

## PHYTOTOXICITY EVALUATION OF MILBEMECTIN ON FERNS

ROBERT H. STAMPS<sup>1</sup>, ANNETTE L. CHANDLER  
AND DIANE K. ROCK  
*University of Florida, IFAS  
Environmental Horticulture Department  
Mid-Florida Research and Education Center  
2725 Binion Road  
Apopka, FL 32703-8504*

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**Abstract.** Milbemectin, a mixture of natural compounds derived from the soil microorganism *Streptomyces hygroscopicus* subsp. *aureolacrimosus*, is a miticide/insecticide used for selective arthropod management. The objective of this study was to test spray applications of an emulsifiable milbemectin formulation (Ultiflora™ 1E) for phytotoxicity on a variety of ferns. Eighteen fern species representing 14 genera were treated just to the point of runoff with aqueous foliar sprays containing 0, 12 or 24 oz Ultiflora™/100 gallons. Plants were held on raised

benches in a double polyethylene-covered greenhouse with additional internal shading. Treatments were applied twice at 14-day intervals in two experiments. Acute phytotoxicity was determined visually for two weeks following each spray application and rated on a scale of 1 = no visible damage to 5 = death. In addition, fresh frond weights were determined 50 days after the initial treatment to assess chronic damage. For many species—*Adiantum raddianum*, *Arachniodes simplicior*, *Athyrium nipponicum*, *Davallia trichomanoides*, *Dryopteris erythrosora*, *Osmunda cinnamomea*, *Pellaea rotundifolia*, *Platycerium bifurcatum*, *Polystichum tsus-sinense*, *Pteris cretica*, *P. vitatta* and *Rumohra adiantiformis*—no damage was detected. For *Didymochlaena truncatula* and *Nephrolepis* spp., significant acute phytotoxicity was observed only at the 24 oz/100 gallons (2×) rate. Both *Polypodium formosanum* and *Pteris quadriaurita* were damaged at the 1× rate. However, despite visual stunting of *Nephrolepis biserrata* 'Macho' at the 2× rate, Ultiflora™ treatments had no effect on fresh frond weights of that fern or any others. Even though this was an EC formulation, Ultiflora™ applied at the 1× rate did not cause significant damage to 16 of the 18 ferns used in this experiment.

Ferns are popular plants for adding texture to landscapes, both indoors and out. These plants are easily damaged by pesticides (Anonymous, 2004; Henley et al., 1991) due to their general herbaceousness and recurring production of new, tender fronds. Many pesticide labels specifically prohibit their use on ferns. Fern authorities recommend using lower than manufacturer recommended dosage rates (Jones, 1987) and avoiding pesticides formulated as emulsions if at all possible (Hoshizaki, 1983).

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<sup>1</sup>Corresponding author; e-mail: rhs@ifas.ufl.edu.

Table 1. *Experiment 1*—phytotoxicity of foliar sprays of milbemectin (Ultiflora™ 1E) to 12 ferns.

Fern	Ultiflora™ application rate (oz./100gal)	Phytotoxicity rating (1–5) <sup>z</sup> x days after treatment (DATx) <sup>y</sup>						Fresh frond weight <sup>t</sup> (g)	
		3 DAT1	7 DAT1	10 DAT1	14 DAT1	3 DAT2	8 DAT2		14 DAT2
<i>Arachniodes simplicior</i> 'Variegata' (variegated shield fern, variegated East Indian holly fern)	0	1.25	1.00	1.00	1.00	1.25	1.25	1.00	21.8
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	24.6
	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	22.9
<i>Athyrium nipponicum</i> 'Pictum' (Japanese painted lady fern)	0	1.00	1.00	1.25	1.00	1.25	1.75	1.38	42.9
	12	1.25	1.25	1.25	1.25	1.25	2.00	1.75	36.5
	24	1.00	1.25	1.75	1.50	1.75	2.25	1.88	36.8
<i>Davalia trichomanoides</i> (rabbit's-foot fern)	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	37.2
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	34.0
	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	32.9
<i>Dryopteris erythrosora</i> (autumn fern)	0	1.00	1.00	1.25	1.25	1.25	1.00	1.00	41.1
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	36.7
	24	1.00	1.25	1.00	1.00	1.00	1.00	1.00	37.9
<i>Nephrolepis biserrata</i> 'Macho' (macho fern)	0	1.00	1.00	1.00	1.00	1.00	1.00	1.13	81.8
	12	1.00	1.25	1.25	1.00	1.13	1.13	1.00	79.4
	24	1.00	2.50 <sup>*w</sup>	2.25 <sup>*</sup>	2.00 <sup>*</sup>	1.75 <sup>*</sup>	2.00 <sup>*</sup>	1.25	79.1
<i>Nephrolepis exaltata</i> 'Montana' (Montana Boston fern)	0	1.00	1.00	1.00	1.00	1.00	1.25	1.25	164.0
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.13	172.9
	24	1.25	1.25	1.75	1.75	2.00 <sup>*</sup>	2.00	1.50	153.3
<i>Nephrolepis falcata</i> 'Furcans' (fishtail fern)	0	1.00	1.00	1.00	1.00	1.00	1.25	1.13	137.7
	12	1.00	1.00	1.00	1.00	1.25	1.00	1.13	128.7
	24	1.75 <sup>*</sup>	1.25	1.25	1.25	1.75	2.00 <sup>*</sup>	1.50	123.8
<i>Osmunda cinnamomea</i> (cinnamon fern)	0	1.00	1.00	1.00	1.00	1.25	1.50	1.63	14.3
	12	1.00	1.00	1.00	1.50	2.00	2.00	1.38	13.2
	24	1.00	1.00	1.00	1.00	1.50	2.00	1.50	15.5
<i>Platyserium bifurcatum</i> (staghorn fern)	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	72.2
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	67.3
	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	53.3
<i>Pteris cretica</i> 'Parkerii' (Parkerii brake fern, Parkerii Pteris fern)	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	22.1
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	22.6
	24	1.00	1.00	1.00	1.00	1.00	1.25	1.25	22.5
<i>Pteris quadriaurita</i> 'Flame' (Flame brake fern)	0	1.00	1.00	1.25	1.25	1.75	2.00	1.75	34.5
	12	1.00	2.67 <sup>*</sup>	2.00	2.67	2.33	3.00	2.33	24.0
	24	1.00	1.75	1.75	1.75	2.25	2.25	2.13	27.1
<i>Rumohra adiantiformis</i> (leatherleaf fern)	0	1.00	1.25	1.25	1.50	2.00	2.50	2.75	33.0
	12	1.25	1.50	1.50	1.50	2.00	2.25	3.00	32.0
	24	1.00	1.75	1.75	2.25	2.25	2.75	2.75	34.3

<sup>z</sup>Phytotoxicity rating: 1 = normal; 2 = slight damage, saleable; 3 = moderate damage, unsaleable; 4 = severe damage; 5 = dead.

<sup>y</sup>Treatments were applied on 9/10/03 (T1) and 9/24/03 (T2).

<sup>t</sup>None of the fern frond fresh weights were affected by treatments (regression analysis at  $P \leq 0.05$ ).

<sup>w\*</sup> indicates that the treatment mean is different for that species from the untreated mean for that date (Dunnett's procedure at  $P \leq 0.05$ ).

Milbemectin, a mixture of natural compounds (milbemycins) derived from the soil microorganism *Streptomyces hygroscopicus* subsp. *aureolacrimosus*, is a miticide/insecticide used for selective arthropod management. It is only available as an emulsion (Ultiflora™ 1E, Gowan Company, Yuma, Ariz.). The objective of this study was to test spray applications of milbemectin for phytotoxicity on a variety of ferns.

### Materials and Methods

These studies were conducted in a double polyethylene covered greenhouse equipped with fan and pad evaporative cooling and natural gas fired forced-air heating at the Mid-Florida Research and Education Center in Apopka, Fla. Temperatures were maintained between 64 ° and 96 ° F [18 ° and 36 ° C]. Ad-

ditional internal shading was provided using a PVC pipe frame covered with 70% shade cloth attached to the expanded metal benches. The resulting shade level was approximately 88%.

Four-inch [10.2-cm] pots of fern and 72-cell fern liners were obtained from suppliers (Casa Flora, Central Florida Ferns, Milestone Agriculture; all in Apopka, Fla.) on 30 June 2003. On 2 July 2003 the liners were potted up into 3.9-inch [10-cm] diameter round plastic pots (Desch 10, APAC Design Ltd., Leicestershire, England) using a *Sphagnum* peat:vermiculite:perlite soilless growing medium (Vergro Container Mix A, Verlite, Tampa, Fla.). A month later, all of the ferns were potted up into 4.7 inch [12 cm] diameter round plastic pots (Desch 12.0H) filled with a soilless growing medium composed of aged pine bark:Canadian *Sphagnum* peat:perlite:vermiculite (3 Mix, Fafard, Apopka, Fla.). Pots were then fertilized with 0.05 oz [1.5 g] of a 15N-4P-10K con-

Table 2. *Experiment 2*—phytotoxicity of foliar sprays of milbemectin (Ultiflora™ 1E) to six ferns.

Fern	Ultiflora™ application rate (oz/100gal)	Phytotoxicity rating (1–5) <sup>z</sup> x days after treatment (DATx) <sup>y</sup>						Fresh frond weight <sup>t</sup> (g)	
		3DAT1	10DAT1	14DAT1	3DAT2	7DAT2	10DAT2		14DAT2
<i>Adiantum raddianum</i> 'Fragrantissimum' (delta maidenhair fern)	0	1.33	1.00	1.00	1.00	1.00	1.00	1.00	46.0
	12	1.33	1.00	1.00	1.00	1.00	1.00	1.00	44.7
	24	1.00	1.00	1.00	1.00	1.00	1.17	1.00	46.2
<i>Didymochlaena truncatula</i> (mahogany fern)	0	1.00	1.67	1.50	1.33	1.33	1.33	1.17	51.6
	12	1.00	2.33	2.50	2.00	2.17	2.00	2.00	58.2
	24	1.33	2.67	3.33**	2.83*	2.83*	2.67*	3.00*	55.8
<i>Pellaea rotundifolia</i> (button fern)	0	1.00	1.00	1.17	1.17	1.17	1.17	1.17	30.5
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	45.4
	24	1.00	1.00	1.17	1.17	1.17	1.17	1.17	31.6
<i>Polypodium formosanum</i> 'Cristatum' (grub fern, caterpillar fern, E.T. fern)	0	1.00	1.00	1.08	1.08	1.08	1.08	1.08	37.5
	12	1.17	1.58*	1.58	1.58	1.50*	1.58*	1.33	37.7
	24	1.17	3.08*	3.00*	2.92*	3.33*	3.33*	3.17*	43.3
<i>Polystichum tsussimense</i> (holly fern)	0	1.00	1.00	1.17	1.17	1.00	1.17	1.00	18.7
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	25.8
	24	1.00	1.50	1.17	1.33	1.00	1.00	1.00	22.8
<i>Pteris vittata</i> (Chinese brake fern, table fern)	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	49.1
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	51.0
	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	49.2

<sup>z</sup>Phytotoxicity rating: 1 = normal; 2 = slight damage, saleable; 3 = moderate damage, unsaleable; 4 = severe damage; 5 = dead.

<sup>y</sup>Treatments were applied on 3/09/04 (T1) and 3/24/04 (T2).

<sup>t</sup>None of the fern frond fresh weights were affected by treatments (regression analysis at  $P \leq 0.05$ ).

\*\* indicates that the treatment mean is different from the untreated mean for that species for that date (Dunnett's procedure at  $P \leq 0.05$ ).

trolled-release fertilizer containing micronutrients (Osmocote® Plus 15-9-12, The Scotts Company, Marysville, Ohio). In addition, pots were fertigated weekly with a soluble 15N-2P-12K fertilizer (Miracle-Gro® Excel, The Scotts Company) applied at a nitrogen concentration of 150 ppm [150 mg L<sup>-1</sup>]. Pots were hand-watered as needed before and after treatment.

Plants with immature fronds were used to maximize the chances of detecting phytotoxicity. Prior to treatment, all senescent or blemished fronds were removed from the plants to facilitate the observation of treatment-caused damage. Plants were treated just to the point of runoff with aqueous foliar sprays of 0× (water only), 1× or 2× rates of Ultiflora™ (0, 12 or 24 oz Ultiflora™ per 100 gal [0, 94 or 188 mL Ultiflora™ per 100 L], respectively) using a hand-held pressure sprayer (1½ quart, Delta Industries, King of Prussia, Pa.) operated at a pressure of 20 psi [138 kPa]. Treatments were reapplied two weeks later using the same methodology.

Phytotoxicity was determined approximately 3, 7, 10 and 14 d after each treatment. Each plant was visually rated for phytotoxicity using a scale where 1 = normal; 2 = slight damage, saleable; 3 = moderate damage, unsaleable; 4 = severe damage; 5 = dead. Photos were made of phytotoxicity symptoms using a digital camera (Nikon Coolpix 5000, Nikon, Tokyo, Japan). In addition, fresh frond weights were determined 50 d after the initial treatment.

The experimental design for both studies was a randomized complete block with three replications. The experimental unit was an individual pot with a fern in it. Ultiflora™ phytotoxicity data were analyzed by analysis of variance and significant treatment differences (compared to the water-only treated controls) were determined for each fern at each rating date using Dunnett's procedure ( $P = 0.05$ ). Treatment effects on fresh frond weights were determined using regression analysis at  $P = 0.05$  (SAS, SAS Institute, Cary, N.C.).

*Experiment 1.* Treatments were applied and reapplied as outlined above to 12 fern species (Table 1) on the mornings of 10 and 24 Sept. 2003. The temperatures in the greenhouse were 82 °F [28 °C] at the time of both Ultiflora™ applications. All fronds were harvested and weighed on 30 Oct. 2003.

*Experiment 2.* Treatments were applied and reapplied to 6 fern species (Table 2) on the mornings of 9 and 24 Mar. 2004. The temperatures in the greenhouse were 86 °F [30 °C] at the time of both Ultiflora™ applications. All fronds were harvested and weighed on 23 Apr. 2004.

## Results and Discussion

*Acute phytotoxicity. Experiment 1.* For many genera—*Arachniodes*, *Athyrium*, *Davallia*, *Dryopteris*, *Osmunda*, *Platynerium* and *Rumohra*—no damage was detected (Table 1). For *Pteris*, *P. cretica* 'Parkerii' was not damaged but *P. quadriaurita* 'Flame' was. Phytotoxicity symptoms progressed from distortion, twisting and chlorosis to eventual necrosis and occurred on fronds that were immature at the time the Ultiflora™ was applied. Fronds of all three *Nephrolepis* species were damaged but only at the 24 oz/100 gal (2×) rate. The predominant symptom was marginal necrosis that developed on fronds that were immature at the time of Ultiflora™ application. The *Nephrolepis* ferns quickly outgrew the damage. *Experiment 2.* No damage was detected for *Adiantum raddianum*, *Pellaea rotundifolia*, *Polystichum tsus-sinense* and *Pteris vittata* (Table 2). For *Didymochlaena truncatula*, significant acute phytotoxicity was observed only at the 24 oz/100 gal (2×) rate. Phytotoxicity symptoms included necrotic spots and pinna margins and deformed and stunted fronds. *Polypodium formosanum* was the most sensitive to Ultiflora™ and was damaged at both the 1× and 2× rates. Damage consisted of brown lesions, some possibly the result of spray material collecting in low areas on horizontally growing frond blades.

*Chronic phytotoxicity. Experiments 1 and 2.* Despite visual stunting of *Nephrolepis biserrata* 'Macho' at the 2× rate, Ultiflora™ treatments had no significant effect on fresh frond weights of this or any of the other ferns (Tables 1 and 2).

Even though this was an EC formulation, which are often phytotoxic to ferns (Hoshizaki, 1983), Ultiflora™ applied at the label (1×) rate caused no significant phytotoxicity to 16 of the 18 ferns used in these experiments. At the 2× rate, two-thirds of the ferns exhibited no significant phytotoxicity symptoms.

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