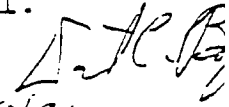



DATA EVALUATION REPORT

1. Chemical: Mycoleptodiscus terrestris
2. Test Material: Mycoleptodiscus terrestris, (10% and 1.0% wet weight) formulated in beads made of alginate/bran mixture
3. Study/Action Type: Nontarget plant testing (154A-22)
4. Study Identification: Toxicity/Pathogenicity Testing of Formulated Mycoleptodiscus terrestris on Nontarget Aquatic, Nontarget Terrestrial, and Endangered Aquatic Plants. By Uma Verma, Ph.D. and R. Charudattan, Ph.D. Prepared By the Biocontrol of Weeds Laboratory, Plant Pathology Department, University of Florida, Gainesville. February, 1991. Project ID. ES-MT-HR-01. Submitted By EcoScience Laboratories, Inc., Amherst, Massachusetts. EPA Acc. No. 418335-11.

5. Reviewed By: David C. Bays  
Microbiologist  
EFED/EEB  
  
Les W. Touart  
Head, Section 1  
EFED/EEB

Signature:   
Date: 7/10/91

Signature:   
Date: 7-16-91

6. Conclusions:

The study is scientifically sound and demonstrated that that Mycoleptodiscus terrestris is pathogenic to a number aquatic plants, and terrestrial crop plants. The study fulfills EPA Guideline requirements for nontarget plant testing.

7. Recommendations: N/A

8. Background:

This study was submitted to meet the requirements for nontarget plant testing which is required for the registration of this microbial pesticide.

10. Materials and Methods:

- A. Test Organisms: The test plants used in this study were chosen because of their economic importance (EPA Subdivision M, Table 3, pg 146) and in the case of the aquatic plants, their likelihood of being present in treated areas. A green algae and diatom were not tested due to difficulties of measuring the effects of the test fungus on these species. Seeds of the terrestrial plants were obtained from a local farm supply store and aquatic plants were collected from various north and northwest Florida counties.

- B. Dosage Form: The test material was a fungal mycelia entrapped in beads made of alginate and was applied at the following rates: for terrestrial plants-0.181 g of beads/inch<sup>2</sup> of exposed soil (70 lb [dry wt.] a.i./acre); for aquatic plants 0.181 g of beads/inch<sup>2</sup> of water surface (70 lb [dry wt.] a.i./acre for nontarget aquatics and 13 lb [dry wt.] a.i./acre for endangered aquatics). The controls consisted of the alginate pellets without the fungus applied at the same rates as the test material.
- C. Referenced Protocol: The aquatic studies were initiated by planting 6 cm long shoots or 6 to 30 cm long plants of the test species in a known quantity of acid-washed sand at the bottom of a glass container. The containers were filled with a known volume of sterilized 5% Hoagland solution containing 0.1% KHCO<sub>3</sub>, adjusted to Ph 6.5, and maintained under diurnal light at 25 ± 2C. One of the test species was a floating plant, which was placed, 30 plants to a container, on the surface of the Hoagland solution. The treatments were applied by dropping the test material onto the surface of the Hoagland solution in which the plants were growing. This allowed the inoculum pellets to randomly settle onto the test plants.

The terrestrial studies were initiated by planting Mycoleptodiscus terrestris free seed, 8-50 seeds/pot, (depending on seed viability, seed size, and/or plant type) in 10 cm diameter clay pots containing twice-autoclaved potting soil mix (150 g/pot). The inoculum (alginate pellets with or without the fungus) was applied to the soil surface of the pots once immediately after planting. The pots were randomly arranged in a split-plot design, with 5 replicates per treatment. The pots were irrigated manually after planting and thereafter with overhead misters. Seedlings were maintained for 4 weeks following germination and observed for phytotoxic or disease damage. Randomly selected symptomatic plants were tested for the presence of the fungus using differential agar media (streptomycin-potato dextrose agar). The test plants were evaluated for seed germination and seedling mortality.

The endangered aquatic plant testing used Sagittaria species, which had been collected from several locations in Florida. Both submerged and emergent species were used. The two submerged species, S. kurziana and S. platyphylla, (along with the positive control, Myriophyllum spicatum) were planted singly in sand at the bottom of glass tubes filled with sterilized 5% Hoagland solution containing 0.1% KHCO<sub>3</sub>. The emergent plants were transplanted singly into 16 cm diameter clay pots filled with 450 g of sterilized soil mix (pH 6.5) 6 weeks before the initiation of the study. The treatments were applied in the same manner as described

in the aquatic plant testing. The experiment was observed daily for 4 weeks and was assessed weekly for disease and phytotoxic damage.

D. Statistical Analysis: The results were calculated as counts, percentages, averages with standard deviations, and based on parametric or nonparametric tests as applicable.

## 12. Reported Results:

Plant Species (#)	Reduced Germination	EFFECT:			Reduced Growth	Death-(%)		
		Symptoms				0	1-37	>37
		None	Mild	Severe				
Terrestrial Plants (17)	0	8 <sup>a</sup>	5 <sup>b</sup>	4 <sup>c</sup>	---	6 <sup>d</sup>	7 <sup>e</sup>	4 <sup>c</sup>
Non-Target Aquatics (7)	---	4 <sup>f</sup>	0	3 <sup>g</sup>	0	7 <sup>h</sup>	0	0
Endangered Aquatics (10)	---	10 <sup>i</sup>	0	0	0 <sup>j</sup>	10 <sup>i</sup>	0	0

<sup>a</sup>Carrot, Soybean (var. Centennial), Tomato, Rice, English Pea, Wheat, Corn, and Wildrice

<sup>b</sup>Cabbage, Soybean (vars. Co 85-453 and Hutton), Crimson Clover, and Red Clover

<sup>c</sup>Birdsfoot Trefoil, Alfalfa, Alyce Clover, and White Clover

<sup>d</sup>Carrot, Rice, English Pea, Wheat, Corn, and Wildrice

<sup>e</sup>Cabbage, Soybean (vars. Centennial, Co 85-453 and Hutton), Tomato, Crimson Clover, and Red Clover

<sup>f</sup>Brazilian Elodea, Duckweed, Southern Naiad, and Strap-leaf Sag

<sup>g</sup>Coontail, Hydrilla, Parrotfeather

<sup>h</sup>"<sup>f</sup>" and "<sup>g</sup>" above

<sup>i</sup>Strap-leaf Sag, Delta Arrowhead, Coastal Arrowhead (3 spp.), Common Arrowhead, California Arrowhead, Unidentified Arrowhead (2 spp.), and Dwarf Arrowhead

<sup>j</sup>Strap-leaf Sag and Delta Arrowhead

Mycoleptodiscus terrestris was found to be highly pathogenic to Myriophyllum spicatum (Eurasian watermilfoil), Hydrilla verticillata, M. aquaticum (parrotfeather), and Ceratophyllum demersum (coontail). All other aquatic plants tested were found to be unaffected by the fungus. M. terrestris was found to have an effect on the following terrestrial plants: strongly pathogenic to birdfoot trefoil, alfalfa, alyce clover and white clover; weakly pathogenic to cabbage, soybean (vars. Co-483 and Hutton), crimson clover, and red clover. All other terrestrial plants tested were found to be unaffected by the fungus. None of the 10 Sagittaria species, which represented the endangered aquatic and wetland plants, were found to be affected by M. terrestris.

13. Study Author's Conclusions/Quality Assurance Measures:

"Report on Project ID ES-MT-HR-01 entitled Toxicity/Pathogenicity Testing of Mycoleptodiscus terrestris on Nontarget Aquatic, Nontarget Terrestrial, and Endangered Aquatic Plants was conducted in compliance with EPA Good Laboratory Practices Standards (40 CFR 160) published in the Federal Register (54[158]:34052-34074) on August 17, 1989, with the following exception: the protocol was not signed by the sponsor." Signed by study director, Uma Verma, Ph.D.

14. Reviewer's Discussion and Interpretation of the Study:

- A. Test Procedures: The procedures used followed those recommended by EPA in Section 158.170 of the EPA Registration Guidelines (Pesticide Testing Guidelines, Subdivision M, Microbial and Biochemical Control Agents).
- B. Statistical Analysis: Results were calculated as counts, percentages, averages with standard deviations, and statistically significant or nonsignificant treatment differences based on parametric or nonparametric tests as applicable.
- C. Discussion/Results: The results of these studies indicate that Mycoleptodiscus terrestris is pathogenic to a number of aquatic and terrestrial plants. This was not unexpected due to the known pathogenic nature of this fungus and that the primary reason this organism is being considered as a biocontrol agent is its ability to be pathogenic on Eurasian watermilfoil. However, the fungus was not pathogenic to the aquatic test plants used to represent endangered species.
- D. Adequacy of the Study:
1. Validation Category: Core
  2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-Liner: