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Utilization of Surfactants for Control of Shot Hole on Almond

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Shot hole disease of almond is caused by the fungus Wilsonomyces carpophilus and can cause significant losses in some years. The disease is currently controlled with fungicides. Consumer concerns and new federal and state regulations indicate the necessity for the development of alternative strategies for disease control. We are trying to use compounds with surfactant properties, currently used either as "agricultural adjuvants" or in skin care products, to control shot hole. These compounds, which are generally regarded as safe, are often economical and could be incorporated into spray programs.

The shot hole fungus spreads throughout a field by spores. These spores are surrounded by material called an extracellular matrix. Although the function of the Wilsonomyces extracellular matrix is unknown, it presumably is important for the fungus' ability in the initial infection process. We postulated that selected surfactants or "spreaders" might have fungicidal or fungistatic activity. First, surfactants contact the fungal extracellular matrix and may be able to disperse critical components. Second, Wilsonomyces spores attach to leaf surfaces, which are very hydrophobic. In addition, Wilsonomyces conidia and germlings attached to a greater extent onto polystyrene, a hydrophobic surface, than onto glass, a comparatively hydrophilic surface (Table 1). Since surfactants make surfaces more hydrophilic, we postulated that the spores may not attach as well to surfactant-treated leaves as to non-treated leaves. That is, adjuvants could function either by disrupting the attachment of spores and germlings onto the host surface, rendering the surface too hydrophilic for attachment to occur or by dispersing the spore extracellular matrix.

Last year, we identified several promising "spreaders" or "spreader-stickers" (Table 2). However, they were not retained well on either polystyrene or on the leaf surface after washing (Table 3). That is, while the surfactant must form a relatively hydrophilic layer over the plant, the applied compounds also must be sufficiently hydrophobic so that it is retained on the plant surface. Therefore, we screened more hydrophobic detergents, including Brij 56, Procetyl 30, and Volpo 3. We also combined materials. For example, we combined water-insoluble surfactants, such as Procetyl 30 and Volpo 3 and emulsifying agents, such as Crovol A-70 and Croval A-40. However, the combinations formed less stable films than the single formulations. We also combined surfactants, such as Tri-Ad 73, Safer Soap, and Brij 56 with compounds with more adhesive properties, including Hold-on, Latron B-1956 and Pemulen; selected examples are also shown in Table 3. While several of these combinations formed stable hydrophilic films on polystyrene (Tables 4 and 5), the materials were not retained well on the leaf surface (data not shown).

While we can demonstrate some control of shot hole disease with the selected formulations, higher concentrations of the materials than desirable are required (Table 6). For example, in lathhouse experiments, 0.5% and 1% Latron B-1956, a "spreader-sticker" reduced disease incidence and severity of shot hole to the same extent as 0.18% Ziram, a standard fungicide (Table 7). Both higher and lower concentrations of Latron B-1956 were ineffective. Please note that Latron B-1956 and other products mentioned above should not be used in spray programs to control shot hole disease at this time. If we can demonstrate that selected adjuvants are useful for shot hole control in the field, we must register them as fungicides before they can be used agriculturally.

Table 1. Attachment of *Wilsonomyces carpophilus* onto polystyrene vs. glass.

Surface	No. of fungal units + SD per mm^2	
	Spores	Germlings
Polystyrene	18.8 ± 1.2	12.8 ± 6.5
Glass	10.4 ± 1.4	2.6 ± 0.4

Table 2. Effect of selected surfactants on adhesion of *Wilsonomyces carpophilus* onto polystyrene.

Surfactant	Reduction of untreated control, %		Effectiveness Ranking
	Spores	Germlings	
RNA Tri-Ad 73	98	99	1
RNA Spreader-Binder	98	87	2
Saturall	84	93	3
Triton CS-7	90	85	4
Stik	29	29	5
Tween 80	13	25	6
Spray Fuse	-8	16	7

[1] The surfactant with the highest reduction in % reduction in spores + germlings was considered to be most effective.

Table 3. Hydrophilicity rating of polystyrene treated with selected agricultural adjuvants before and after washing.

Adjuvant 1	Adjuvant 2	Hydrophilicity Rating 2: Unwashed control	Hydrophilicity Rating 2: Washed
Untreated	-	5	5
RNA Tri-Ad 73	-	13	6
RNA Spreader-Binder (RSB)	-	7	5
Triton CS-7	-	6	5
RSB	Tri-Ad 73	15	6
RSB	RNA Resin	11	5
RSB	Hold-on	11	10
Tri Ad 73	Hold-on	17	17
Triton CS-7	Hold-on	5	5
RSB	Triton B-1956	14	5
Hold-on	Triton B-1956	13	8
Triton CS-7	Triton B-1956	7	5
Tri-Ad 73	Triton B-1956	15	13

1 Tri-Ad 73, Spreader Binder (RSB), and Triton CS-7 are primarily surfactants; the resin, Hold-on, and Triton B-1956 are primarily adhesives. All compounds were tested at 0.12%.
2 Diameter in mm of droplet of dye. For comparison, glass has a hydrophilicity rating of 7.

Table 4. Hydrophilicity rating of polystyrene treated with varying concentrations of RNA Hold-on and RNA Tri-Ad 73 before and after washing.

Concentration, %		Hydrophilicity Rating [1]	
Hold-on	Tri-Ad 73	Unwashed control	Washed
0	0	5	5
0.62	0.12	8	6
0.25	0.12	16	14
0.12	0.12	17	17
0.12	0.25	16	16
0.12	0.62	11	11

[1] Diameter in mm of droplet of dye.

Table 5. Hydrophilicity rating of polystyrene treated with varying concentrations of RNA Hold-on and RNA Spreader Binder before and after washing.

Concentration, %		Hydrophilicity Rating [1]	
Hold-on	Spreader Binder	Unwashed control	Washed
0	0	5	5
0.62	0.12	7	7
0.25	0.12	16	13
0.12	0.12	14	12
0.12	0.25	14	14
0.12	0.62	14	13
0.03	0.12	15	6
0.03	0.62	15	5

[1] Diameter in mm of droplet of dye.

Table 6. Effect of selected agricultural adjuvants on shot hole disease of almond.

Treatment[1]	Infected leaves (%)	Disease index[2]
Control	53.1 a[3]	0.59 ab
1% Triad	56.4 a	0.67 a
1% Triton B 1956	51.6 ab	0.60 ab
Triad+Hold+Triton B	40.1 bc	0.45 bc
1% Hold on	36.9 c	0.42 c
0.18% Ziram	17.9 d	0.18 d

1 Five shoots per treatment were treated with the adjuvants and then inoculated with *Wilsonomyces carpophilus*.
2 Disease index was based on three categories: 0 = healthy; 1 = 1-5, and 2 = ≥6 shot hole lesions per leaf.
3 Significant differences according to DMRT ($P < 0.05$).

Table 7. Control of shot hole disease (*Wilsonomyces carpophilus*) of almond with Latron B-1956 in the lathhouse.

	Exp. #1		Exp. #2	
Treatment	Disease incidence (%)	Disease index[1]	Disease incidence (%)	Disease index*
Control	55.0 a[2]	0.68 a	47.0 a	0.51 a
0.18% Ziram	26.1 bc	0.29 bc	29.8 bc	0.30 c
1.5% Latron B	38.3 b	0.39 b	45.9 ab	0.48 ab
1% Latron B	24.9 bc	0.25 bc	22.1 c	0.24 c
0.5% Latron B	18.0 c	0.19 c	29.0 c	0.30 c
0.25% Latron B	-	-	32.6 abc	0.34bc
0.125% Latron B	-	-	46.7 a	0.53 a

1 Disease index was based on: 0 = 0 lesion/leaf; 1 = 1-5 lesions/leaf and 2 = $\geq$6 lesions/leaf.

2 Significance according to DMRT at ($P < 0.05$).