically using low range PAA colorimetric detection paper (Hydrion low range, 0 – 160); when the thiosulfate was added, the assay paper indicated immediately no PAA was present. Preliminary tests indicated the acetic acid concentration remaining after thiosulfate addition was apparently below a concentration that inhibited conidial germination, since conidia placed in it for one hour or more germinated normally when transferred to potato dextrose agar.

The assay was conducted as follows: 1) 0.4 ml of the sporocide solution was placed in a 1.5 ml capacity polycarbonate centrifuge tube. 2) at Time = 0 seconds, a 0.05 ml volume of a dense conidial suspension (containing 2.75 x 10^4 conidia) was added, the tube was capped and inverted several times; 3) at Time = x seconds of exposure, 0.10 ml of the thiosulfate solution was added, the tube was capped and inverted several times; 4) the centrifuge tube was spun at 2000 x g in a micro-centrifuge for 3 minutes; 5) the pellet of conidia was removed in a 0.2 ml volume and deposited on the surface of potato dextrose agar; 6) the agar surface dried under sterile conditions for several hours, the plate lid was closed, and after a total of 16-18 hours at 20-22^{o}C the conidia were examined with a compound microscope at 200x on the surface of the agar; and 7) not less than 200 conidia were examined per plate, and the number germinated was divided by the number examined to determine the percentage of survivors. If few or no germinated conidia were observed, the plate was examined after an additional 24 hours to determine if germination was stopped or just delayed by the treatment.

Data analysis. Actual percentages were recorded and shown in tables and figures. LT_{50} times were used to compare the potency of the assay solutions when PAA was present. They were determined using probit regression where the probit of survivors versus the log of the time in the assay solution was used to construct a linear regression.

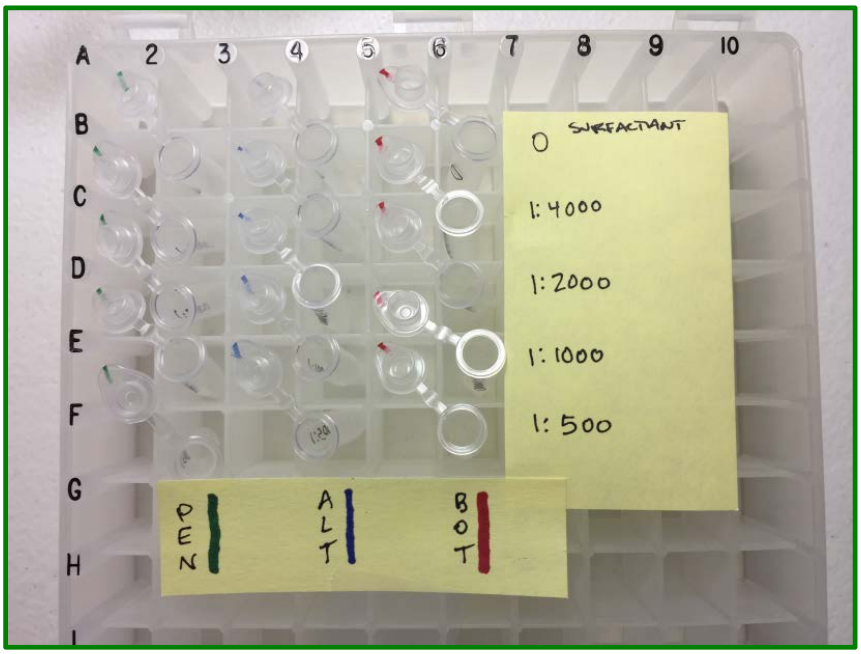

Figure 2. Micro-centrifuge tubes placed and organized in a carrier just before an assay was conducted to determine **Jet+Wash** toxicity to the conidia of several fungi.

Results and discussion

Jet+Wash toxicity alone to fungi. Survival of conidia in **Jet+Wash** in various dilutions after immersion for one hour followed by incubation on potato dextrose agar is shown in Table 1. **Jet+Wash** (400:1 dilution) alone inhibited the germination of condia of *Alternaria alternata,* but not those of *Penicillium digitatum* or *Botrytis cinerea.* This result is like that of a prior test where *Alternaria alternata* conidial germination was inhibited by **Jet+Surf**.

Table 1. Jet+Wash inhibited the germination of conidia of *Alternaria alternate* alone, but not those of *Penicillium digitatum* or *Botrytis cinerea* after 1-hour immersion in a 400:1 dilution of **Jet+Wash**.

	Survival (Germination %) of the conidia		
Dilution	*P. digitatum*	*B. cinerea*	*A. alternata*
Water alone	>95	>95	98.0
1:3200	>95	>95	96.8
1:1600	>95	>95	94.7
1:800	>95	>95	41.1
1:400	>95	>95	13.0

Influence of Jet+Wash and Jet+Surf on peracetic acid toxicty to *Penicillium digitatum*. Both products enhanced the kill power(toxicity) of PAA.

The effect of **Jet+Wash** (400:1) was greater than that of **Jet+Surf** (500:1) (Tables 2 and 3). LT_{50} times were determined using probit regression where the probit of survivors versus the log of the time in the assay solution was used to construct a linear regression, which was then solved to 50% mortality. Zero values were excluded and R^2 values exceeded 0.95 in all cases. When **Jet+Wash** was present during the PAA spore mortality assay, the LT_{50} (time to kill 50% of the conidia) was 55 seconds, while if the PAA was in water alone the LT_{50} was about 9 minutes (Figure 3). When it was present during the PAA assay, the LT_{50} was about 4 minutes,

while if the PAA was in water alone the LT_{50} was about 12 minutes (Figure 4). Comparing the time for 50% of the conidial population to be inactivated, **Jet+Wash** increased the rate of conidial mortality in PAA about ten-fold (Figure 3), while **Jet+Surf surfactant** increased the rate of PAA conidial mortality in PAA about three-fold (Figure 4).

Table 2. Germination of *Penicillium digitatum* spores from lesions on infected oranges after exposure to 30 parts per million (mg/L) peracetic acid in water alone or in a 1:400 dilution of **Jet+Wash**. To terminate the exposure to peracetic acid, sodium thiosulfate was added, the solution was centrifuged (2000 x g for 2 minutes), then the conidia were plated on potato dextrose agar and germination (n ≥ 200 conidia) was counted 18 hours later with a compound microscope at 100x.

	Germination (%) after immersion in 30 ppm PAA		
Time (minutes)	PAA in water	PAA in **Jet+Wash**	
Control	…	97.1	…
0	94.9		93.7
2.5	78.5		19.7
5.0	66.7		6.2
10.0	42.9		5.1
20.0	28.4		0.5
30.0	13.5		0.0

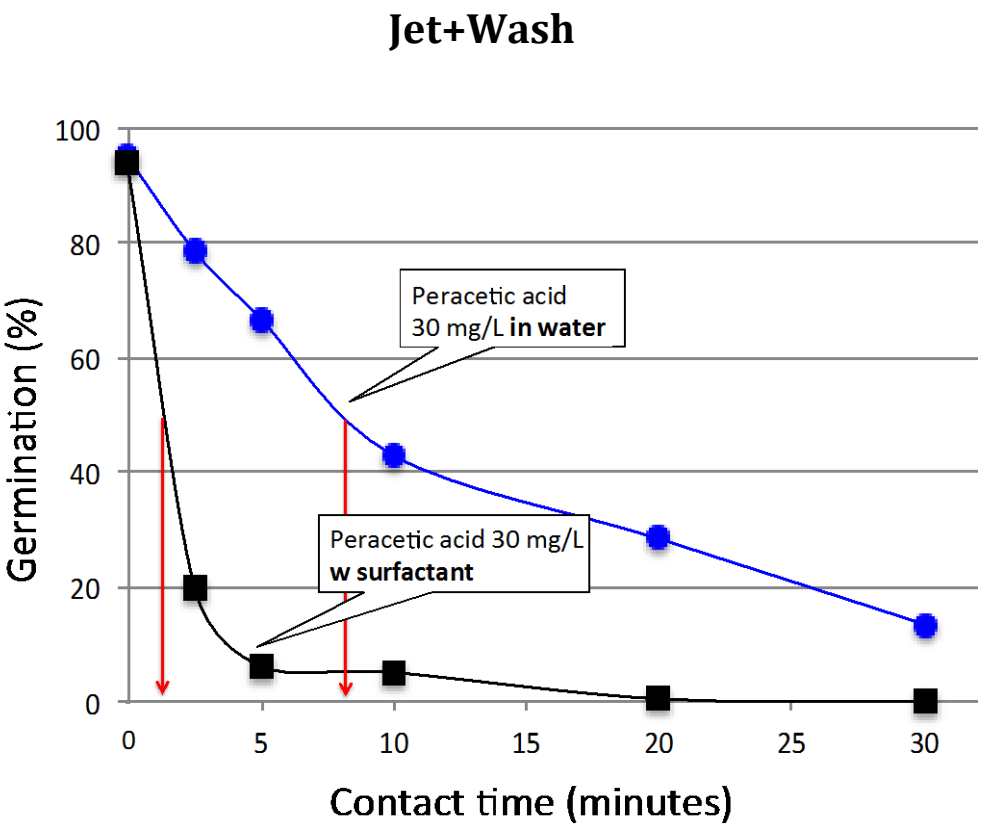

Figure 3. Germination of *Penicillium digitatum* spores from lesions on infected oranges after exposure to 30 parts per million (mg/L) peracetic acid in water alone or in a 1:400 dilution of **Jet+Wash**. Red arrows indicate when 50% of the conidial population was inactivated.

Table 4. Germination of *Penicillium digitatum* spores from lesions on infected oranges after exposure to 30 parts per million (mg/L) peracetic acid in water alone or in a 1:500 dilution of **Jet+Surf**. To terminate the exposure to peracetic acid, sodium thiosulfate was added, the solution was centrifuged (2000 x g for 2 minutes), then the conidia were plated on potato dextrose agar and germination (n=150 spores) was counted 18 hours later with a compound microscope at 100x.

	Germination (%) after immersion in 30 ppm PAA		
Time (minutes)	PAA in water		PAA in **Jet+Surf**
Control	…	93.1	…
0	91.8		92.0
2.5	72.7		63.5
5.0	63.6		47.1
10.0	59.1		20.8
20.0	44.6		13.6
30.0	32.3		10.7

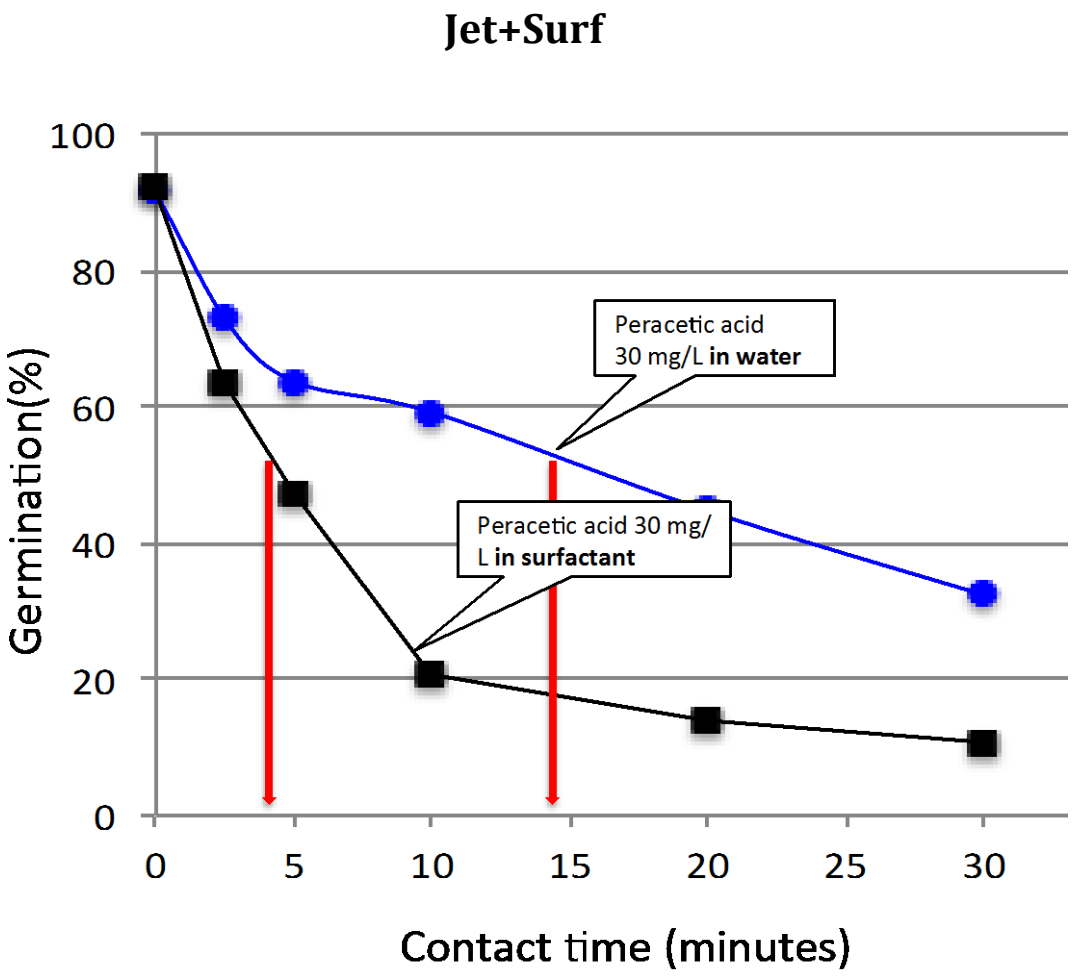

Figure 4. Germination of *Penicillium digitatum* spores from lesions on infected oranges after exposure to 30 parts per million (mg/L) peracetic acid in water alone or in a 1:500 dilution of **Jet+Surf**. Red arrows indicate when approximately 50% of the conidial population was inactivated.