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Control of Milk Weed (*Euphorbia heterophylla*) with *Helminthosporium* sp.

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Abstract

In the 1980/81 crop season the National Soybean Research Center of EMBRAPA, Brazil, initiated a biological control program for milk weed, *Euphorbia heterophylla*. It is estimated that *ca.* 200.000 ha of cropland, mainly soybeans, is infested, demanding an expenditure of *ca.* US$ 3.7 million worth in chemicals for 1987/88 season. A naturally-occurring *Helminthosporium* species was found to be virulent on most populations of the weed. The majority of the populations are uniformly susceptible, some are uniformly immune and others are mixed-susceptible, immune and plants with varying degrees of infection. Germination of spores in mixtures with herbicides bentazon, sethoxydin and surfactant was unaffected, but was severely affected by fomesafen and the surfactant energic at the recommended rates. Germination was not affected when in mixtures with *Bacillus thuringiensis*, *Baculovirus anticarsia* and carbaryl. Dried spores kept for 14 months in sealed test tubes were slow to germinate in water but reached 83% after 24 h. When spores were dry-formulated in lactose and stored for six months under laboratory conditions, germination reached 93% after 4 h incubation, and 99% after 24 h. Time of field application (8:00, 12:00 and 16:00), with temperatures varying from 23.8, 27.7 and 32.3°C, respectively, did not interfere with degree of infection, indicating that the fungus can be applied at any time of the day. Field application was adversely affected by low relative humidity but timely application under adequate conditions has shown that *Helminthosporium* can be as effective as the best post-emergence herbicide.

Introduction

The extensive use of herbicides in annual crops, especially for soybeans, has selectively favored the expansion of milk weed, *Euphorbia heterophylla* L. (Euphorbiaceae). From a minor weed about 15 yrs ago it has become one of the most troublesome and expensive weed to control. Currently it is estimated that *ca.* 200,000 ha of cropland, mainly soybean fields, are infested, demanding an expenditure of *ca.* US $ 3.7 million for chemicals in the 1987/88 season. Herbicides such as acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitro-benzoic acid), fomesafen, lactofen and imazaquin are extensively used (Gazziero *et al.* 1985) but represent one of the most expensive items in the cost of production.

In the 1980/81 crop season the National Soybean Research Center of EMBRAPA, Brazil, started a biological control program for milk weed.

Previous work (Yorinori 1985) showed that among seven pathogenic agents found associated with the weed, a *Helminthosporium* species was a potential candidate for biological control. It had the advantage of being easily cultured under laboratory conditions, was specific and highly virulent on the weed and occurred naturally in the soybean ecosystem (Yorinori 1985). It was found that certain populations of the weed were unaffected by the fungus, leaving the possibility of resistant plants existing among susceptible plants.

This paper reports recent findings on: a) distribution of milk weed populations which are resistant and susceptible to *Helminthosporium* sp.; b) compatibility of mixtures of herbicides, insecticides and surfactants with the fungus; c) effect of time of inoculation; d) spore longevity; and e) inoculum concentration.

Materials and Methods

Distribution of Resistant and Susceptible E. heterophylla *Populations*

Seeds of *E. heterophylla* were collected from States of Mato Grosso do Sul (1), Minas Gerais (1), Parana (20), Rio Grande do Sul (1), Santa Catarina (1) and Sao Paulo (1) and artificially-inoculated on potted plants in the greenhouse.

Inoculations were done using a spore suspension of 2.0 x 10^5 conidia/ml on 30- to 35-d-old plants. Reaction was based on presence or absence of susceptible type lesions (lesions varying in size from 3 to 6 mm).

Compatibility with Insecticides and Herbicide Mixtures

Conidia of *Helminthosporium* sp. were suspended in herbicides, insecticides and surfactants at the recommended rates for application in soybean fields. The following herbicides and insecticides were tested: bentazon (3-(1-Methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide) (0.72 kg a.i./ha), bentazon + surfactant assist (0.72 + 1.5), sethoxydin + surfactant assist (0.23 + 1.5); surfactant assist (1.5), fomesafen + energic (0.25 + 0.2); fomesafen (0.25); energic (0.2%), *Bacillus thuringiensis* (8 x 10^9 I.U.), *Baculovirus anticarsia* (50 caterpillar equivalent/ha) and carbaryl (1-Naphthyl N-methylcarbamate) (0.50). The effect of the mixtures on *Helminthosporium* sp. was measured by percent germination after 6 h of incubation under laboratory conditions. Spores were considered germinated when germ tubes had extended to twice or more the spore length.

Time of Inoculation

To asses the effect of time of application on the efficiency of infection of milk weed by *Helminthosporium* sp. inoculations were done at 8:00, 12:00 and 16:00 h using an inoculum concentration of 2.0 x 10^5 conidia/ml. The weather at each inoculation was sunny, with the following temperatures and RH: 0800 = 23.8°C and 88% RH; 1200 = 27.7 and 66; 1600 = 32.2 and 64. During the first two applications the wind speed varied from 4 to 6 mph; there was no wind during the third application.

The effect of time of application was determined by visual assessment of percent defoliation and degree of infection (scale of 0 = no infection to 10 = > 90% leaf area infected) at 6, 11 and 12 d after field inoculation.

Spore Longevity

Helminthosporium sp. spores collected directly from culture plates (14 months old), or formulated in lactose (6 months old) both stored either at room temperature or at 10 ±1°C in the refrigerator were tested for longevity. The longevity was based on germination after spores were suspended in sterile distilled water, plated on water-agar and observed under a stereoscopic microscope at hourly intervals from 1 to 8 h and at 24 h after plating. Spores were considered germinated when the germ tube had extended to twice or more the spore length.

Inoculum Concentration

To establish an adequate concentration of inoculum for the expected bioherbicidal effect of the fungus, field experiments were carried out using 0.5, 1.0, and 3.0 x 10^5 conidia/ml. The effect of each treatment was assessed based on disease severity (0 = no symptoms to 10 = >90% of leaves are infected), percent defoliation (leaf shed) assessed at 8, 26 and 36 d after inoculations, and fresh weight determined at 12 and 76 d following the inoculations.

Results and Discussion

Distribution of Resistant and Susceptible E. heterophylla Populations (Table 1)

Out of 20 sites from which *E. heterophylla* seeds were collected for tests for reaction to *Helminthosporium* sp. in the State of Parana, nine had the entire population with susceptible plants, eight had mixtures of resistant and susceptible plants and three had all resistant plants. Seeds collected from the State of Minas Gerais (Lavras) gave all susceptible plants; seeds collected at Sao Domingos, State of Santa Catarina (SC), gave 92% of susceptible plants; and seeds from Sao Gabriel D'Oeste, Mato Grosso do Sul (MS) gave all susceptible plants. The majority of the populations of *E. heterophylla* seem to be susceptible to *Helminthosporium* sp. From the results, four types of populations can be distinguished: a) those that have all susceptible plants; b) those with majority of susceptible plants; c) those with majority of resistant plants; and d) those with all resistant plants. Further tests need to be carried out with more sites and more seeds/site.

Table 1. Reaction of *Euphorbia heterophylla* L. populations to *Helminthosporium* sp. Plants from seeds collected at different sites in Brazil and inoculated in the greenhouse (EMBRAPA-CNPSo, Londrina, PR. 1985).

Localities (State)	No. of plants tested	% Susceptible plants
Assis Chateaubriand (PR)	13	92.3
Cascavel (PR)	7	0
Cascavel-OCEPAR (PR)	5	80
Castro - A (PR)	10	100
Castro - B (PR)	9	77.8
Castro - C (PR)	12	100
Castro - D (PR)	12	91.7
Cornelio Procopio (PR)	7	100
Guaira (PRO)	8	100
Guarapuava - Col. Vitoria (PR)	11	100
Londrina - CNPSo (PR)	31	100
Londrina - Guaravera (PR)	94	64
Mangueirinha (PR)	11	100
Manguerinha - COAMO (PR)	10	100
Marialva - SPSB (PR)	9	78
Ponta Grossa - FT-Pesq. Sem. (PR)	12	100
Terra Boa - Faz. Palmital - A (PR)	32	0
Terra Boa - Faz. Palmital - B (PR)	40	10
Toledo (PR)	4	0
Ubirata - Juranda (PR)	3	33.4
Lavras (MG)	6	100
Sao Domingos (SC)	12	92
Sao Gabriel D'Oeste (MS)	10	100

Compatibility with Insecticide and Herbicide Mixtures (Table 2)

Insecticides. When *Helminthosporium* sp. spores were suspended in the two bioinsecticides *(Bacillus thuringiensis* at 8 x 10^9 International Units, and *Baculovirus anticarsia* at 50 caterpillar equivalents/ha) and insecticide carbaryl (at recommended rates), only *B. anticarsia* slightly inhibited germination.

Herbicides. Among three herbicides (bentazon, sethoxydin and fomesafen) and two surfactants (assist and energic), only fomesafen and energic affected the germination of *Helminthosporium* sp. when suspended at the recommended dosage. These results indicate the possibility of mixing the fungal spores to control *E. heterophylla* with herbicides and insecticides to control other weed species and insects.

Table 2. Germination of *Helminthosporium* sp. suspended in herbicides, insecticides and surfactant solutions at the recommended dosages (EMBRAPA-CNPSo, Londrina, PR. 1986).

Chemicals	Dosage g or l a.i./ha	*Helminthosporium* sp. % germination[1]
Bentazon	0.72	93.8
Bentazon + assist	0.72 + 1.5	93.2
Sethoxydin + assist	0.72 + 1.5	91.8
Assist	1.5	87.8
Fomesafen + energic	0.25 + 0.2%	1.6
Fomesafen	0.25	49.6
Energic	0.2%	0
Bacillus thuringiensis	0.5 (= 8 x 10^9 I.U.)	93.8
Baculovirus anticarsia	50 LE[2]	68.6
Carbaryl	0.19	95.6
Control (water)	-	96.8

[1] % germination determined after 6 h incubation; average of five counts of 100 spores. Spores were considered germinated when germ tube had twice or more the spore length.
[2] Extraction of 50 caterpillar equivalent/ha.

Time of Inoculation (Table 3)

Inoculations of *Helminthosporium* sp. at three different hours in the day did not affect pathogenicity of the fungus. The levels of defoliation and degree of infection were the same whether inoculations were done at 0800, 1200 or 1600. Evaluations made at 6, 11 and 27 d after inoculation did not show progress of the disease beyond the initial infection. The weather condition following the inoculations was not favorable for further disease development.

Spore Longevity (Table 4)

Pure spores remained viable for 14 months when stored either in the refrigerator or at room temperature, but germination of spores kept for 14 months was slower and lower than those formulated in lactose and stored for 6 months. In both cases (6 months or 14 months storage), there was no difference between the storage in the refrigerator and at room temperature. Fourteen-month-old spores had 35% germination after 8 h of incubation and a maximum of 83% germination after 24 h. Germination of six-month-old spores kept at room temperature reached 49% after 2 h of incubation, 95% after 8 h and 99% after 24 h. Spores kept in the refrigerator had 70% germination after 2 h incubation, 94% after 8 h and 98% after 24 h. Spores kept in the refrigerator tended to germinate faster than those maintained at room temperature. These data show that the spores of *Helminthosporium* sp. offer great flexibility for handling and storing at room temperature.

Table 3. Effect of time of inoculation with *Helminthosporium* sp. on disease severity, percent defoliation and fresh weight of *Euphorbia heterophylla* L. evaluated at 6, 11 and 27 d after inoculation (EMBRAPA-CNPSo, Londrina. 1987).

Parameter	Hour of Inoculation				
	0800	1200	1600	Control	CV (%)
Weather conditions					
Temp. °C	23.8	27.7	32.2	-	-
RH (%)	88	66	64	-	-
Disease severity (0 to 10)[1]					
6[2]	7.7	6.6	7.4	-	16.42 ns
11	6.6	5.4	6.4	0	32.20 ns
27	7.7	7.3	7.3	0	-
% defoliation[3]					
6	51	42	53	0	26.35 ns
11	52	43	52	0	29.90 ns
27	54	50	58	0	25.40 ns
Fresh wt. (g) of 50 plants	317 *b*	390 *b*	361 *b*	550 *a*	22.12

[1] Disease severity: 0 = no symptoms to 10 = > 90% leaf area infected.
[2] Days after inoculation.
[3] Defoliation (%) = percent leaf shed as compared to the control by visual observation.

Effect of Inoculum Concentration

Disease severity assessed at 8 d following inoculation showed similar levels of infection for the two lower (0.5×10^5 and 1.0×10^5 conidia/ml) and the two higher (2.0×10^5 and 3.0×10^5) concentrations of inocula (Table 5). No differences were observed among concentrations when disease severity was assessed at 26 and 36 d following inoculations. Even the control plots had similar level of infection, showing that spores produced on inoculated plants had spread to the whole experimental field. Percent defoliation (leaf shed) was the same for the lower two concentrations (0.5×10^5 and 1.0×10^5 conidia/ml) but differed from the higher concentrations at 8 d following inoculations. Assessment at 26 and 36 d following inoculations showed similar levels of defoliation for the two lower and the two higher concentrations. Leaf shed reached the maximum of 73% at 36 d following inoculations. The control plots did not differ in disease severity at 26 and 36 d, but differed in percent defoliation (43%) from all the treatments, indicating a slower leaf shed from natural infection.

When fresh weight was compared among treatments at 12 d after inoculations, the treatment with 0.5×10^5 conidia/ml gave the same weight as the control, and both were significantly different from the other treatments (Table 5). At 76 d after inoculations, there were no differences among inoculated plots, but all gave less fresh weight than the control.

From the results obtained here, the following conclusions can be drawn: a) the populations of *E. heterophylla* differ in their reaction to *Helminthosporium* sp. varying from immunity to high susceptibility, with many populations with both types of plants; b) the majority of the populations seem to be susceptible, but further work is needed with more samples and plants to be tested/site; c) compatibility with certain herbicides and insecticides could reduce cost of application, allowing for tank mixtures for control of other weed species and insects in a single application; d) the spores of *Helminthosporium* sp. seem to be unaffected by solar

radiation, allowing for application at any time of the day; e) disease progress beyond the artificial inoculation is dependent upon continuous favorable weather conditions; f) the viability of the spores for at least 14 months from the date of collection from culture offers great flexibility in handling the fungus; g) under weather condition favorable for a fast disease establishment and production of secondary inoculum, concentration of the initial inocula, can be lower (e.g., 0.5 x 10^5 spores/ml) as compared to a less favorable condition.

Table 4. Germination of *Helminthosporium* sp. stored for 6 months (formulated in lactose) and 14 months (pure spores) under room temperature and at 10 ±1°C (EMBRAPA-CNPSo, Londrina, PR. 1986).

Duration and storage condition		Incubation period (hour) and % germination									
		0	1	2	3	4	5	6	7	8	24
Pure spores (14 months)	A[1]	0	0	0	1.4	5	5	20	28	35	83[1]
	B	0	0	0	0.2	16	16	21	35	35	78
Lactose formulated (6 months)	A	0	0	49	71	93	94	96	94	95	99
	B	0	0	70	85	95	97	97	97	94	98

[1] Storage condition: A = room temperature; B = 10°C ± 1°C
[2] Average of five counts of 100 spores; spores were considered germinated when germ tube had twice or more the spore length.

Table 5. Effect of inoculum concentration of *Helminthosporium* sp. on the control of milk weed, *Euphorbia heterophylla* L. (EMBRAPA-CNPSo, Londrina, PR. 1987).

Inoculum concentration (conidia/ml) x 1,000	Disease severity (0 to 10)[1]			% defoliation[2]			Fresh weight (g/m²)	
	8[3]	26[3]	36[3]	8	26	36	12	76
50	3.2 *b*[4]	8.8 *a*	9.0 *a*	30 *c*	48 bc	56 b	880 a	552 b
100	2.8 *b*	8.9 *a*	9.0 *a*	35 *c*	45 *c*	58 *b*	572 *b*	368 *bc*
200	6.3 *a*	8.8 *a*	9.0 *a*	45 *b*	62 *ab*	73 *a*	596 *b*	376 *bc*
300	6.6 *a*	9.0 *a*	9.0 *a*	55 *a*	65 *a*	73 *a*	612 *b*	304 *bc*
Control	0 *c*	8.7 *a*[5]	9.0 *a*[5]	0 *d*	36 *c*	43 *c*	936 *a*	844 *a*
C.V. (%)	18.8	2.4	-	14.9	20.6	14.4	31.9	54.2

[1] Disease severity (0-10): 0 = no infection to 10 = > 90% of leaf area infected.
[2] % defoliation: % leaf shed.
[3] Days after inoculation.
[4] Means of four replicates. Each plot (replicate) assessed as a whole. Means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's Multiple Range Test.
[5] Infection of control plots.

References

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