Effects of Spray Adjuvants on Development of *Botrytis cinerea* on *Vitis vinifera* Berries

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ABSTRACT

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Ten agricultural spray adjuvants, including spreaders, stickers, and a penetrator, were sprayed on mature grape berries (cultivar Thompson Seedless or Emperor) at 0.125% product. Twenty microliters of a suspension of conidia of *Botrytis cinerea* containing 2×10^4/ml was applied to the surface of each berry after the berries dried. Water and chloroform treatments were used as controls. The overall mean of disease incidence and slope of disease increase over time were increased significantly by the treatments, with the chloroform treatment being affected most. Application of Penetrator 3 actually reduced the slope of the disease increase. The effects of Triton B1956, Ortho X-77, and Penetrator 3 on spore germination, sugar exudation, and berry water uptake and loss were studied. Germination of conidia increased significantly in the water droplets on the treated berries or when the water droplets were removed after 6 hr and the conidia added. The rate of exudation of reducing sugars was not affected significantly by the adjuvants; however, the rate of water uptake and loss by treated berries was increased, indicating that the epicuticular wax was affected by the spray adjuvants.

Additional key words: resistance, surfactants.

A spray adjuvant is a chemical added during an application of a pesticide mixture to alter its physical properties and enhance its effectiveness. Because of the development of pesticides with low effective dose values, systemic activities, and reduced numbers of required applications for economic control, there has been a tremendous increase in the production and use of spray adjuvants to aid spray tank mixing and spray coverage. Most spray adjuvants have nonionic or anionic surfactants as their major active ingredients and can be classified as spreaders, stickers, penetrators, or compatibility agents.

Spreaders usually contain the nonionic surfactants alkyl-aryl-polyoxyethylenate or alcohol polyoxyethylenate. About 10% of the spreader-type adjuvants contain an anionic surfactant such as a fatty acid or a linear alkyl sulfonate (19).

Stickers typically combine nonionic and anionic surfactants and may also contain resin acids, methene polymers, polyethylene plastic, or latex. Spreader-stickers are stickers with surfactants added.

Penetrators, many of which contain petroleum oils, can enhance the penetration of some pesticides through the cuticle. Some surfactants used in spreaders or stickers also may increase penetration of pesticides into plants because of their specific molecular configuration. This type of penetration activity is affected by plant species and pesticide used; thus, the plant/surfactant/pesticide combination must be considered for each application (19).

Compatibility agents contain emulsifiers, a group of specific petroleum oils, designed to prevent pesticides from breaking down when combined with the strong salt solutions of liquid fertilizers. The types of spray adjuvants considered in this paper are classified as spreaders, stickers, or penetrators.

The main function of spray adjuvants is to increase the wettability of plant parts, increase penetration of pesticides, and increase adhesion of particles to plant parts. All of the compounds act on the outermost layer of the plant cuticle, the epicuticular wax, which provides an important barrier between a plant and its environment. Epicuticular wax may also protect plants from disease caused by organic and inorganic agents. Swiecki et al (17) found an indirect relationship between the quantity of epicuticular wax and plant sensitivity to the air pollutant hydrogen chloride. Davies (2) reported that the physical configuration and chemical composition of surface wax affected the deposition of inoculum. Plant surface waxes also may contain chemicals inhibitory to fungal spores after deposition. An ether-soluble, acidic fraction of apple leaf wax was toxic to *Podosphaera leucotricha* (12), and linoleic and linolenic acids present in leaf wax extracts of rye plants inhibited several fungi (5). The amount of epicuticular waxes was correlated with resistance to *Alternaria brassicae* (16) and *Pyrenopeziza brassicae* (14) on rape and to several fungi on raspberry canes (7).

The role of epicuticular waxes in plant disease development can be altered by agricultural chemicals. Rawlinson et al (14) reported that the herbicides dalapon, carbetamide, and propyzamide decreased the amount and altered the form of epicuticular wax of rape leaves. The decreased wax was associated with an increase in wettability of the leaves and an increase in spore deposition, resulting in an increase in infection by *P. brassicae*. However, the effect of spray adjuvants on epicuticular wax and on plant disease has not been investigated.

The objective of this research was to determine the effects of spray adjuvants on the development of Botrytis bunch rot of grape (*Vitis vinifera* L.) caused by *Botrytis cinerea* Pers. A portion of this research was reported elsewhere (9).

MATERIALS AND METHODS

Spray adjuvants used. Witt (19) documented the spray adjuvants registered for agricultural use in the northwestern United States. He found that they could be classified as activators or special-purpose adjuvants. All of the spray adjuvants used in this study were classified as activators (Table 1).

Effects of spray adjuvants on disease development. In laboratory tests, mature berries of the table grape cultivars Thompson Seedless or Emperor were sprayed until runoff with a 0.125% solution of each spray adjuvant. The surface of each berry was inoculated with *B. cinerea* by placing 20 μl of a suspension containing 2×10^4 conidia per milliliter. Conidia were obtained from 2-wk-old cultures of *B. cinerea* on potato-dextrose agar. The suspension was amended with 0.2% water agar (Difco-Bacto agar) to prevent the droplet from sliding off the berry. A water treatment and a chloroform treatment, where the berries were dipped in chloroform for 10 sec to remove the epicuticular wax, were used as

controls. The berries were then placed in sealed plastic containers that had 1 cm of water in the bottom to maintain a high relative humidity (about 97% at 23 C). Berries were inspected daily for 7 days for mycelium of *B. cinerea*. The experiments were repeated four times with 30 berries used in each treatment. Data were transformed by the multiple-infection transformation, $\ln 1/1-y$, where y is the proportion of diseased fruit before statistical analysis was performed. The LSD test was used to compare the overall mean of each treatment with the water-treated control. The slope of disease progress for each treatment was compared with the water control by testing for equality of slopes with analysis of variance procedures.

In field tests, the spray adjuvants Triton B1956, Ortho X-77, and Penetrator 3 were applied to Thompson Seedless vines managed for the production of table grapes. These adjuvants were chosen for the remainder of the experiments because they represented the range of responses observed in the earlier experiments; Triton BI956- and Ortho X-77-treated berries developed high levels of disease and Penetrator 3-treated berries developed the least disease. The adjuvants were applied at 0.125% solution at 300 L/ha, the recommended volume for the applications of wettable sulfur for the control of severe outbreaks of powdery mildew on grape (15). The nonsprayed treatment was used as the control. After the spray had dried, 150 berries were randomly selected from each treatment and inoculated with conidia of *B. cinerea* as described. Susceptibility of the berries to infection was quantified by determining the percentage of diseased berries after 7 days. Four vines were used for each treatment 3 wk before harvest in a completely random design, and the experiment was repeated once. The mean of each treatment was compared with the nonsprayed control by the LSD test.

Effects of spray adjuvants on germination of *B. cinerea* conidia. Berries were treated as in the laboratory tests with the spray adjuvant Triton BI956, Ortho X-77, or Penetrator 3. Water and chloroform treatments were used as controls. Five hours after inoculation, the percentage of germination was determined by examining 100 conidia. The experiments were replicated three times with 30 berries used in each treatment. The means of the treatments were compared by Duncan's multiple range test.

To determine if an increase in germination was due to an interaction between the conidia and the surface of the berry or an interaction with substances exuding into the water droplet, berries were treated as in the germination experiments except that 20 μl of 0.2% water agar without conidia was placed on the treated berries. Water droplets were removed after 6 hr, and the conidia were added at 2×10^4/ml. The percentage of germination was recorded for 100 conidia after 5 hr at 23 C. The means of the treatments were compared by Duncan's multiple range test.

To identify the presence of sugars in the water droplets on the berries, berries were treated as before and placed in test tubes (25× 200 mm) with 1.5 ml of double-distilled water so that the stem end of a berry was not submerged. Reducing sugars in the water were analyzed after 6 hr with paper chromatography using Whatman 3MM paper and a developing solvent mixture consisting of *n*-butanol, pyridine, and water (6:4:3, v/v). Reducing sugars were detected by spraying the chromatogram with a chloroform solution containing 0.5% triphenyl tetrazolium chloride followed by saturated ethanolic KOH (18).

A separate, nonsprayed chromatogram was sectioned at 2-cm intervals, and the sections were eluted with 400 μl of distilled water for 7 hr. Twenty microliters of an eluate were placed in a microtiter well, and 10 μl of suspension containing 2×10^4 conidia of *B. cinerea* per milliliter was then added to each well. Percent germination was determined after 22 hr at 23 C for 100 conidia per well. The experiment was repeated twice with the water from five berries combined from each treatment for each replicate. The data were analyzed by analysis of variance procedures.

Effects of spray adjuvants on grape berry exudation. Berries were treated with the spray adjuvants and misted with water every 20 min. The 20 berries in each of six replicates were washed in 20 ml of double-distilled water. The phenol-sulfuric acid assay according to Dubois et al (3) was used to quantify the sugars in the wash solution, using glucose as the standard. This was repeated five times with 20 berries per replicate per treatment. The means of the treatments were compared by Duncan's multiple range test.

Effects of spray adjuvants on water absorption of grape berries. Individual berries were weighed and treated with adjuvants as described before. Berries were then placed in 1.5 ml of double-distilled water in 25 200-mm test tubes so that the water did not cover the stem ends. After 22 hr, 0.5 ml of water was added to compensate for evaporation and absorption by the berries. After 48 hr, the berries were extracted, the surface water removed, and the berries reweighed to quantify percent weight gain caused by water absorption. The number of berries that had split open because of swelling was also recorded; however, these berries were not used in determining the rate of water absorption. The means of the treatments were compared by Duncan's multiple range test.

Effects of spray adjuvants on water loss of harvested berries. Thompson Seedless vines were sprayed as in the field experiments. Twenty-eight berries were harvested from each treatment and dried at 21 C and 30% relative humidity. The stem ends of the berries were sealed with wax to restrict the loss of water from nonsurface areas. Weight loss was recorded every 8 hr for 96 hr. The experiment was repeated three times, and the slopes of the weight loss over time were compared with the nonsprayed control by testing for equality of slopes with analysis of variance procedures. Also, the overall mean of percent weight loss after 72 hr was compared with the nonsprayed control treatment by the LSD test.

RESULTS

In the laboratory tests, the overall mean and slope of the disease progress curves were affected significantly by the application of the spray adjuvants (Table 2). Fruit treated with the spray adjuvants Ortho X-77, Triton B1956, Amway All Purpose Spray Adjuvant, Surfix, and No-Foam A had significantly ($P \leq 0.01$) more disease than did the water control treatment. Penetrator 3 was the only

TABLE 1. Classification and properties of spray adjuvants tested

Company	Product name	Class	Major compound[a]	Percent active ingredient
Amway	All Purpose Spray Adjuvant	Spreader	AAPOE	23.6
Chevron	Ortho X-77	Spreader	AAPOE, FA, IPA	<90.0
Creative Marketing Research	No-Foam B	Spreader	AAPOE, IPA, LAS	<25.0
	No-Foam Adjuvant	Spreader	AAPOE, SI	25.0
FMC	Widespread	Spreader	AAPOE	80.0
Thompson Hayward	Activator 3	Spreader	APOE, PG, SI	9.0
Miller Chem. Co.	Nu-Film 17	Sticker	DM	96.0
Rohm & Haas	Triton B1956	Sticker	AR	77.0
Helena Chemical	Surfix	Sticker	APOE, FA, PR	78.0
	Penetrator 3	Penetrator	PO, POPOE	98.0

[a] APPOE = Aalkyl-aryl-polyoxyethylenate, APOE = alcohol polyoxyethylenate, AR = alkyl resins, DM = dimethene, FA = fatty acids or salts, IPA = isopropyl alcohol, LAS = linear alkyl sulfonate, PG = propylene glycol, PO = petroleum glycol, POPOE = propylene oxide polyoxyethylenate, PR = polymerized resins, and SI = silicon polymer, dimethyl polysiloxane (after Witt [19]).

spray adjuvant that resulted in less disease than the water control, 65.4 vs. 53.1%; however, the difference was not significant. The slope of the transformed disease over time was also affected by the treatments. Applications of Ortho X-77, Oilspreader, No-Foam A, Triton B1956, Amway All Purpose Spray Adjuvant, and Surfix all resulted in significant increases in the rate of disease development. Berries treated with Penetrator 3 developed disease at a significantly ($P \leq 0.02$) slower rate than did the water control (Fig. 1, Table 2).

In the field tests, the percentage of diseased berries from the vines treated with Triton B1956 (84.0%) or Ortho X-77 (70.5%) was significantly greater ($P \leq 0.05$) than that from vines treated with Penetrator 3 (14.0%) or water (12.7%) and nonsprayed controls (10.0%).

The mean of the percent germination of conidia of *B. cinerea* on the berries treated with chloroform was significantly greater ($P \leq 0.05$) than that for all other treatments (73.5%). Percent germination of conidia on berries treated with Ortho X-77 (14.8%), Penetrator 3 (14.0%), or Triton B1956 (10.7%) did not differ significantly but was significantly greater than that on the water-treated control berries (4.7%). Similar results were obtained when the water droplets were removed from the berries before the conidia were added. Germination of conidia in water droplets from chloroform-treated berries was significantly greater than in all other treatments (80.2%). Germination of conidia in water droplets from berries treated with Ortho X-77 (17.84%), Penetrator 3 (14.2%), or Triton B1956 (15.3%) did not differ significantly but was significantly greater than on the water-treated control berries (4.7%).

When *B. cinerea* conidia were added to solutions eluted from paper chromatograms, the greatest percentages of germinated conidia (14–16%) were in the solutions eluted from a zone that reacted with the triphenyl tetrazolium chloride reagent and chromatographed with glucose (Rf = 0.40) (Table 3). Germination in the solutions eluted from other zones of the chromatograms varied from 0 to 6%. Analysis of variance indicated that there was not a significant effect from the treatment ($P \leq 0.1$), but there was a significant effect by the Rf value ($P \leq 0.01$).

The rate of exudation of glucose equivalents per berry was not significantly affected by the application of the spray adjuvants. Sugar exudation was increased significantly ($P \leq 0.01$) by the chloroform treatment (274.5 μg/berry) compared with the other treatments (91–125 μg/berry) including the water control (Table 4).

The amount of water absorbed by the berries was determined by comparing their weights before and after partial submergence in distilled water for 48 hr. The chloroform, Triton B1956, Penetrator 3, and Ortho X-77 treatments resulted in a significant increase in water absorption compared with the control. The chloroform-treated berries had the largest increase in weight (3.18%). None of the berries split in the water treatment, whereas all of the other treatments had split berries resulting from the absorption of the water after 22 hr (Table 4).

The rate of water loss and the overall mean water loss after 72 hr were affected by the spray applications. Berries from vines treated with Triton B1956, Ortho X-77, or Penetrator 3 had a significantly ($P \leq 0.01$) different rate of water loss (0.12, 0.11, and 0.11 g/berry, respectively) than the water-sprayed or nonsprayed vines (0.10 and 0.10 g/berry, respectively). The overall mean water loss after 72 hr

TABLE 2. Development of *Botrytis cinerea* on Thompson Seedless berries

Treatment[a]	Mean of percent diseased	Slope of percent diseased
Chloroform	100.0	0.17*[b]
Ortho X-77	86.4*	0.14*
Amway	83.9*	0.14*
No-Foam A	80.2*	0.13*
Triton B1956	79.0*	0.13*
Surfix	74.1*	0.12*
No-Foam B	71.6	0.12
Widespread	70.4	0.12
Nufilm 17	69.1	0.11
Activator 3	67.9	0.11
Water	65.4	0.11
Penetrator 3	53.1	0.09*

[a] Berries were sprayed until runoff with a 0.125% product solution. Berries were dipped for 10 sec in the chloroform treatment.
[b] Value is significantly ($P \leq 0.05$) different from the control.

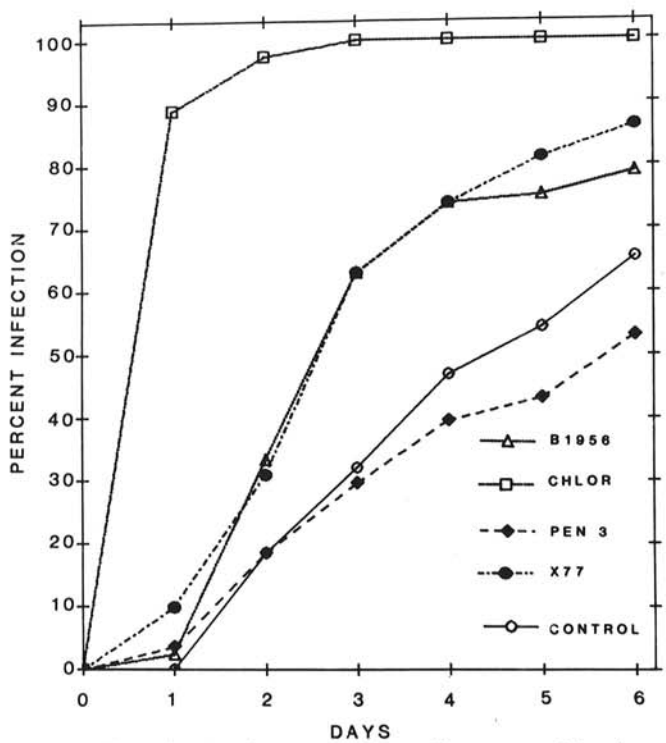

Fig. 1. Effect of chloroform- and spray adjuvant-treated berries on infection incidence over time. Each surfactant was applied at 0.125% product. B1956 = Triton B1956, Chlor = chloroform, Pen 3 = Penetrator 3, X77 = Ortho X-77, and control = water.

TABLE 3. Percent germination of *Botrytis cinerea* conidia from reelutriated chromatography separations after 22 hr in solution at 23 C

	Rf values								
Treatment[a]	0.05	0.17	0.28	0.39	0.50	0.61	0.72	0.83	0.94
Chloroform	2	2	3	14	2	3	2	3	4
Ortho X-77	2	3	3	12	4	2	3	0	2
Penetrator 3	2	4	3	15	3	2	0	3	1
Triton B1956	3	2	6	14	5	3	2	4	4
Water	3	3	4	16	3	2	3	0	1

[a] Berries were sprayed until runoff with a 0.125% product solution. Berries were dipped for 10 sec in the chloroform treatment, then submerged in 1 ml of water for 6 hr. The water solution (100 μl) was developed in *n*-butanol, pyridine, and water (6:4:3, v/v) and applied to Whatman 3MM chromatography paper.

TABLE 4. Effects of surfactant applications to grape berries on rate of sugar exudation and absorption of water

Treatment[y]	Glucose equivalents per berry	Percent weight gain	Percent split
Chloroform	274.5 a[z]	3.18 a	16.7
Ortho X-77	91.0 b	2.44 b	17.2
Penetrator 3	106.1 b	1.94 b	6.9
Triton B1956	108.2 b	2.17 b	2.8
Water	125.0 b	1.49 c	0.0

[y] Berries were sprayed until runoff with a 0.125% product solution. Berries were dipped for 10 sec in the chloroform treatment.
[z] Means followed by same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

was significantly greater in the Triton BI956 (9.01%) or Ortho X-77 (8.56%) ($P \leq 0.01$) and Penetrator 3 (7.92%) or water (7.99%) ($P \leq 0.05$) than in the nonsprayed control (7.49%). The rate of water loss and the percent disease were linearly correlated ($r = 0.96$ and $P \leq 0.01$).

DISCUSSION

The application of several spray adjuvants to grape berries significantly increased the development of disease caused by *B. cinerea* in both laboratory and field tests. These findings may have a significant effect on present disease control strategies. Although spray adjuvants are used to increase the efficacy of a fungicide, it is possible that their use may increase the susceptibility of a plant part to other pathogens that are not controlled by the specific fungicide applied. This is an important factor when one considers the limited specificity of many fungicides.

Adjuvants increased the germination of conidia of *B. cinerea* on treated berries, water absorption, and water loss of berries. Spray adjuvants did not significantly affect the rate of exudation of sugars. Berries dipped in chloroform to remove the epicuticular wax developed the most disease, induced the highest rate of germination of conidia, had the highest rate of exudation of sugars, and absorbed the most water when partially submerged. These observations, combined with the significant correlation observed between water loss and disease, indicate that the mechanism responsible for the increase in disease was the disruption of the function of the epicuticular waxes on the berry.

Brown (1) found that conidia of *B. cinerea* increased their rate of germination when exposed to water taken from the surfaces of grape berries. Kosuge and Hewitt (8) determined that water leached glucose and fructose from grape berries and was responsible for the increase in germination, elongation of germ tubes, and increase in formation of appressoria. The increase in disease incidence when the spray adjuvant Ortho X-77 or Triton BI956 was applied to the grape berries (59 and 50%, respectively) was supported by a corresponding increase in germination of conidia (216 and 128%, respectively). However, an increase in the exudation of reducing sugars by the surfactant-treated grapes was not observed. The possibility that compounds other than sugars led to the increase in germination of conidia and disease after application of spray adjuvants needs to be investigated.

The only emulsifiable oil tested, Penetrator 3, was also the only adjuvant that significantly decreased the rate of disease development, although the overall mean was not significantly different from the water control (Table 2). Hoy and Ogawa (6) determined that the anionic surfactant sodium dodecylbenzene had fungicidal properties toward conidia of *B. cinerea*. Forsyth (4) concluded that the primary effects of the spray adjuvants he tested were caused by disruption of the plasmalemma with a secondary effect on respiration. In preliminary experiments, we did not observe an effect of the adjuvants on the germination of conidia.

There was no correlation between the amount of active ingredient in the spreader adjuvants and their effects on disease development (Tables 1 and 2), indicating that other unidentified compounds added to the products affect the disease process. Petroleum oils, common components of spray adjuvants that are not identified as active ingredients, may be involved. Radler and Horn (13) reported that 30% of the epicuticular wax of grape can be classified as a soft wax composed chiefly of long-chain alcohols that are readily removed by petroleum oils.

The increase in the rate of water absorption by the grape berries may have been due to the alteration of the wax structure or concentration of wax on the berry surface. Wortmann (in Rawlinson et al [14]) showed that a spray adjuvant changed wax structure and the wettability of rape leaves. However, the effect was only temporary because the wax was regenerated. The regeneration of wax would not have been observed in our experiments because we used mature berries. Martin (11) reported that A. M. S. Fernandes confirmed that epicuticular wax provides an important barrier to the passage of water from the surface through the cuticular membrane, similar to our experiments with water uptake in the spray adjuvant- and chloroform-treated berries.

The role of epicuticular wax in protection of grape berries from infection of *B. cinerea* and water exchange was suggested by the fact that disease development, conidial germination, sugar exudation, water uptake, and loss of water from berries treated with chloroform were greater than in those treated with spray adjuvants or water. Also, Marois et al (10) found that the surface area of grape berries that was in contact with other berries in the cluster had altered epicuticular wax and was more susceptible to infection by *B. cinerea* than were noncontact areas of the berries, which had normal wax development. Although Martin (11) concluded that the cuticle was not a formidable barrier to the development of the disease, these studies indicate that, in the case of *B. cinerea* on grape berries, the epicuticular waxes of the plant may not only provide protection from adverse climatic conditions but also from plant pathogens.

The role of epicuticular wax in plant disease resistance may explain why many greenhouse experiments do not reflect results obtained in the field. The less severe temperature extremes, higher humidity, and reduced light intensity may all alter the development of waxes on plants. The subsequent effects on disease development may be significant.

Results obtained in these investigations indicate that epicuticular wax is an important barrier to infection of grape berries by *B. cinerea*. The interference of the function of epicuticular waxes may result in a plant part being more susceptible to infection. The adverse effects that some spray adjuvants have on the disease resistance associated with epicuticular wax are shown here. How this phenomenon may affect actual disease control practices needs to be investigated further; however, from a research consideration, it indicates the necessity for considering the impact spray adjuvants may have on the experimental design and the possibility of using spray-adjuvant-alone treatments as necessary controls. This research also attests to the need for one to consider the types of spray adjuvants included in pesticides formulated as emulsifiable concentrates, wettable powders, or flowables and how they may affect the interactions of the plant with target and nontarget pathogens. Finally, it is apparent from this research that the importance of epicuticular wax as a mechanism of resistance to plant pathogens needs further investigation and may have potential as a tool for disease management.

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