

## Biological Control of Milk Weed (*Euphorbia heterophylla*) with Pathogenic Fungi

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### Abstract

The milk weed (*Euphorbia heterophylla*) is widely distributed in most of the crop land in Brazil and is more important in the southern soybean-producing states. In the State of Paraná alone, it is estimated that about \$1.6 million worth of herbicide will be spent in 1984 to treat 70,000 ha of badly infested areas. The main damage to soybean production is caused by the green stems of the weed that clog harvesting machines and also transfer moisture to harvested beans. *E. heterophylla* has been found naturally infected by seven pathogenic agents (*Euphorbia* mosaic virus; *Uromyces euphorbiae*; *Sphaceloma* sp.; *Alternaria* sp.; *Helminthosporium* sp.; *Rhizoctonia solani*; and *Sclerotinia sclerotiorum*). Under experimental conditions, *Alternaria* sp. and *Helminthosporium* sp. were highly efficient and are promising as biological agents for the control of *E. heterophylla*. They have the advantage of being easily cultured under laboratory conditions.

### Le Biocontrôle de la Mauvaise Herbe *Euphorbia heterophylla* L. au Moyen de Champignons Pathogéniques

La mauvaise herbe *Euphorbia heterophylla* est bien répartie dans la plupart des terres agricoles du Brésil, mais elle est plus importante dans les états du sud, où se trouvent les plantations de soja. On estime que, dans le seul état de Paraná, on dépensera 1.6 million de dollars environ en 1984, en herbicides, pour traiter 70,000 ha des régions gravement infestées. Le dommage principal au rendement du soja résulte des tiges vertes de la mauvaise herbe, qui bouchent la moissonneuse et qui en même temps transfèrent de l'humidité à la récolte. On a observé sept agents pathogènes qui infectent *E. heterophylla* de façon naturelle: le virus mosaïque d'*Euphorbia*; *Uromyces euphorbiae*; *Sphaceloma* sp.; *Alternaria* sp.; *Helminthosporium* sp.; *Rhizoctonia solani*; et *Sclerotinia sclerotiorum*. Dans des conditions expérimentales, *Alternaria* sp. et *Helminthosporium* sp. se sont révélés hautement efficaces et promettent bien pour le biocontrôle d'*E. heterophylla*. Ces agents ont l'avantage de se prêter facilement à la culture en laboratoire.

### Introduction

Most weed plants of crop land in Brazil are controlled by chemical mechanical, or manual means, but a few remain that are more difficult to handle. One of the most difficult to control has been the milk weed, *Euphorbia heterophylla* L. (Euphorbiaceae).

The weed is widely distributed in most agricultural land in Brazil but is more important in the southern soybean-producing states.

Being resistant to most herbicides it has selectively survived chemical control and has become economically important in the past 5-6 yrs. If weather conditions are favorable, the weed produces seed and germinates all year round, frequently requiring more than one herbicide application during a crop season. Available herbicides are expensive, and more than one application could be economically unfeasible. Of several commercial herbicides currently used only one (Blazer) (sodium 5-[2-chloro-4-

(trifluoromethyl) phenoxy]-2-nitrobenzoate) (Rohm & Haas Co.) is effective against *E. heterophylla* (Gazziero *et al.* 1983).

For control of *E. heterophylla* alone, and only in the State of Paraná where about 70,000 ha of crop land will require chemical control, an estimated \$1.6 million worth of herbicide will be spent in 1984.

In recent years, increasing awareness of undesirable effects of agricultural pesticides have resulted in stronger legislation regulating use and recommendation of chemicals for crop protection. As a consequence, more effort and funds have been directed toward biological control of crop pests.

Biological control of soybean and sugar-cane insects with pathogens are routinely used in Brazil but research on weed control is at the embryonic stage. The only reference on pathogens associated with *E. heterophylla* is in Viegas (1961).

Preliminary studies with biological agents have shown excellent results under experimental conditions.

Research carried out to date (Yorinori 1984) has dealt with: studies on the biology of *E. heterophylla*; surveys of pathogens associated with the weed; and evaluation of efficiency of the pathogens in control of *E. heterophylla* as compared with the herbicide Blazer.

Observations on the biology of *E. heterophylla* have shown that most infestation occurs from early December to February (Fig. 1) coinciding with early development to pod-filling stages of soybeans. The highest incidence of pathogenic agents has occurred from late December to end of February (Fig. 2).

In general, association of pathogens in the field does not prevent the weed from producing seed. They may cause severe defoliation, but leave the green stems. Stems cause clogging of harvesting machines and are most damaging to soybeans for conferring moisture to harvested beans.

Disease surveys on *E. heterophylla* have shown that the weed is naturally infected by at least seven pathogenic agents: *Euphorbia* mosaic virus; the rust, *Uromyces euphorbiae* (Ke. & Pk.) (Uredinales); the scab, *Sphaceloma* sp. (*Sphaceloma kruggii* Bitanc. & Jenkins?) (Coelomycetes); the leaf spot, *Helminthosporium* sp. (Hyphomycetes); the leaf spot and stem canker, *Alternaria* sp. (Hyphomycetes); a damping-off and root rot fungus, *Rhizoctonia solani* Kühn (Agonomycetes); and *Sclerotinia sclerotiorum* (Lib.) de Bary (Agonomycetes).

The first five pathogens were found consistently associated with *E. heterophylla*, and occur simultaneously when there was maximum infestation of the weed. *R. solani* and *S. sclerotiorum* occur in certain soybean producing regions but are also very destructive to soybean.

Under experimental conditions, only *Alternaria* sp. and *Helminthosporium* sp. have been shown to be highly efficient and promising as biological control agents. They have the advantage of being easily cultured in the laboratory. Under natural conditions these two fungi are generally found causing minor disease.

Greenhouse inoculations with isolates of *Alternaria* sp. and *Helminthosporium* sp., showed that both fungi caused significant reduction of fresh weight of *E. heterophylla* 72 h after inoculation (Table 1). *Helminthosporium* sp. was as efficient as the herbicide Blazer.

*Helminthosporium* sp. was extremely virulent in greenhouse tests. It was capable of colonizing leaf tissue in less than 6 h of incubation time (Fig. 3); with 12 h or more it caused complete defoliation within 72 h when inoculated on 32-day-old plants with inoculum containing  $1 \times 10^4$  spores/ml. The first lesions were visible within 12–14 h after inoculation.

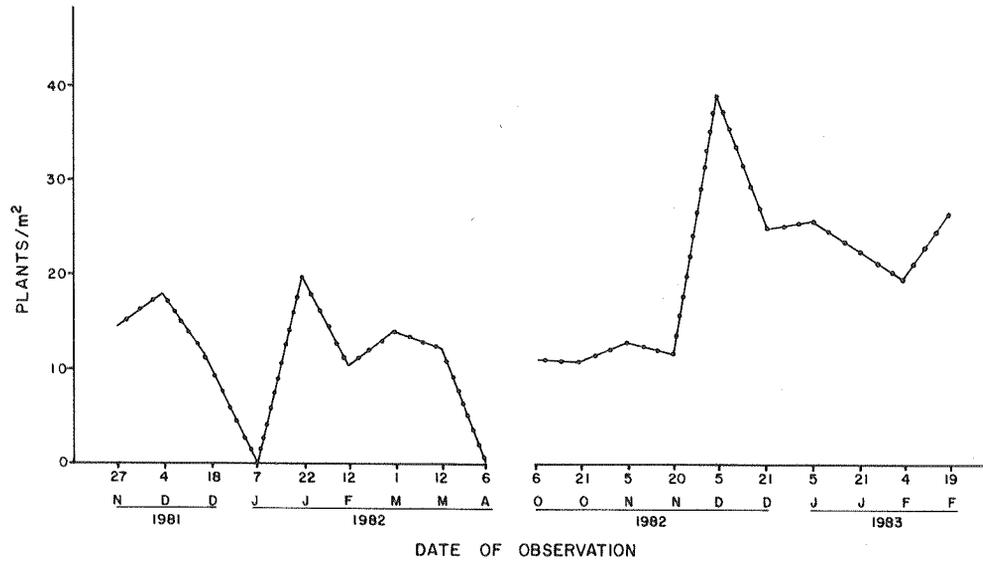


Fig. 1. Fluctuation of *Euphorbia heterophylla* L. population (plants/m<sup>2</sup>) in the field. Period of observation: 27 November 1981 to 19 February 1983.

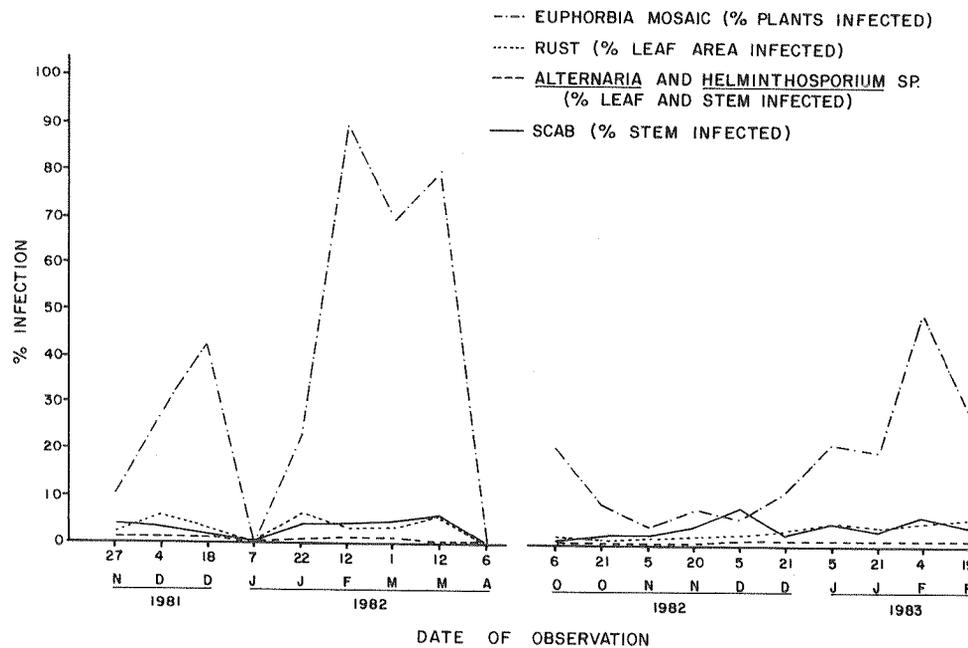


Fig. 2. Disease incidence on *Euphorbia heterophylla* L. under field conditions. Period of observation: 27 November 1981 to 19 February 1983.

**Table 1. Effect of pathogenetic fungi and herbicide Blazer on control of *Euphorbia heterophylla* L. in a greenhouse experiment conducted in 1982.**

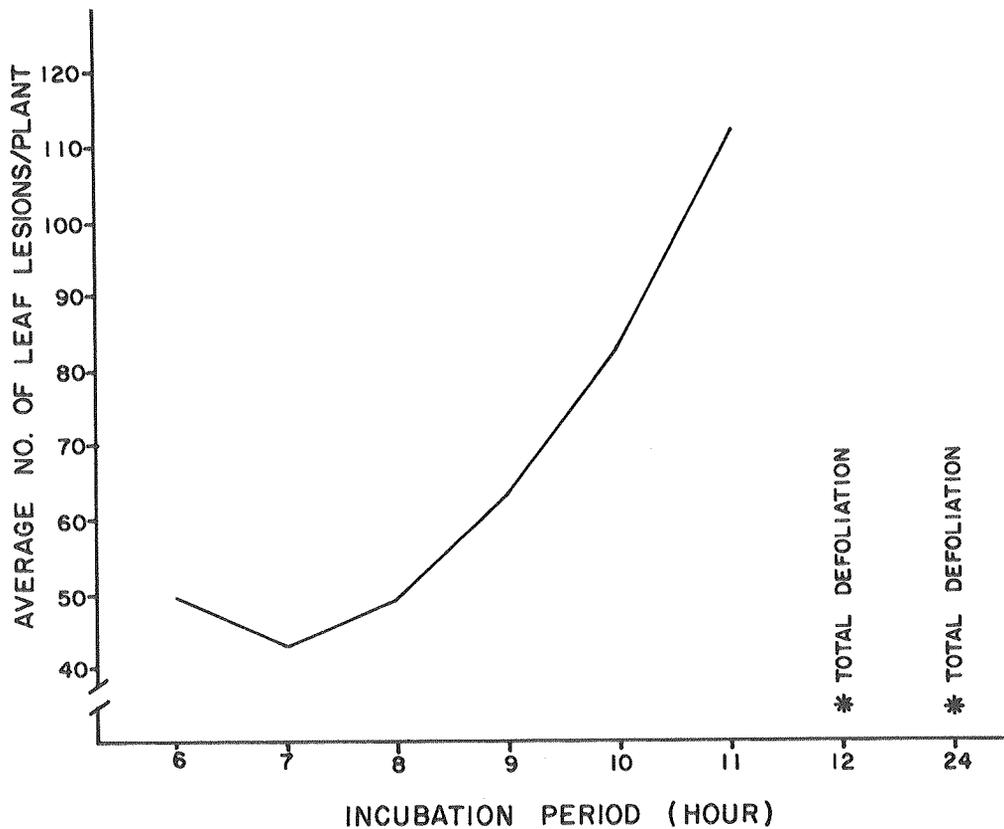
| Treatment <sup>1</sup>               | Fresh weight <sup>2</sup><br>(g) | % reduction<br>from control |
|--------------------------------------|----------------------------------|-----------------------------|
| Control (Water)                      | 27.5 a <sup>3</sup>              | 0                           |
| EMG-1 ( <i>Alternaria</i> sp.)       | 24.6 ab                          | 10.5                        |
| EMG-2 ( <i>Alternaria</i> sp.)       | 23.1 ab                          | 16.0                        |
| EPR-2 ( <i>Alternaria</i> sp.)       | 18.8 bc                          | 31.6                        |
| C95-A ( <i>Helminthosporium</i> sp.) | 17.7 bc                          | 35.6                        |
| Blazer (0.75 l/ha) <sup>4</sup>      | 14.4 cd                          | 47.6                        |
| C95-A ( <i>Helminthosporium</i> sp.) | 8.7 d                            | 68.4                        |

<sup>1</sup> Treatment at 32 days after planting.

<sup>2</sup> Avg. of 5 replicates, 72 h after treatment.

<sup>3</sup> Numbers followed by the same letter are not significantly different (P<0.05).

<sup>4</sup> Blazer: Sodium 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoate.



**Fig. 3.** No. of leaf lesions on 32-day-old *Euphorbia heterophylla* L. (plants 72 h after inoculation with *Helminthosporium* sp. and 6–24 h of incubation period. Experiment conducted in 1983.

Under field experiments, *Alternaria* sp. was less efficient requiring longer time to cause infection, but *Helminthosporium* sp. was as efficient as Blazer (Table 2). Inoculations were made when plants were flowering, thus under a condition when Blazer would not be recommended. Both *Helminthosporium* sp. and Blazer caused severe defoliation, preventing treated plants from producing seeds.

Almost all plants in the field experiment became infected by Euphorbia mosaic virus, but apparently seed production was not affected by the virus. *E. heterophylla* plants seem to be adapted to infection by the virus.

*Alternaria* sp. is less effective than *Helminthosporium* sp. in causing defoliation, but is effective in killing the plant by stem infection. A single stem infection at seedling stage can kill the plant. On the other hand, *Helminthosporium* sp. is efficient in causing defoliation, but causes only minor stem spots. A mixed inoculation with both *Alternaria* sp. and *Helminthosporium* sp. may result in greater control. This has not been tested.

An attempt to test the *Helminthosporium* sp. as a biological control agent of *E. heterophylla* in c. 2 ha of heavily infested soybeans did not reproduce the experimental results. The reason for this failure remains unknown.

Table 2. Effect of pathogenic fungi and herbicide Blazer on control of *Euphorbia heterophylla* L. in the field during 1982.

| Treatment <sup>1</sup>               | Dry weight <sup>2</sup><br>kg/8 m <sup>2</sup> | % variation<br>from control |
|--------------------------------------|--|-----------------------------|
| EMG-2 ( <i>Alternaria</i> sp.)       | 6.6 a <sup>3</sup>                             | 11.86                       |
| C95-A ( <i>Helminthosporium</i> sp.) | 6.2 a  | 5.10                        |
| EPR-12 ( <i>Alternaria</i> sp.)      | 6.0 a  | 1.69                        |
| Control (Water)                      | 5.9 ab   | 0                           |
| EMG-1 ( <i>Alternaria</i> sp.)       | 5.9 ab   | 0                           |
| Blazer (0.75 l/ha) <sup>4</sup>      | 5.0 bc   | -15.25                      |
| C95-B ( <i>Helminthosporium</i> sp.) | 4.3 c  | -27.12                      |

<sup>1</sup> Treatment at early flowering stage.

<sup>2</sup> Avg. of 4 replicates; harvested 72 h after application.

<sup>3</sup> Numbers followed by the same letters are not significantly different (P<0.05).

<sup>4</sup>Blazer: Sodium 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoate.

Current research is being conducted on: selection of more virulent isolates of *Alternaria* sp. and *Helminthosporium* sp.; development of culture media for mass production of spores; and evaluation of effects of environmental conditions on field infection by both fungi.

In addition to the naturally occurring pathogens, an application of *Alternaria* sp. and *Helminthosporium* sp. inocula at the early stage of weed development is expected to keep the weed population at a manageable level. Neither fungus has been shown to be pathogenic to crop plants.

## References

- Gazziero, D.L.P., Almeida, F.S., Rodrigues, B.N., and Oliveira, V.F. 1983. Recomendações para o controle de plantas daninhas na cultura de soja. Londrina, EMBRAPA/CNPS, Comunicado Técnico, 21, 1983, 10 p.
- Viegas, G.P. 1961. *Índice de Fungos da América do Sul*. Instituto Agrônômico — Campinas, 921 p.
- Yorinori, J.T. 1984. Controle biológico de amendoim bravo (*Euphorbia heterophylla* L.). In: Seminário Nacional de Pesquisa de Soja, 3, Campinas, SP, 1984. Resumos, Londrina, EMBRAPA/CNPS, 1984, p. 40.