

Evaluation of Fungus-Chemical Compatibility for *Melaleuca (Melaleuca quinquenervia)* Control¹

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Abstract: Integration of a fungal pathogen with herbicides may enhance melaleuca control efforts in South Florida. Hyphal inoculum of four *Botryosphaeria ribis* Gross & Duggar isolates were evaluated in vitro for compatibility with imazapyr, glyphosate, and a surfactant. Imazapyr at 12 to 60 mg ai/ml did not cause significant loss of inoculum viability in all four isolates within 2 h after mixing. After 24 h, inoculum viability of isolate BR-4 remained unchanged at these imazapyr concentrations, but viability of BR-1 through BR-3 was reduced. Glyphosate at the lowest concentration (32 mg ai/ml) significantly reduced inoculum viability of all isolates within 2 h. Initially, the inoculum viability of all isolates remained unaffected by 1, 5, and 10% (v/v) surfactant concentrations. After 24 h, the surfactant reduced inoculum viability of BR-2, BR-3, and BR-4 inconsistently between experiments, while the inoculum viability of BR-1 was reduced significantly at all concentrations. Mixing of the lowest concentrations of imazapyr, glyphosate, and surfactant significantly reduced inoculum viability within 2 h. This corresponded to the results obtained for glyphosate alone. These results show that hyphal inoculum of *B. ribis* may be mixed with imazapyr and surfactant for field applications, but mixing the fungus with glyphosate may not be as efficacious.

Nomenclature: Glyphosate, *N*-(phosphomethyl) glycine; imazapyr, (\pm)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid; triclopyr, [(3,5,6-trichloro-2-pyridyl)oxy]acetic acid; melaleuca, *Melaleuca quinquenervia* (Cav.) Blake #3 MLAQU.

Additional index words: Biological control, weed control, fungus, herbicide, surfactant, glyphosate, imazapyr, triclopyr, *Botryosphaeria ribis*, MLAQU.

Abbreviations: CFU, colony forming units; PDA, potato-dextrose-agar; PDB, potato-dextrose broth; SDW, sterile deionized water.

INTRODUCTION

Melaleuca is an aggressive weed in the Everglades ecosystem of South Florida (Bodle et al. 1994). It not only displaces native vegetation but aids in deterioration of wildlife habitat and creates fire hazards and human health problems (Diamond et al. 1991; Morton 1969). *Melaleuca*'s vigorous reproductive (Hofstetter 1991; Meskimen 1962) and invasiveness (Laroche and Ferriter 1992; Myers 1983, 1984) potential warrant integrated use of biological, chemical, and mechanical methods to achieve satisfactory control (Bodle et al. 1994).

Chemical and mechanical methods evaluated for melaleuca control have provided only limited success (Balciunas and Center 1991; Bodle et al. 1994) and usually

require repeated treatments of the infested site. Recently tested herbicides for melaleuca control are imazapyr, glyphosate, and triclopyr (Bodle et al. 1994). Small populations of melaleuca trees are felled or girdled and the fresh wounds are individually treated with one or more of these herbicides; monocultures are aerially treated with the mixture of one or more of these herbicides (F. Laroche, personal communication). Several investigators reported that the efficacy of some bioherbicides can be enhanced by integration with chemical herbicides and additives (Altman et al. 1990, Christy et al. 1993; Klein and Auld 1995; Klein et al. 1995). Thus, synergistic use of chemicals with biological control agents may reduce the chemical dosage and minimize effects on nontarget plants and the environment (Christy et al. 1993). The interactions of plant pathogens and chemicals may result in antagonistic, synergistic, or additive effects in weed control, with the additive and synergistic effects desirable for weed control purposes (Grant et al. 1990). The chemical herbicides may operate by weakening the host

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³ Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA.

defenses, thus enhancing host colonization by plant pathogens (Altman et al. 1990, Charudattan 1993).

Previous work has shown that an indigenous pathogen *Botryosphaeria ribis* may be developed as a potential biological control agent for melaleuca (Rayachhetry et al. 1996a). The fungus was found to be more aggressive when inoculated on trees stressed by drought, low temperature, or defoliation (Rayachhetry et al. 1996b). Stress imposed by low rates of herbicides may improve the tree-killing efficacy of this fungus. The purpose of this research was to determine the compatibility of hyphal inoculum of *B. ribis* isolates with herbicides and a surfactant commonly used for melaleuca control.

MATERIALS AND METHODS

Inoculum. Four isolates of *Botryosphaeria ribis* (BR-1, BR-2, BR-3, and BR-4) were obtained from the canker margins of melaleuca stems in South Florida (Rayachhetry et al. 1996a). These isolates were grown on full strength potato-dextrose-agar (PDA)⁴ without light at 27 C for 3 to 5 d, and only fresh cultures were used for all experiments.

Chemicals. Commercial formulations of imazapyr (Arsenal[®],⁵ 28.7% ai), glyphosate (Rodeo[®],⁶ 53.8% ai), triclopyr (Garlon[®],⁷ 3A, 44.8% ai), and a surfactant (Dyne-Amic[®]) were selected for this study based on the list of chemicals previously tested (Bodle et al. 1994) or commonly used for melaleuca control in South Florida. A preliminary study demonstrated fungistatic activity of triclopyr to all *B. ribis* isolates at concentrations as low as 4 mg ai/ml. Therefore, triclopyr was not included in the fungus-chemical compatibility and inoculum viability tests.

Fungus-chemical Compatibility. Autoclaved PDA was cooled to 50 C, amended with either imazapyr at 2, 12, or 24 mg ai/ml, or glyphosate at 7, 32, or 65 mg ai/ml, and dispensed into petri dishes. These concentrations represented 10, 50, and 100 ml/L formulated material. Nonamended PDA was used as the treatment control. The surfactant was not included in compatibility tests since it prevented PDA from solidifying.

For each *B. ribis* isolate, four replicate plates of each herbicide by treatment rate and the control were inoculated with a 3-mm-diam PDA disk taken from an ac-

tively growing 3-d-old colony. Plates were incubated for 66 h at 27 C in the dark, after which the colonies were measured to determine their radial growth. The radial growth of the colonies on herbicide-amended treatments was compared with radial growth of colonies on nonamended PDA (control). The experiment was conducted twice.

Inoculum Viability in Individual Chemicals. Each *B. ribis* isolate was grown in potato-dextrose broth (PDB)⁴ in 250-ml flasks inoculated with five 3-mm-diam disks taken from the colonies of respective isolates. The broth-cultures were continuously shaken (120 rpm) for 3 to 5 d under 10 h fluorescent light and room temperature. The mycelial residues (ca 20 gm fresh wt) obtained by filtering each broth culture through sterile cheesecloth were macerated separately for 20 to 25 s using a sterile food blender. The slurry of macerated hyphae was diluted with an equal volume of sterile deionized water (SDW) and was used to determine inoculum viability in imazapyr, glyphosate, or surfactant. Surfactant was included in the test since it is mixed with herbicides in aerial applications.

A final volume of 5.0-ml fungus-herbicide or fungus-surfactant mixture was prepared for each isolate and chemical concentration. Each of these mixtures contained 1.5 ml of hyphal slurry mixed with the required volume of imazapyr, glyphosate, or surfactant plus SDW to obtain the respective chemical concentrations. The chemical concentration in the mixture was adjusted to 12, 24, 60, and 120 mg ai/ml for imazapyr and 32, 65, 162, and 324 mg ai/ml for glyphosate; concentration of surfactant was adjusted to 1, 5, and 10% (v/v). The herbicide concentrations represented 5, 10, 25, and 50% formulated solutions (v/v). These concentrations were chosen to include the upper limit of the concentrations used by the South Florida Water Management District for aerial spray as well as stump treatments. The control contained the hyphal slurry diluted with SDW to a final volume of 5.0 ml.

Within 0 to 2 h (referred to as 0 h) after mixing, the fungus-herbicide mixtures were used to prepare a dilution series. Four replicate dilution series were prepared for each isolate (BR-1 through BR-4) and the chemical concentration. A 0.1-ml aliquot of each dilution was spread onto PDA. Plates were incubated in the dark at 27 C for 24 h for imazapyr and surfactant but at 48 h for glyphosate because colony initiation by viable fragment was delayed in the glyphosate-amended PDA. Then, culture plates were evaluated for number of colonies initiated from individual hyphal fragments; each

⁴ Difco Laboratories, Detroit, MI 48232.

⁵ American Cyanamid Co., Wayne, NJ 07470.

⁶ Monsanto Co., St. Louis, Missouri 63167.

⁷ The Dow Chemical Co., Midland, MI 48674.

⁸ Dyne-Amic, a blend of 80% methylated seed oils with 20% polyalkylene oxide, modified polydimethyl siloxane, and non-ionic emulsifiers. Helena Chemical Co., Memphis, TN 38119.

Table 1. Radial growth inhibition of four *Botryosphaeria ribis* isolates on potato-dextrose-agar amended with three herbicides, each at three concentrations.

Herbicide	Percentage of radial growth ^a							
	BR-1		BR-2		BR-3		BR-4	
	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
mg ai/ml								
Imazapyr								
2	81 ± 0	80 ± 2	94 ± 1	99 ± 1	91 ± 2	99 ± 2	83 ± 1	98 ± 1
12	71 ± 2	81 ± 1	94 ± 1	98 ± 0	92 ± 1	95 ± 2	84 ± 1	96 ± 1
24	52 ± 2	60 ± 3	77 ± 1	86 ± 1	73 ± 1	83 ± 2	59 ± 3	80 ± 2
Glyphosate								
7	37 ± 2	36 ± 1	54 ± 1	56 ± 2	54 ± 1	55 ± 1	40 ± 5	46 ± 2
32	12 ± 1	13 ± 1	14 ± 0	13 ± 1	13 ± 1	11 ± 2	13 ± 1	13 ± 1
65	11 ± 0	9 ± 1	10 ± 0	8 ± 1	10 ± 1	10 ± 0	10 ± 0	10 ± 0

^a Percentage of radial growth = (radial growth of isolate on herbicide amended PDA/radial growth of isolate on non-amended PDA) × 100. Values are means of four replicate samples ± the standard error.

viable fragment was considered a colony forming unit (CFU). The remaining mixtures were incubated 24 h at room temperature under 10-h fluorescent light. These mixtures were again evaluated for CFUs using the same procedure as for 0 h. All experiments were conducted twice.

The CFUs per milliliter mixture of fungus and herbicide, surfactant, or SDW (control) were transformed to log₁₀ values. The log₁₀ CFUs of the control were compared with the log₁₀ CFUs of the herbicides and the surfactant for both 0- and 24-h treatments.

Inoculum Viability in Chemical Mixtures. Hyphal inoculum of isolates BR-1 through BR-4 were combined with mixtures of chemicals to evaluate their effect on fungal viability. Chemical types and rates used in this experiment were chosen to simulate the chemicals tested by South Florida Water Management District for aerial spray to control melaleuca. The concentration of each herbicide in the mixture was adjusted to 0.5% (1.2 mg ai/ml of imazapyr plus 3.3 mg ai/ml glyphosate), 1.5% (3.6 mg ai/ml of imazapyr plus 10.0 mg ai/ml glyphosate), 4.5% (10.8 mg ai/ml of imazapyr plus 29.3 mg ai/ml glyphosate), or 7.5% (18.0 mg ai/ml of imazapyr plus 48.8 mg ai/ml glyphosate) by volume. Treatments were supplemented with the surfactant at 0.5% (v/v).

 Table 2. Mean log₁₀ CFUs of *Botryosphaeria ribis* isolates in the control (SDW treatment) at 0 and 24 h after mixing.

Experiments	Treatment time (h)	Log ₁₀ CFUs/ml ^a			
		BR-1	BR-2	BR-3	BR-4
I	0	6.11	6.18	6.08	6.09
	24	6.05	6.16	6.09	6.05
II	0	5.88	6.41	6.39	6.25
	24	5.89	6.44	6.31	6.29

^a Values are means of four replicate samples.

The method of inoculum production—mixing macerated hyphae with chemicals and evaluating CFUs—was the same as described previously. All experiments were conducted twice.

Data Analyses. Mean and standard error of fungus-herbicide compatibility in vitro and Dunnett's test for inoculum viability in different concentrations of herbicides and surfactant was determined using SAS (1985).

RESULTS AND DISCUSSION

Fungus-chemical Compatibility. Growth of *B. ribis* isolates on PDA amended with different herbicide concentrations compared with growth on nonamended PDA (control) is presented in Table 1. Except for isolate BR-1 in experiment I, imazapyr reduced growth of all four *B. ribis* isolates by less than 20% at the two lowest concentrations. At the highest imazapyr concentration, growth was reduced by < 48% across all isolates. Glyphosate reduced the growth of all four *B. ribis* isolates by greater than 85% at the two highest concentrations. At the lowest glyphosate concentration, growth was reduced by at least 45%.

Overall, an increased herbicide concentration resulted in reduced hyphal growth of *B. ribis* isolates. Hyphal growth reduction with increased herbicide concentration was also observed in *Chondrostereum purpureum* Fr. Pouzar, a potential bioherbicide for hardwood vegetation control in forests (Prasad 1994). *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *malvae*, a bioherbicide of round-leaved mallow (*Malva pusilla* Sm. # MALPU), showed a differential compatibility with different herbicide groups (Grant et al. 1990).

Inoculum Viability. The integration of mycoherbicides and chemical herbicides in weed control programs will probably require tank-mixing for field applications.

Table 3. Differences in the *Botryosphaeria ribis*-inoculum viability (viability in herbicide mixture – viability in SDW = viability difference) in herbicide and surfactant concentrations with respective control at 0 h (0 to 2 h) and 24 h after mixing.

Chemicals	Treatment time	Differences in log ₁₀ CFUs/ml ^a							
		BR-1		BR-2		BR-3		BR-4	
		Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
Herbicides									
mg ai/ml									
Imazapyr									
12	0 h	0.01	-0.06	0.01	0.04	0.03	0.01	0.03	0.01
	24 h	-0.02	-0.08	-0.01	-0.07	-0.02	-0.06	-0.04	-0.01
24	0 h	0.02	-0.13	0.03	0.02	-0.02	0.03	0.05	0.03
	24 h	-0.01	-0.18	-0.06	-0.09	-0.02	-0.06	-0.04	-0.03
60	0 h	-0.05	0.03	0.05	0.04	-0.06	-0.07	-0.11	0.03
	24 h	-0.13 ^b	-0.24 ^b	-0.07	-0.13 ^b	-0.11 ^b	-0.13 ^b	-0.05	-0.06
120	0 h	-0.09	-0.18	-0.15 ^b	-0.08	-0.15 ^b	-0.12 ^b	-0.15 ^b	-0.11 ^b
	24 h	-0.31 ^b	-0.98 ^b	-0.38 ^b	-0.31 ^b	-0.42 ^b	-1.16 ^b	-0.11 ^b	-0.12 ^b
Glyphosate									
32	0 h	-1.72 ^b	-1.20 ^b	-1.09 ^b	-1.33 ^b	-0.94 ^b	-0.45 ^b	-1.12 ^b	-2.82 ^b
	24 h	-1.80 ^b	-1.21 ^b	-1.30 ^b	-1.64 ^b	-1.24 ^b	-0.72 ^b	-1.39 ^b	-3.07 ^b
65	0 h	-1.69 ^b	-1.51 ^b	-1.18 ^b	-1.43 ^b	-1.24 ^b	-1.17 ^b	-1.43 ^b	-3.24 ^b
	24 h	-2.96 ^b	-2.55 ^b	-2.06 ^b	-1.72 ^b	-2.53 ^b	-1.60 ^b	-2.81 ^b	-3.51 ^b
162	0 h	-1.77 ^b	-2.78 ^b	-1.64 ^b	-1.55 ^b	-1.55 ^b	-1.69 ^b	-2.72 ^b	-6.29 ^b
	24 h	-3.26 ^b	-3.20 ^b	-5.44 ^b	-2.19 ^b	-3.20 ^b	-3.73 ^b	-6.06 ^b	-6.25 ^b
324	0 h	-3.17 ^b	-3.26 ^b	-1.90 ^b	-3.05 ^b	-2.84 ^b	-2.39 ^b	-3.09 ^b	-6.29 ^b
	24 h	-6.04 ^b	-5.88 ^b	-6.16 ^b	-6.44 ^b	-6.09 ^b	-6.31 ^b	-6.06 ^b	-6.25 ^b
Surfactant									
% by vol									
1	0 h	0.11	-0.08	0.04	0.03	0.00	0.00	0.03	-0.02
	24 h	-0.38 ^b	-0.25 ^b	-0.02	-0.03	-0.11	-0.01	-0.04	-0.08 ^b
5	0 h	0.25	-0.09	0.01	0.02	0.01	-0.02	0.04	-0.04
	24 h	-0.61 ^b	-0.26 ^b	-0.10	-0.07	-0.17 ^b	-0.09	-0.02	-0.05 ^b
10	0 h	0.23	-0.09	0.01	0.05	-0.05	-0.01	-0.02	0.01
	24 h	-0.63 ^b	-0.40 ^b	-0.16	-0.14 ^b	-0.09	-0.09	-0.06	-0.04

^a Values are the differences between the log₁₀ CFUs of the control (SDW) and the respective herbicide or surfactant treatment.

^b These comparisons are significant at P = 0.05 according to Dunnett's *t*-test. Control values, presented in Table 2, were used for statistic comparisons.

Also, there may be a time lapse between mixing the fungus with an herbicide and field application of the mixture. Fungal inoculum may lose viability during this time. Therefore, integrated use of a fungus with com-

patible herbicides and surfactants will require that the fungal inoculum be capable of surviving in the chemicals before application to melaleuca trees.

Inoculum viability of *B. ribis* in SDW, imazapyr, gly-

Table 4. Differences in *Botryosphaeria ribis*-inoculum viability in SDW and mixtures of herbicide and surfactant treatments (viability in herbicide mixture – viability in SDW = viability difference) at 0 h (0 to 2 h) and 24 h after mixing.

Chemical treatments ^b	Treatment time	Differences in log ₁₀ CFUs/ml ^a							
		BR-1		BR-2		BR-3		BR-4	
		Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II	Expt. I	Expt. II
1	0 h	-0.02	-0.01	-0.04	-0.04	-0.02	-0.04	-0.02	-0.02
	24 h	-0.02	-0.01	-0.03	-0.04	-0.02	-0.03	-0.03	-0.06
2	0 h	-0.02	-0.06	-0.06	-0.09	-0.04	-0.02	-0.01	-0.01
	24 h	-0.11 ^c	-0.02	-0.25 ^c	-0.17	-0.09	-0.06	-0.01	-0.14 ^c
3	0 h	-0.16 ^c	-0.10	-0.42 ^c	-0.37 ^c	-0.16 ^c	-0.43 ^c	-0.10 ^c	-0.22 ^c
	24 h	-0.44 ^c	-0.36 ^c	-1.21 ^c	-1.30 ^c	-0.52 ^c	-1.16 ^c	-0.32 ^c	-0.41 ^c
4	0 h	-0.63 ^c	-0.32 ^c	-1.17 ^c	-1.37 ^c	-0.61 ^c	-0.99 ^c	-0.19 ^c	-0.55 ^c
	24 h	-1.18 ^c	-1.16 ^c	-2.32 ^c	-2.22 ^c	-1.46 ^c	-2.09 ^c	-1.31 ^c	-1.10 ^c

^a Mean log₁₀ CFUs of *Botryosphaeria ribis* isolates in the control (SDW treatment) at 0 and 24 h after mixing were comparable to the corresponding isolates presented in Table 2.

^b Chemical treatments: 1 = mixture of 1.2 mg ai/ml imazapyr and 3.3 mg ai/ml glyphosate; 2 = mixture of 3.6 mg ai/ml imazapyr and 10.0 mg ai/ml glyphosate; 3 = mixture of 10.8 mg ai/ml imazapyr and 29.3 mg ai/ml glyphosate; 4 = mixture of 18.0 mg ai/ml imazapyr and 48.8 mg ai/ml glyphosate. Treatments 1 to 4 were supplemented with surfactant to a final concentration of 0.5% (v/v).

^c These comparisons are significant at P = 0.05 according to Dunnett's *t*-test.

phosphate, and surfactant was evaluated over a period of 24 h. For all the isolates, the inoculum viability in SDW was reduced by less than \log_{10} 0.1 CFUs/ml between 0- and 24-h sampling times (Table 2).

When compared to the control, imazapyr concentrations of 12, 24, and 60 mg ai/ml did not cause significant reduction of inoculum viability across isolates at 0 h (Table 3). After 24 h, the inoculum viability reduction for 12 and 24 mg ai/ml imazapyr concentration was not significant across isolates, but for 60 mg ai/ml imazapyr, inoculum viability reduction was significant for all but isolate BR-2. At 120 mg ai/ml of imazapyr, inoculum viability of all four isolates was significantly decreased in both experiments. After 24 h, the maximum CFUs reduction by imazapyr was 1% and 17% for 12 and 120 mg ai/ml concentrations, respectively.

Even at the lowest concentration (32 mg ai/ml), glyphosate caused significant loss of inoculum viability across all isolates (Table 3). At 324 mg ai/ml concentration, glyphosate had significantly reduced the viability across isolates. After 24 h, the maximum CFUs reduction by glyphosate was 44% and 100% for 32 and 324 mg ai/ml concentrations, respectively.

The surfactant concentrations at 1, 5, and 10% did not reduce inoculum viability at 0 h (Table 3). After 24 h, all surfactant concentrations caused a significant viability loss for BR-1 in both experiments while other isolates showed significant reduction of inoculum viability in only one of the experiments. After 24 h, the maximum CFUs reduction by the surfactant was 6% and 10% for 1% and 10% surfactant concentrations, respectively.

Isolates BR-1 through BR-4 were not affected by a mixture of 1.2 and 3.3 mg ai/ml of imazapyr and glyphosate, respectively, and 0.5% (v/v) surfactant (Table 4), even 24 h after mixing. As concentrations of the herbicides in the mixture were increased, inoculum viability was reduced significantly even within 2 h after mixing. The effect of the herbicide mixture is similar to the effect of glyphosate alone.

Information generated from this study will be useful in designing field inoculations that will integrate fungus with appropriate concentrations of imazapyr, glyphosate, and surfactant for melaleuca control. For example, the highest concentration of imazapyr used in melaleuca control is 120 mg ai/ml for stump treatment for regrowth control (F. Laroche, personal communication). Our study indicates that up to 60 mg ai/ml imazapyr would be the best rate to use in herbicide-*B. ribis* mixtures. However, if all four *B. ribis* isolates are mixed together for application purposes, the higher rate may be acceptable since

normally less than \log_{10} 0.5 CFUs/ml was lost per isolate.

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