Efficacy of Fatty Acid Chemistry: Candidate Mold and Decay Fungicides

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ABSTRACT

Although organic, lipophilic acids, such as acetic, propionic, sorbic and benzoic, have a long history as preservatives in the food industry, relatively high concentrations are required and their bioactivities generally pertain to retarding microbial growth rather than eliminating pathogens. Moreover, exclusive use of organic acids such as lactic or citric acid, alone, as broad-spectrum biocides, is generally not sufficient for long term control of mold fungi. However, specific organic acids (i.e., glycolic and L-lactic), even at low concentrations, are now known to be potent synergists with certain fatty acids as broad-spectrum, environmentally compatible fungicides. Specifically, low molecular weight, aliphatic fatty acids (mono-carboxyl compounds such as pentanoic to decanoic acids or C5 to C10) at various application rates +/- selected adjuvants have been examined as candidate mold and decay fungicides. Substantial evaluation of formulation homogeneity, stability of dilutions in water and fungicide activity of stable formulations, both for dip and pressure treated wood products, has been accomplished. Antimicrobial formulations having multiple mechanisms of action with greater formulation potency would be considerably more effective. The research effort is under a joint venture agreement between Summerdale, Inc. and Forest Products Laboratory (FPL), Madison, WI.

Keywords: fatty acid, mold fungi, decay fungi, fungicide

INTRODUCTION

Summerdale, Inc. has developed novel, fungicide formulations, containing synergistic combinations of low molecular weight, aliphatic mono-carboxylic acids and selected adjuvants, highly effective against a wide range of fungal pathogens. Earlier studies focused on control of crop pathogens with more recent work applied to wood preservation. Much of the multi-year fungicide development projects were supported by competitive, Phase I & II research grants to Summerdale, Inc. from the NSF and USDA SBIR programs.

Numerous independent studies, pertaining to safer, effective fungicide chemistry, have been conducted in collaboration with Michigan State University, University of Wisconsin, University of California at Davis, North Carolina State University, USDA ARS (Beltsville, MD), University of Idaho, University of Nebraska and more recently, the Forest Products Laboratory in Madison, WI. Brief trial results relating to crop fungicides as well as efficacy for control of wood pathogens is presented.

MATERIAL AND METHODS

Application via dip and/or vacuum-pressure treatments were evaluated for treated, kiln-dried southern yellow pine (SYP) and the composite hardboard. Moldicide activity of leached versus unleached, formulation-treated SYP were evaluated to determine bioactivity of a fatty acid formulation +/- selected

adjuvants. The leach procedure was conducted according to Standard AWPA Ell-06 (2009). The mold assay was conducted as follows:

Test organisms and inoculum preparation

Mold fungi, *Aspergillus niger* 2.242, *Penicillium chrysogenum* PH02, *Alternaria alternata* and *Trichoderma viride* 20476 were grown on 2% malt agar and individual spore suspensions prepared by washing the surface of a 2-wk old culture of each fungus with 10 mL of sterile deionized (DI) water according to ASTM standard D4445-91 (1998). Spore suspensions were transferred to a spray bottle and diluted to 100 mL with DI water to yield approximately 10^7 spores ml^{-1}. The spray bottle was adjusted to deliver 1 mL inoculum/spray. The sapstain fungus, *Aureobasidium pullulans,* was grown on 2% potato dextrose agar and an individual spore suspension was prepared from a 2-wk old culture.

Mold and sapstain tests on southern pine stakes and hardboard composite

Specimens (7 x 20 mm cross section by 7 cm long and 75 x 100 mm by €2.5 mm thick) were cut and conditioned at 27°C, 70% relative humidity (RH). Twelve random replicate specimens were dip-treated for −15 seconds in each moldicide formulation and held overnight according to the ASTM standard test method D4445-91 (1998) or ASTM D3273-00 (1986). Vacuum treatments were conducted according to AWPA E10-09 (2009). For the stake test, treated specimens were arranged over 4 layers of blotting paper saturated with 30 mL DI water and a polyethylene mesh spacer in sterile disposable Petri dishes (150 x 25 mm) (B-D Falcon, Los Angeles, CA, USA). Untreated specimens dipped in DI water served as controls. Specimens were sprayed with 1 ml of individual mold or sapstain spore inocula, sealed in polyethylene bags to prevent drying and incubated at 27°C and 70% RH for 8 - 12 weeks.

Decay fungi test

The experimental approach for the soil-block test followed AWPA E10-01: Standard Method for Testing Wood Preservatives by Laboratory Soil-block Cultures. Three-fourth inch cubes were treated in a vacuum desiccator. Blocks were air dried, then equilibrated to a constant weight before being placed in a soil-block test. Five replicates per wood species per retention were treated with experimental preservative formulations. Both treated and untreated sapwood blocks were exposed to decay caused by two brown-rot and one white- rot fungi. The softwoods were exposed to the brown-rot fungi and the hardwoods were exposed to the white-rot fungus. After twelve weeks the blocks were brushed free of mycelium and then allowed to air-dry overnight. Blocks were reconditioned to a constant weight at 6% equilibrium moisture content (EMC) before determining the percentage of weight loss due to fungal decay. Test fungi and wood species were as follows:

Test fungi

Brown-rot Fungi: *Gloeophyllum trabeum* (Pers. ex Fr.) Murr.: MAD 617
Postia placenta (Fries) Larsen and Lombard: MAD 698
White-rot Fungi: *Trametes versicolor* (L.:Fr.) Pilat: MAD 697

Wood species

Softwoods: Southern Pine – *Pinus, elliotii, palustris, or taeda.*
Hardwoods: Sweet gum – *Liquidambar styraciflua*

Commercial woodprotectant products

Chlorothalonil (Sostram Corporation, Roswell, GA)
DDAC (didecyl dimethyl ammonium chloride) (Lonza Group, Allendale, NJ)
DDAC/IPBC (didecyl dimethyl ammonium chloride/ 3-iodo-2-propynyl butyl carbamate) (Kop-Coat, Inc., Pittsburgh, PA)
Potassium sorbate (Bendiner Technologies, LLC, Pinehurst, NC)

RESULTS AND DISCUSSION

Early development as an agricultural fungicide

Recent application of fatty acid chemistry in wood preservation was established from many years of extensive formulation development and dozens of independent, greenhouse and field studies comparing commercial agricultural chemicals and experimental pesticides. A brief description of chemistry found efficacious against fungal diseases in agriculture provides a better understanding of strategy adopted for combating wood fungi.

Earlier research pertained to fungicides for controlling a broad spectrum of crop pathogens Issued patents are: Coleman, U.S. patents U. S. #6,218,336, #6,509,297, #6,812,190, # 6,969,696, #7,741,244 and Chinese patent, issued 2006, and a pending patent application: Pesticide Compositions and Methods For Their Use, a continuation-in-part of Fungicide Compositions (#7,741,244). Publications include: Coleman and Penner 2002, 2006, 2008, Clausen and Coleman, 2010 (in press).

Trials comparing fatty acids (C-6 through C-10) against a broad range of fruit pathogens revealed that octanoic (caprylic) acid or C-8 performed significantly better than other fatty acid species. To evaluate control of *Botrytis cinerea* (grey mold) on raspberry fruit (Table 1), aqueous dip treatments, each comprised of a single fatty acid species (0.35%, v/v), Cognis Emsorb 6915 (0.10%, v/v) and mineral oil (0.05%, v/v) in water, were compared. Reductions of fungal infection (4 replicate berries/ treatment group) were determined 1,2 and 3 days after treatments. All treated and untreated (control) fruit were incubated at ambient room temperature (90 - 100% relative humidity).

Table 1. Comparison of aliphatic, saturated fatty acids (C6 through C10): Post-harvest treatments (low application rates)

	Infected berries (% of total) **		
Treatment*	1	2	3
1 = Water	19%	58%	92%
2 = caproic acid (C-6)	3	39	75
3 = heptanoic acid (C-7)	0	0	6
4 = caprylic acid (C-8)	0	0	3
5 = pelargonic acid (C-9)	0	3	14
6 = capric acid (C-10)	0	31	69

*0.35% fatty acid/ 0.10% Emsorb 6915/ 0.05% mineral oil in water

** Days after treatment (fatty acid is C6, C7, C8, C9 or C10)

Summary: C8 > C7 > C9 > C10 > C6

Numerous fungal pathogens (*B. cinerea, Armillaria, Alternaria, Colletotrichia and Pestalotia*) were inhibited at 0.20 – 0.40%, v/v, by a caprylic acid-based formulation: 10% C8/ 20% Emsorb 6915/ 10% mineral oil (Figure 1). Potato dextrose agar plates with and without C8 formulation were inoculated with fresh fungal cultures followed by plate incubation for several days. Complete inhibition of all pathogens occurred at 0.40%, v/v, with C8 demonstrating a broad pathogen specificity.

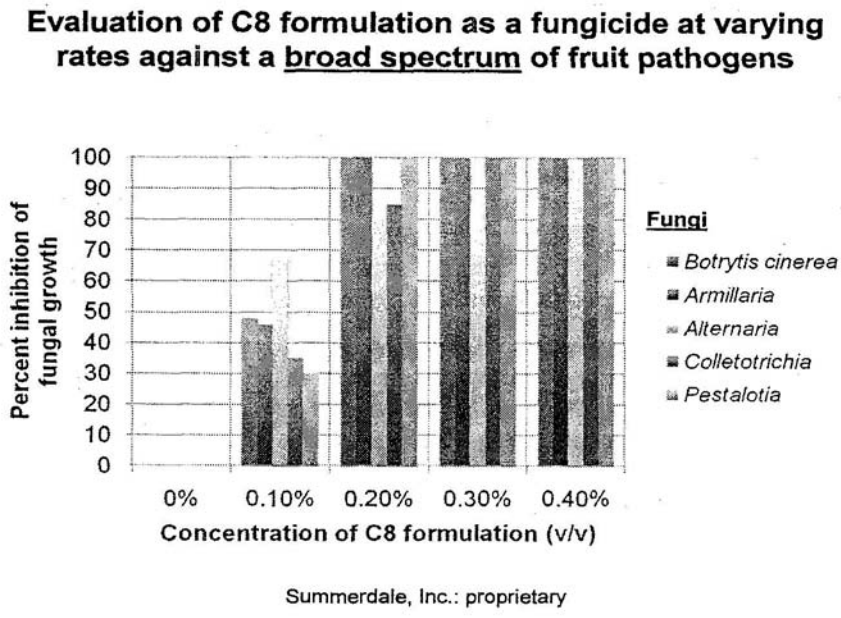


Figure 1: Efficacy of C8 formulation against numerous fungal pathogens:

Table 2: Synergistic relationship between a fatty acid and glycolic acid

Post-harvest treatments: Synergy between caprylic and glycolic acids as a fungicide for control of strawberry pathogens

	Pathogen inhibition (days after treatment)		
Treatment Solutions	Day 2	Day 3	Day 4
1. Water	0%	0%	0%
2. 0.35% glycolic acid	7%	0%	0%
3. 0.35% glycolic acid + (0.70% caprylic acid, 0.20% emulsifier, 0.10% diluent)	100%	100%	95%
4. 0.70% caprylic acid 0.20% emulsifier 0.10% diluent	67%	67%	5%

Summerdale, Inc.: proprietary

Post-harvest treatments, as fungicides for small hit, were consistent, highly effective and often, superior to commercial fungicide treatments. In one example, post-harvest treatments of strawberries (60 sec immersion), followed by incubation of berries for several days at 100% humidity (room temperature)

revealed that glycolic acid greatly enhanced fungicide activity of a C-8 formulation, from 5% inhibition of *Botrytis cinera* by C-8, alone, to 95% inhibition by C-8 + glycolic acid at 4 days after application of treatments (Table 2). Glycolic acid, alone, had little or no fungicidal activity. The degree to which a combination of caprylic and glycolic acids would manifest such potent synergy was not anticipated. Furthermore, fatty acid treatments were very effective in pathogen reduction on amorphous surfaces such as raspberry and strawberry, akin to what would be encountered in treating rough, irregular wood or wood composite structures.

Hypothesized mechanisms of action:

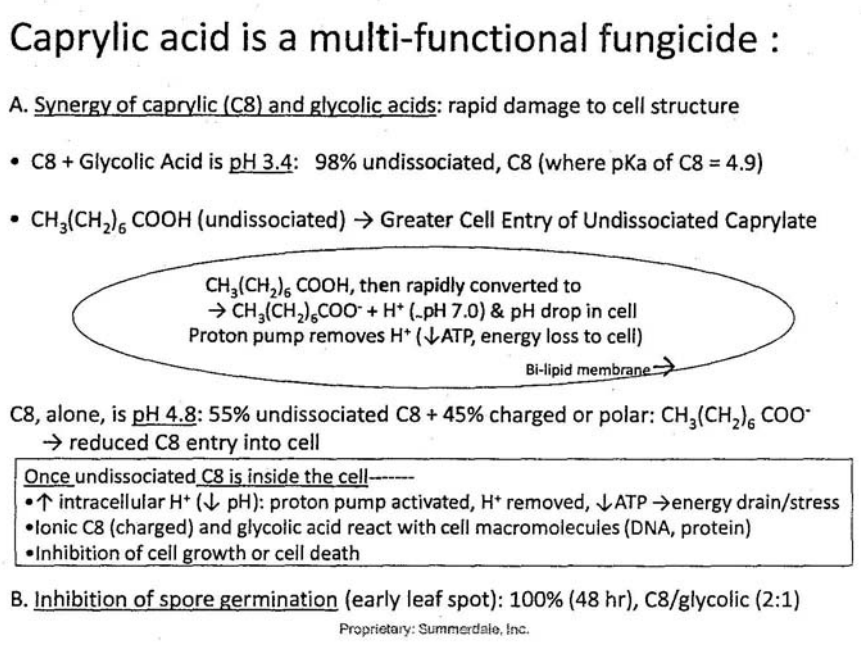
It is likely that the addition of selected organic acids (such as glycolic acid), based on their pKa values, increase the proportion of undissociated fatty acids over anionic species, thereby promoting greater fatty acid penetration through microbial, bi-lipid cell membranes (Figure 2). Once undissociated fatty acids enter cells, intracellular, proton pump activity is increased requiring more energy by the cell for electrolyte balance, thus placing more stress on the cell (Coleman, Penner, 2006, Coleman, 2007). Energy expenditure occurs via hydrolysis and loss of cellular ATP (adenosine triphosphate).

Moreover, high intracellular concentrations of dissociated (charged) fatty acid and organic acid (glycolic) most likely cause a high level of damage to organelle membranes and protein structure (Samelis, 2003, Barbosa-Canovas, 1998, Ecklund, 1989).

The enhancement of undissociated C8 by selected organic acids has been demonstrated. The pH values at application rates of 0.2%, v/v, C-8 formulation and 0.1%, v/v, organic acid, each in water, were measured. These particular application rates were effective in numerous field trials for control of various fungal crop pathogens. The pH values of the C-8 formulation +/- organic acid (2:1, C-8 formulation to organic acid), pKa values of each acid and the Henderson-Hasselbach equation were used to calculate the degree of ionization and percent undissociation for C-8 and organic acid in all combinations. The most effective organic acids with C-8 were: L- lactic, glycolic, succinic and propionic, elevating undissociated C-8 from 56% (C-8, alone) to 97-98% (C-8 + organic acid). It is very likely that a certain threshold of intracellular, undissociated C8 is required for significant damage to and stress on microbial cells (Figure 2). Trial data suggests that such a threshold may be achieved when the rate of undissociated C8 entering cells, facilitated by the presence of a selected, organic acid, is nearly doubled.

There is evidence that inhibition of spore germination occurs. In a study conducted by Dr. B. Shew, North Carolina State University, foliar disease of peanut, *Cercospora arachidicola* (early leaf spot), was chosen as a model test system. Spore and conidia treated with a 1%, v/v, aqueous solution of 70% C8/ 20% Emsorb 6915/ 10% mineral oil and 0.5%, v/v, glycolic acid (70%, v/v) resulted in 93% inhibition of germination at 24 hrs and 100% at 48 hours, when compared to untreated or control samples. The presentation of caprylic acid as a multi-functional fungicide is illustrated and summarized in Figure 2.

Figure 2. Fatty acid as a fungicide: mechanisms of action



As candidate preservatives for wood products:

Recent adoption of effective fungicide technology in agriculture to applications in wood protection has led to the development of novel wood preservatives. Specifically, formulations, combining low molecular weight monocarboxylic acids with selected adjuvants (organic acids, essential oils, surfactants, sticking agents, methylated seed oils, other natural compounds, etc.) as synergists, suggest potential use against mold and decay fungi. This work continues through a joint venture agreement between Summerdale, Inc. and Forest Products Laboratory (FPL), Madison, WI. Evaluation of formulation homogeneity, stability of dilutions in water and moldicide activity of stable formulations, both for dip and pressure treated wood products, is in progress. Formulation development and stability testing by R. Coleman (Summerdale, Inc.) has been followed by an extensive moldicide testing program and preliminary decay tests at FPL. Specifically, low molecular weight, aliphatic fatty acids (propionic to decanoic acids or C3 to C10) at various application rates +/- selected adjuvants have been or are being examined in mold and decay fungicide studies.

Creating fatty acid-based formulations including C-5 to C-10 (pentanoic, hexanoic, heptanoic, octanoic (caprylic) nonanoic (pelargonic), decanoic acids) that are highly stable as emulsions in water can be a challenging task (personal communication, D. Ferguson, formulation chemist, Huntsman Chemical). Summerdale, Inc. (Coleman 2001; 2003; 2004; 2005) has identified two excellent classes of emulsifiers, sorbitan esters and phosphate esters for emulsifying most low molecular weight, aliphatic fatty acids in water. Evidence for their exceptional emulsification properties was observed for fatty acid formulations (C-7 to C-10) as fungicides. Both emulsifiers are approved for use on food crops and hence, are relatively safe for general use and handling. Therefore, these two particular emulsifiers have been adopted and are the basis for stabilizing wood fungicide formulations and formulation dilutions (micro-emulsions) in water.

Since numerous studies revealed that combining an organic acid with a fatty acid functioned synergistically against crop pathogens, similar trials were conducted to determine efficacy against mold fungi. L-lactic, as an organic acid amendment, greatly enhanced several fatty acid species (C4, C5 and a C8, C10 combination) in controlling mold fungi over 12 weeks; i.e., moldicide ratings improved by 2 to 10-fold when L-lactic acid was present (Table 3). All moldicide and sapstain assays were conducted via ASTM standard D4445-91 (1998) and AWPA E-24-06 (2007). Test fungi were *Aspergillus niger 2.242,*

Penicillium chrysogenum PH02 and Trichoderma viride 20476 and the sapstain fungus was *Aureobasidium pullulans.* More recently, *Alternaria alternata* has been tested. The exception to the moldicide method entailed use of 10 or 12 replicates/ treatment group (in lieu of 5). Many of the trials were replicated. Statistical analysis of treatment differences in many of the trial data was conducted at FPL.

Table 3: Enhancement of fatty acid-based formulations with L-lactic acid

Formulation treatments in water	Rating*
6% butyric acid (C-4)	2.1
5.4% butyric acid/ 0.6% L-lactic acid (88%, v/v)	0.2
6% pentanoic acid (C-5)	3.0
5.4% pentanoic acid/ 0.6% L-lactic acid (88%, v/v)	0.3
4.2% caprylic, capric (C-8, C-10)/ 1.8% Emsorb 6915	2.3
4.2% caprylic, capric/ 1.5% Emsorb 6915/ 0.3% L-lactic (88%)	1.3
Water (control)	3.3

Ten replicates were used for each treatment. Mold ratings were done at 12 weeks.

***Rating of 0 - 5 where 0 and 5 were zero and 100% mold infections, respectively.**

Identifying moldicide activity associated with expected "inert" ingredients, such as emulsifiers (s) and/or diluents, would likely have ramifications for future registration of fatty acid formulations. Hence, it's preferable to evaluate that possibility prior to further formulation development and testing. Table 4 shows that the emulsifier PE 1198LA and mineral oil (diluent) were truly "inerts" in formulations applied at 6 and 12%, v/v, having no moldicide activity when compared to water (control). When C8, C10 fatty acids were added to the formulation inerts, moldicide activity at 8 weeks was good.

Table 4. Determine if formulation "inerts" have moldicide activity

	Rate: %, v/v	Avg**	FPLSD P @0.05
50% C8, C10/20% PE 1198LA/ 30% mineral oil	6	**0.9**	a
50% C8, C10/20% PE 1198LA/ 30% mineral oil	12	**0**	a
50% water/ 20% PE 1198LA/ 30% mineral oil*	6	2.8	b
50% water/ 20% PE 1198LA/ 30% mineral oil*	12	3.2	b
Water, control		3.0	b

*Formulations having only "inerts"; i.e., no fatty acid
**Moldicide ratings at 8 weeks
12 replicate southern yellow pine stakes/ treatment group
FPLSD: Fisher's Protected Least Significant Difference

Moldicide and anti-sapstain activities of a fatty acid formulation (C8, C10) were compared with commercial products (Table 5). Fungicide activities of the C8, C10 formulation at 6 and 12%, v/v, were compared with existing preservatives applied at manufacturer recommended rates (MRR). The fatty acid formulation had similar control of a mold consortium and sapstain. There were no meaningful, statistically significant treatment differences (P at 0.05) between any of the commercial products and the C8, C10

formulation. It should be noted that although the application rates for the C8, C10 formulation are higher than commercial preservatives, the cost of synthetic active ingredients in commercial products can be 5 to 10-fold higher than bulk cost of a fatty acid such as C8/C10 (i.e., $1 - 2/lb).

Table 6 compared commercial preservatives with the same fatty acid formulation as Table 5 but only using C8/C10 formulation at 6%, v/v. Both southern yellow pine and hardboard composite were inoculated with a 3-strain, mold consortium. DDAC/IPBC, Chlorothalonil and the C-8 formulation were very similar with no significant treatment differences noted except for DDAC/IPBC at the MRR lower rate (0.33%, v/v) which failed on hardboard at 8 weeks. Moldicide activity of potassium sorbate at 100%, v/v, was comparable at 12 weeks to the C8/C10 formulation applied at 6 and 12%, v/v (Table 7). Although statistical treatment differences were not evident, all treatments were significant over the control.

Table 5. Comparison of C8, C10 formulation with commercial products

			Moldicide assay		Anti-sapstain assay	
		Rates	FPLSD			FPLSD
	Treatments:	v/v:	Avg	P0.05	Avg	P0.05
1	Water, control		1.92	a	2.67	C
2	DDAC/IPBC	0.33	0.58	a	0.17	a
3	Chlorothalonil	0.5	0.17	a	0.08	a
4	Potassium sorbate	25	0.08	a	0	a
5	Potassium sorbate	10	0.42	a	0.25	a
6	DDAC	0.25	0.67	a	1.42	b
7	DDAC	1	0	a	0	a
8	C8, C10*	6	0.08	a	0.75	ab
9	C8, C10*	12	0	a	0	a

12 replicate southern yellow pine stakes/treatment group

Recommended rate for potassium sorbate is 100% (efficacy at lower rates was determined in this trial)
*50% C8, C10/ 20% PE 1198 LA/ 30% mineral oil (experimental formulation)
MRRs of formulated, commercial products (including active ingredients shown above)

Table 6. Compare experimental treatment with DDAC/IPBC and chlorothalonil as moldicides on southern yellow pine and hardboard (composite)

	Treatment solution	Rates (v/v):	Wood	8wk Avg	FPLSD P @ 0.05	FPLSD P @ 0.10
1	Water, control		SYP	2.67	c	d
2	C8/C10 formulation*	6%	SYP	0.75	ab	ab
3	DDAC/IPBC	0.33%	SYP	0.67	ab	ab
4	DDAC/IPBC	1.00%	SYP	0.17	a	a
5	Chlorothalonil	0.50%	SYP	0.25	a	a
6	Water, control		**Hardboard**	1.08	b	b
7	C8/C10 formulation*	6%	**Hardboard**	0.17	a	a
8	DDAC/IPBC	0.33%	**Hardboard**	2.0	c	c
9	DDAC/IPBC	1.00%	**Hardboard**	0.42	ab	a
10	Chlorothalonil	0.50%	**Hardboard**	0.33	ab	a

12 replicate southern yellow pine stakes/ treatment group
*50% C8, C10/ 20% PE 1198/ 30% mineral oil
(experimental formulation)
MRRs of formulated, commercial products
(including active ingredients shown above).

Table 7. Compare experimental formulation with potassium sorbate at 12 weeks.

Mold assay	Rate	12wk Avg	FPLSD P @ 0.05
Water, control		3.33	b
Potassium sorbate	100%	0.33	a
Potassium sorbate	25%	1.08	a
C8, C10 formulation*	6%	0.33	a
C8, C10 formulation*	12%	0.42	a

*50% C8, C10/ 20% PE 1198LA/ 30% mineral oil
12 replicate southern yellow pine stakes/ treatment group

A 3 month soil block test (AWPA E-10) was conducted and the results are summarized in Table 8. Although a C8 formulation (treatment #2) only had a modest reduction of weight loss for the brown-rot (*G. trabeum, P. placenta*) and white rot (*T. versicolor*) fungi, small amounts of proprietary amendments to C-8 (treatments #3, 4) further reduced decay. Weight loss for all three strains of decay fungi was substantially reduced by a pre-treatment of adjuvant C, followed by the C8 formulation (treatment #1).

Table 8. Examine efficacy of C-8 formulations: control of decay fungi.

	Weight loss (%)					
Formulation	***G. trabeum***		***P. placenta***		***T. versicolor***	
	Avg.	P @ 0.05	Avg.	P @ 0.05	Avg.	P@0.05
Untreated control	35.12	c	22.13	a	64.05	e
Treatment						
1	3.6	a	4.25	a	3.98	a
2	11.63	b	10.57	a	50.80	d
3	5.1	a	3.35	a	40.12	C
4	13.22	b	4.14	a	28.95	b

Treatment #1: adjuvant C (5%, v/v) → 6%, v/v, C8 formulation
Treatment #2: 6%, v/v, C8 formulation
Treatment #3: 6%, v/v, C8-adjuvants formulation
Treatment #4: 6%, v/v, C8-adjuvants formulation

Trt 1,2: 50% C8/ 20% PE 1198/ 10% L-lactic/ 20% mineral oil
Trt 3: 50% C8/14.8% mineral oil/ 10% PE 1198/10% 6915/ 2% adjuvant A/8% glycerol/2% adjuvant B/3.2% water
Trt 4: 50% C8/10% mineral oil/10% PE 1198/10% 6915/ 14.8% adjuvant C/2% adjuvant B/3.2% water

As a means to determine cost and also obtain formulation improvements to match industry needs and requirements, the following criteria are important. That is, in preparation for collaborator and potential licensee participation (i.e., company-specific testing prior to commercialization), the following general areas are receiving considerable attention.

A. Improved use characteristics: To obtain broader pest protection (decay fungi and insects) and for potential applications in both interior and exterior residential (above-ground) markets, develop and test moldicide formulations that include other functionalities. Current test results indicate that this approach may be feasible but requires further development. Demonstrate longer-term trials: outdoor, multi-year stake (E7-09) and above-ground (El 8-06) conducted by FPL.

B. Minimal leaching/depletion of active ingredients (i.e., fatty acids) from treated wood (Standard AWPA El 1-06). Together with leach trials, extensive, multi-year performance is important to demonstrate product consistency and credibility within the wood products industry including potential licensees. Trials will include long-term field stake and above-ground trials, the latter in MS and WI as mentioned in A, above.

C. Cost and performance of fatty acid-based formulations compared to existing product and treatment methodologies: 1) increase formulation potency such that lower rates are feasible, thereby reducing costs, 2) use multi-functional formulations containing synergistic combinations, resulting in lower cost and broader pesticide activity.

There will be continued interaction with the wood products industry including trade associations (e.g. American Wood Protection Association, American Plywood Association, Coalition for Advanced Wood Structures) to further identify needs and requirements.

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PROCEEDINGS

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