UNIVERSITY OF CALIFORNIA | KEARNEY AGRICULTURAL CENTER

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO | SANTA BARBARA • SANTA CRUZ

9240 S. Riverbend Avenue
Parlier, CA 93648
(209) 891-2500

December 26, 1990

Ms. Susan McCloud
Research Director
P. O. Box 15920
Sacramento, CA 95852-1920

Dear Sue:

Enclosed is a report on our research done in 1990. You will realize after reading the report that the surfactant (Tri-ad 73) and the anti-transpirant (Red Spider Gel) used in our studies were not as effective as we expected them to be. Because there are some funds left from last year's grant, we plan to do additional experiments especially early in the spring when Tri-ad 73 showed effectiveness in reducing shot hole disease of almond ('NonPareil').

Have a Happy and Prosperous New Year!

Sincerely,

Themis

Themis J. Michailides
Research Plant Pathologist

TJM/dln
Enclosure
cc: Lynn Epstein
Beth Teviotdale

Research Report
December, 30, 1990
Project No. 90-ZF1

Utilization of Surfactants for Control of Shot Hole Disease of Almond

Themis Michailides[1], Lynn Epstein[2], Beth Teviotdale[1], David P. Morgan[1] and Anjali Vaidya[2]

[1]Kearney Agricultural Center, Parlier, California and [2]Department of Plant Pathology, University of California, Berkeley, California

Shot hole disease of almond can cause significant losses in some years. The disease is currently controlled with fungicides. Consumer concerns and new federal and state regulations demand the development of alternative strategies for disease control. We postulated that selected agricultural adjuvants, which are generally regarded as safe, might directly inhibit pathogen attachment and development on the host surface.

Stigmina carpophila, the causal agent of shot hole, spreads throughout a field by spores called conidia. These spores are surrounded by material called an extracellular matrix. Although the function of the Stigmina extracellular matrix is unknown, it presumably is important for the fungus' ability in the initial infection process. We postulated that agricultural surfactants or "spreader-stickers" might have fungicidal or fungistatic activity. First, surfactants contact the fungal extracellular matrix and may be able to disperse critical components. Second, Stigmina spores attach to leaves. Since some surfactants alter the surface wetting properties of plant leaves, we postulated that the spores may not attach as well to surfactant-treated leaves as on non-treated leaves.

In our laboratory assays for spore attachment, we used polystyrene as a model for a hydrophobic surface and glass as a model for a hydrophilic surface; aerial plant surfaces are hydrophobic and surfactant-treated plant surfaces are comparatively hydrophilic. As we predicted, Stigmina conidia and germlings attached to a greater extent onto polystyrene than onto glass (Table 1). Furthermore, significantly fewer conidia and germlings attached to polystyrene dishes treated with selected surfactants: RNA Tri-Ad 73, RNA Spreader Binder (RSB), Triton CS-7, and Saturall than to untreated controls (Table 2). In comparison, other surfactants, i.e., Spray Fuse, Stik, and Tween 80, were ineffective in reducing attachment of spores and germlings onto polystyrene. Tri-Ad 73 was selected for further experiments.

To determine if Tri-Ad 73 effected spore viability, Stigmina spores were washed in either TRI-AD 73 or distilled water and adjusted at a concentration of $5X10^4$ spores/ml. Spores were washed by centrifuging the suspensions three times at 8,200 rpm for 3-5 minutes and 200-300 µm were spread on the surface of each of five dishes containing water agar. Germination of spores was recorded 5 hr later. The experiment was repeated twice. Spores washed with Tri-Ad 73 germinated less than those washed in water, suggesting that the surfactant either causes leakage of cellular metabolites or more effectively removes the extracellular matrix than water (Fig. 1).

To determine if a selected surfactant could reduce disease incidence, one pint Tri-Ad 73 per 100 gallons was sprayed onto 'Nonpareil" almond plants in the lath house, allowed to dry, and then shoots sprayed with a suspension of 10^5 conidia/ml of Stigmina. The shoots were immediately covered with a 10 mil plastic bag which had been sprayed inside with distilled water. Bags were secured to the shoot with masking tape. A brown paper bag was placed over the plastic bag to protect the shoots and leaves from sunburn. In another set of trees, shoots were sprayed with the spore inoculum of Stigmina, allowed to dry, and then sprayed with a solution of Tri-ad 73. In an experiment on 19 April 1990, plants treated with Tri-Ad 73 and then inoculated with Stigmina had reduced incidence and severity of disease than the controls without surfactant (Fig. 2A). Disease incidence was not reduced when plants were inoculated and then sprayed with Tri-Ad 73 (Fig. 2B). In a second experiment on 30 April 1990, Tri-Ad 73 did not reduce disease when the plants were either treated with the surfactant and then inoculated with Stigmina or inoculated and then treated with the surfactant (Table 3). In fact, plants which were inoculated and then sprayed with 1 pint Tri-Ad 73/100 gallons had significantly more symptomatic leaves than the untreated control. We caution that in the lath house experiments, we did not include a treatment with surfactant on non-inoculated plants. Since we monitored shot-hole-like symptoms and did not attempt to reisolate the fungus from apparently infected tissue, it is possible that some of the lesions which we attributed to Stigmina infections could actually have been due to phytotoxicity of the Tri-Ad 73. Alternatively, Tri-Ad 73 could have enhanced disease. Experiments are in progress to determine whether the components in Tri-Ad 73 can degrade the leaf surface.

In a field experiment, incidence of shot hole was too low to determine efficacy (Table 4).

Although anti-transpirants generally do not increase plant surface hydrophilicity, we tested one anti-transpirant since it has been reported to control some pathogens. However, the anti-transpirant Red Spider Gel actually increased the incidence of shot hole when almond leaves were first treated and then inoculated with Stigmina (data not shown).

Since TRI-AD 73 was effective in preventing spore and germling attachment, but relatively ineffective in reducing disease in lath house trials (except in one experiment on 19 April), we postulated that Tri-Ad 73 was not retained on the leaf surface. We monitored retention of selected surfactants on polystyrene by measuring the diameter of a 25 μl droplet of dye. The diameter of the droplet on unwashed, surfactant-treated polystyrene was compared to washed, surfactant-treated and untreated polystyrene (Table 5). All of the surfactants were rapidly removed.

In order to improve retention, the selected surfactants were combined with more adhesive adjuvants, primarily RNA Hold-on and Triton B-1956. Retention of the surfactant was monitored by measuring the surface hydrophilicity, as described above. Addition of an adhesive, especially Hold-on, significantly improved retention on polystyrene. Varying concentrations of Hold-on with either Tri-Ad 73 or Spreader-Binder were tested (Tables 6A and 6B) to determine maximum retention and hydrophilicity. The quantity and concentrations of material required for retention on polystyrene (0.53 ml containing 0.12% Hold-on and 0.12% Tri-Ad applied onto a 60 mm diameter polystyrene petri plate) are sufficiently small that they could be used agronomically. That is, our application rates would be roughly equivalent to 1 pint each of Hold-on and Tri-Ad 73 per 100 gallons with 200 gallons sprayed per acre.

Experiments are in progress to determine whether Tri-Ad 73 in combination with Hold-on disrupts subsequent attachment of S. carpophila spores and germlings onto polystyrene. In addition, we should investigate the possibility whether surfactants affect the waxy cuticle of almond leaves. Marois et al. showed that surfactants increased incidence of bunch rot disease of grapes caused by Botrytis cinerea because the surfactants interfered with the removal of epicuticular wax of grape berries [Phytopathology 75: 1329 (1985)].

Table 1. Attachment of *Stigmina carpophila* onto polystyrene vs. glass.

	No. of fungal units + SD per mm^2	
Surface	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 *Stigmina carpophila* onto polystyrene.

	Reduction of untreated control, %[1]		Effectiveness
Surfactant	Spores	Germlings	ranking
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. Effects of surfactant RNA TRI-AD 73 on shot hole disease of almond in the field.

Inoculation	Treatments	Symptomatic leaves(%)[1]	Disease index[2]
Inoculated, then treated	RNA TRI-AD73 (1pt/100)	43.3 a[3]	0.5 a
	RNA TRI-AD73 (0.5pt/100)	26.9 ab	0.3 b
	Control	22.9 b	0.2 b
Treated, then inoculated	RNA TRI-AD73 (1pt/100)	33.8 a	0.4 a
	RNA TRI-AD73 (0.5pt/100)	29.2 a	0.3 a
	Control	22.9 a	0.2 a

[1] Average of four 46-112 leaf replications.

[2] Disease index was based on five disease severity categories: "0"=0, "1"=1-5, "2"=6-10, "3"=11-15, and "4"≥16 lesions/leaf.

[3] Numbers in each column followed by the same letter are not significantly different, according to Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

Table 4. Effects of RNA TRI-AD 73 on shot hole disease on almond fruit in an experimental orchard at Kearney Agricultural Center.

	Mean number of spots[1] per fruit on:	
Treatment	2 May	18 May
RNA TRI-AD 73/noninoculated	0.18 b[2]	0.08 bc
RNA TRI-AD 73/inoculated (10^4)[3]	0.18 b	0.42 b
Inoculated (10^4)	0.57 ab	0.32 bc
Inoculated (10^5)	1.07 a	0.82 a
Noninoculated control	0.33 b	0.05 c

[1] Mean of four 20-25 fruit replications inoculated on 2 and 18 May.

[2] Significance at $P < 0.05$ (DMRT).

[3] Conidia of _S. carpophila_ were applied with a hand sprayer.

Table 5. Hydrophilicity rating of polystyrene treated with selected agricultural adjuvants before and after washing.

		Hydrophilicity Rating[2]	
Adjuvant 1	Adjuvant 2	Unwashed control	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 6A. 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

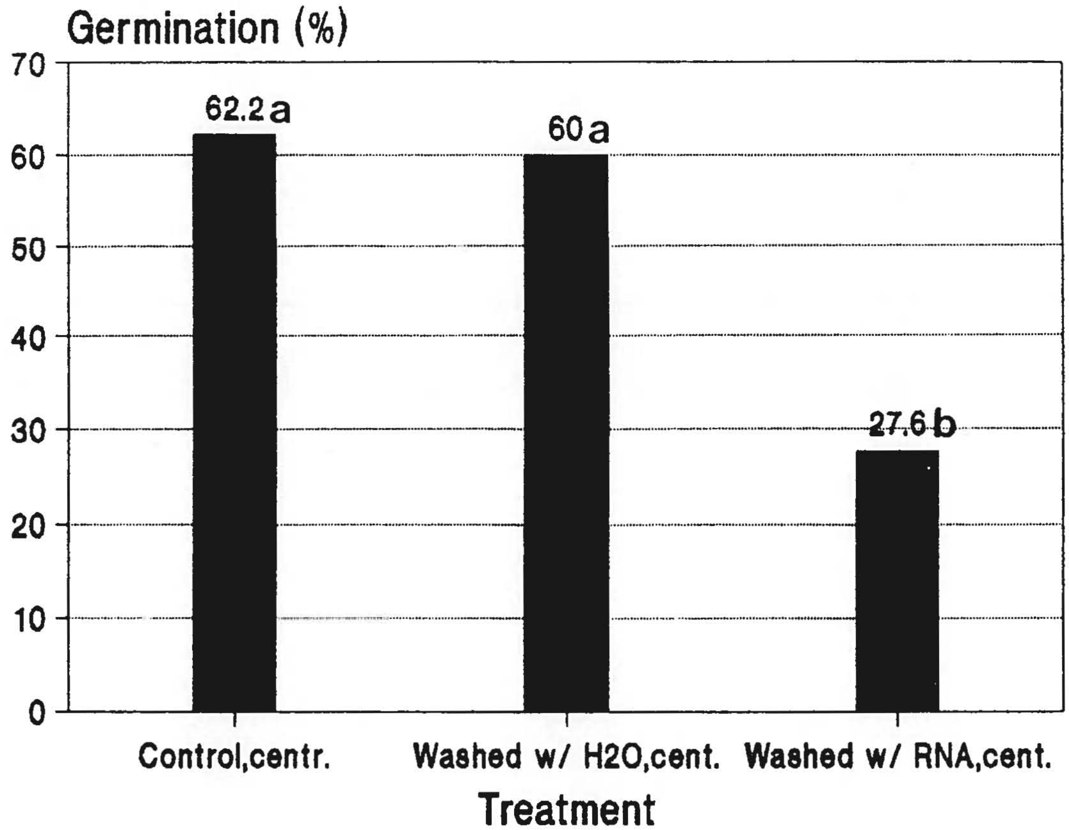
[1] Diameter in mm of droplet of dye.

Table 6B. 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	Tri-Ad 73	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.

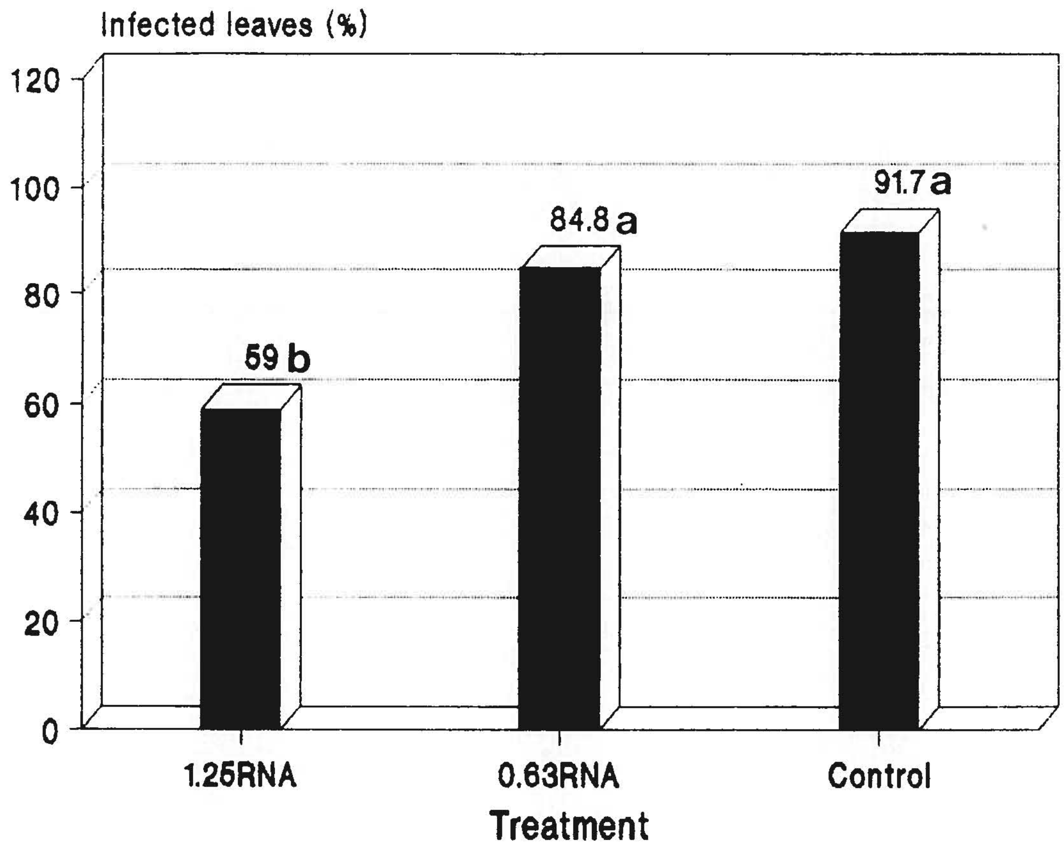
Effect of RNA TRI-AD 73 on Spore Germination of *Stigmina carpophila*



*Average of four replications.

Figure 1

Effect of RNA-TRIAD* ON SHOT-HOLE DISEASE (*Stigmina carpophila*)



*Treated and then inoculated

Figure 2A