

GERMINATION AND MYCELIAL GROWTH OF *BIPOLARIS EUPHORBIAE* MUCHOVEJ & CARVALHO AS INFLUENCED BY HERBICIDES AND SURFACTANTS

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ABSTRACT

Bipolaris euphorbiae Muchovej & Carvalho can be combined with herbicide in order to control a large spectrum of weed species, being a strong candidate for the biocontrol of *Euphorbia heterophylla* L. (milk weed). The fungus release can be combined with herbicide in order to control a broader spectrum of weed species. Thus, laboratory experiments were set up to study the feasibility of using tank mixes of *B. euphorbiae* spores with herbicides or surfactants recommended for soybean. Mycelial growth and conidia germination were evaluated in PDA medium enriched with the herbicides oxasulfuron (80 g/ha), glyphosate (4 L/ha), bentazon (1.5 L/ha), fomesafen (1 L/ha), chlorimuron-ethyl (80 g/ha), lactofen (1 L/ha) and imazetaphyr (1 L/ha), and the surfactants Energic (2 ml/L), Aterbane (2.5 ml/L), Silwet L-77Ag (1 ml/L), Herbitensil (2 ml/L) and Natur L'óleo (10 ml/L). Dilution of the herbicides at 50% and 25% were evaluated based on solution consumption of 300 L/ha. The surfactants were evaluated only in the recommended concentrations. Mycelial growth was not affected by bentazon and fomesafen and slightly by oxasulfuron. However, glyphosate and the surfactants Energic, Herbitensil and Aterbane strongly reduced its growth. The reduction observed on imazetaphyr enriched medium was intermediate and the Natur L'óleo promoted mycelial growth. All of the surfactants allowed *B. euphorbiae* conidia germination equivalent to that reached in the presence of water. Energic and Herbitensil caused an expressive retardation on spore germination. The germinative process only began after 120 minutes in the presence of Herbitensil. In relation to the herbicides, it was observed that only in the presence of glyphosate and imazetaphyr the conidia germination did not follow the trend of the treatment with water.

Key words: *Bipolaris euphorbiae*, mycelial growth, conidia germination, herbicides, surfactants

INTRODUCTION

The fungus *Bipolaris euphorbiae* Muchovej & Carvalho is considered an ideal candidate for the biological control of *Euphorbia heterophylla* L. (milkweed), a very important weed in soybean, through the inundative strategy (bioherbicides). This fungus has been the subject of studies that will allow its use in the management of milkweed in areas densely populated by this species (5,9,12).

The introduction of *B. euphorbiae* in weed management programs is dependent on the basic studies of the biology of the organism and its interactions with the host plant. The knowledge of its compatibility with other control agents commonly used for this target plant in each agroecosystem is fundamental (6). Toffanelli (12) determined that the herbicides chlorimuron-ethyl, fomesafen, glyphosate and imazetaphyr, and the surfactants Energic, Triton x-100 and Herbitensil, were inhibitory to the mycelial growth of *B. euphorbiae*.

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Many chemical products can enhance the efficacy of weed control when applied in combination with biocontrol agents, causing either a synergistic or additive effect. There is a great availability of herbicides and, thus, many combinations can be tested for the interactions pathogen-herbicide that would have a better cost: benefit ratio. Such combinations can result in the reduction of the amount of chemical products and microorganisms applied for the effective control of weeds (8).

The objective of the present study was to determine the effects of several post-emergence herbicides and surfactants on *B. euphorbiae* mycelial growth and spore germination. The answer to these questions may promote the combined use of these agents for the control of *E. heterophylla*.

MATERIALS AND METHODS

Production of *Bipolaris euphorbiae* inoculum

B. euphorbiae isolate used in all experiments was kindly provided by the Plant Pathology Laboratory of the “Centro Nacional de Pesquisa de Soja da EMBRAPA, Londrina, Paraná/ Brazil” and stored at 4°C in test tube slants containing PDA (Potato-Dextrose-Agar). The fungus was transferred to Petri plates with PDA and grown for 7 days in growth chambers (FANEM 624) adjusted for 25°C and 12 h photoperiod provided by four 20W fluorescent lamps, according to Marchiori's (9) modified methodology. After this period, two 5 mm diameter sections of the colony were transferred to erlenmeyers (1000 mL) containing 200 g parboiled rice and 120 ml distilled water, previously autoclaved for 20 min at 120°C. The flasks were maintained on the bench in the laboratory for fungal growth. The flasks were shaken daily to ensure uniform growth of the fungus in the substrate. After 7 days of incubation, the content of the two flasks was transferred to trays (34 cm x 22 cm x 4 cm) lined with paper foil, and kept in an acclimatized room, at 25°C (\pm 3) and 60% RH, until dry (7 days). The inoculum was then ground in a Wiley mill, with a 0.5 mm screen, and stored in plastic bags at 4°C in a refrigerator. The inoculum had 9.1×10^7 conidia/gram.

Mycelial growth of *B. euphorbiae*

Mycelial growth of the fungus was evaluated on Potato-Dextrose (PD) broth amended with different herbicides and surfactants, considering a flow rate of 300 L/ha. The surfactants Energic® (Zêneca do Brasil Ltda; 2 ml/L), Aterbane® (Rohm and Hass Química Ltda; 2.5 ml/L), Silwet L-77Ag® (Witco do Brasil Ltda.; 1 ml/L), all spreader adhesives; Herbitensil® (Hoechst Schering Agro do Brasil Ltda.; 2 ml/L), a spreader and Natur L'óleo® (Arbore; 10 ml/L) an adhesive, were used in the recommended doses of the commercial product. The test followed a completely randomized design with 4 replications. The herbicides oxasulfuron (Du Pont do Brasil Ltda.; 80g/ha), glyphosate (Monsanto do Brasil Ltda.; 4 L/ha), bentazon (BASF Brasileira S.A.; 1.5 L/ha), fomesafen (Zeneca Brasil Ltda.; 1 L/

ha), chlorimuron-ethyl (Novartis Biociências; 80 g/ha), lactofen (BASF Brasileira S.A.; 1 L/ha) and imazetaphyr (Cyanamid Química do Brasil Ltda.; 1 L/ha) were tested at the recommended doses (100%) and diluted to 50% and 25% with the medium broth. Test the herbicides glyphosate, fomesafen and imazetaphyr were evaluated in the first test and, bentazon, oxasulfuron, chlorimuron-ethyl and lactofen, in the second. The treatments followed a 3x3 (1st test) or a 4x3 (2nd test) factorial design with herbicides and doses as variables, with one treatment as control (no herbicide). Two 0.5 cm diameter sections of mycelium-agar from the margins of 12-day old colonies of *B. euphorbiae* were seeded in erlenmeyer flasks, containing 100 ml PD amended with the herbicides or surfactants. The flasks were kept in an incubator (FANEM 624), at 25°C and 12 hours photoperiod for 10 days, which was the time required for the fungus to cover the surface of the broth in the control treatment. The contents of each flask were filtered on Whatman #1 filter paper, the mycelium was dried at 80°C until constant weight, and the weight was recorded.

B. euphorbiae conidia germination

Conidia germination was evaluated using Nakamura's (10) modified procedure for incubation in microscope glass slides. The glass slides were washed with neutral detergent, placed in a sulfochromic solution for removal of all impurities, and rinsed with deionized water. Four milliliter of melting water-agar (1%) were carefully pipetted on each slide. Stock solutions (10x) of the herbicides and surfactants were prepared in sterilized water. One milliliter of each stock solution was pipetted into test tubes containing 9 ml sterile distilled water and 5 mg of the stored inoculum were added to the solution. The suspension was homogenized in a vortex, and 4 drops were pipetted equidistantly on the water-agar slide. Four repetitions of each treatment were made, and the statistical design was completely randomized.

The conidial germination speed was determined in 8 groups of slides, for incubation times of 45, 60, 90, 120, 180, 240, 360 and 720 min for herbicides and 45, 60, 90, 120, 150, 310, 370 and 490 min for surfactants. The prepared slides were placed in Petri plates containing a moist cotton ball and kept on the laboratory bench, at 26°C (\pm 3°C). The germination process was interrupted by adding a drop of lactic blue dye (1.2 g cotton blue in 100 ml lactic acid) to each drop of spore suspension. All germinated and non-germinated conidia in each sample were counted in an optical microscope, at 400x. The maximum germination of the conidia was determined from the glass slides kept for 720 min (herbicides) or 490 min (surfactants). A conidium was considered as germinated when the germ tube was at least one half as long as the length of the spore.

Statistical analysis

The data from mycelial growth was submitted to the analysis of variance and the averages compared by the Tukey test. The

data on percent germination was analyzed according to Boltzman sigmoidal model (MicroCal Origin, Version 4.00, Microcal Software, Inc., 1991-95).

RESULTS AND DISCUSSION

Mycelial growth

Glyphosate was strongly inhibitory to *B. euphorbiae* mycelial growth, independently from the dose used, causing a reduction of 96.5%, on average, in relation to the control (Fig. 1A). Fomesafen was not inhibitory at any of the doses tested. However, imazetaphyr reduced the dry biomass of the fungus as a function of the concentration, with 44.9%; 37.4% and 27.6% for the recommended, 50% and 25% of the recommended dose, respectively.

The herbicides evaluated on the second test did not present statistical differences in relation to the doses applied (Fig. 1B). Bentazon and oxasulfuron did not affect mycelial growth of *B. euphorbiae*. However, chlorimuron-ethyl and lactofen reduced mycelial growth by 93.3 and 85.3%, respectively.

In a more general analysis, the herbicides chlorimuron-ethyl, lactofen, glyphosate and imazetaphyr were markedly antagonistic for the mycelial growth of *B. euphorbiae* (Figs. 1A and 1B). Thus, the use of the fungus with these herbicides in tank mixes is not viable. Similar responses were found by Toffanelli (12). Alves Jr. (1) observed that the mycelial growth of *B. euphorbiae* in liquid media amended with chlorimuron-ethyl was reduced by 84% in relation to the control, while the media amended with imazetaphyr, imazaphyr or flumetsulan increased biomass production. This could reflect the ability of the fungus to degrade or use nutrients provided by these products. The application of more than 10 ppm glyphosate in liquid media inhibited the growth of the ectomycorrhizal fungi *Suillus tomentosus*, *Thelephora americana* and *T. unistis* (2). An inhibitory effect of Goltix and Igran (triazines) on the mycelial growth of *Aspergillus fumigatus*, *Fusarium oxisporum*, *F. oxisporum* f. sp. *vasinfectum*, *Helminthosporium oryzae* and *Verticillium agaricum* was observed, possibly due to the chemical structure of the herbicides, their concentration and the characteristics of the organisms studied (4). However, the herbicides bentazon, oxasulfuron and fomesafen, at all the doses tested, did not affect mycelial growth of the fungus which, apparently, would not forbid the use of the two plant control agents in tank mixes.

Several herbicides, such as bentazon, acifluorfen, among others, presented a synergistic interaction with *Alternaria cassiae*, *Colletotrichum coccodes*, *C. truncatum* and *Fusarium lateridium* (8), indicating that herbicides with different modes of action and chemical groups can interact synergistically with microorganisms to improve weed control. However, the knowledge of the type of interaction between the biological and chemical control agents does not imply that the physiologic and biochemical basis for these mechanisms are known.

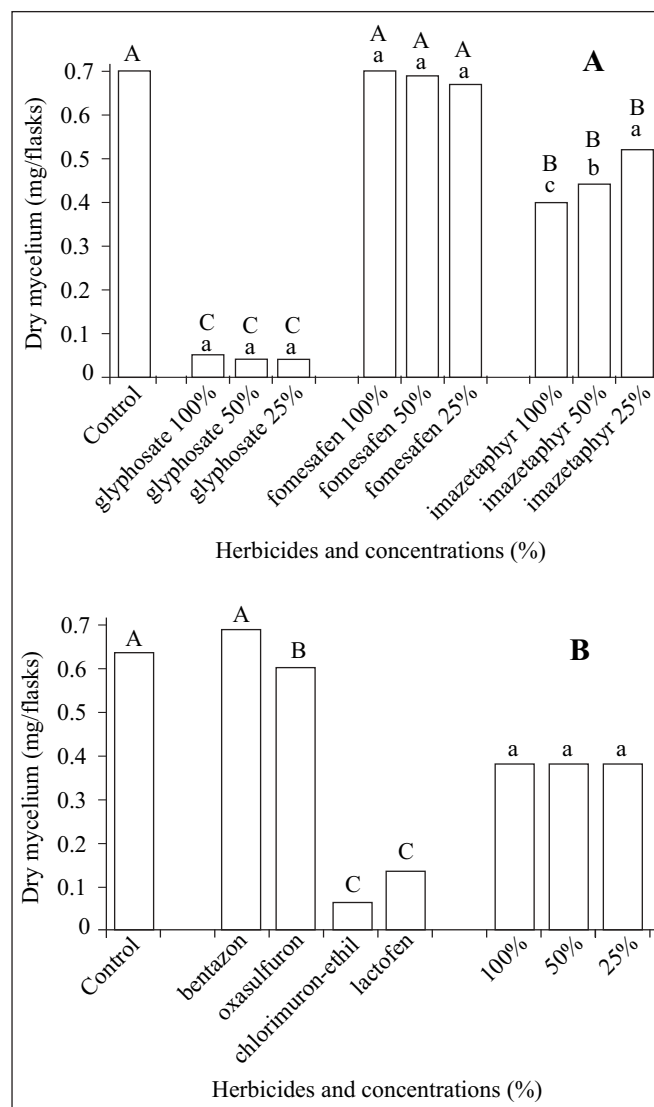


Figure 1 A, B. *B. euphorbiae* mycelium dry biomass from PD broth amended with herbicides. Average of 4 replications. Bars with the same letter do not differ by the Tukey Test. Small cap letters compare doses and capital letters the products.

The surfactants Energic, Herbitensil, Silwet and Aterbane suppressed *B. euphorbiae* mycelial growth (Fig. 2). An opposite effect was observed with Natur L'óleo, which promoted twice (91.3%) as much mycelial growth as the control. Steiner & Watson (11) stated that non-ionic surfactants generally are harmless to bacteria. Other surfactants can, depending on their concentration, completely inhibit mycelial growth of certain fungi. This difference can be explained by the chemical formula of the surfactant. According to these authors, surfactants containing more than 15 moles of ethylene oxide, for example, did not inhibit colony growth of *Curvularia*

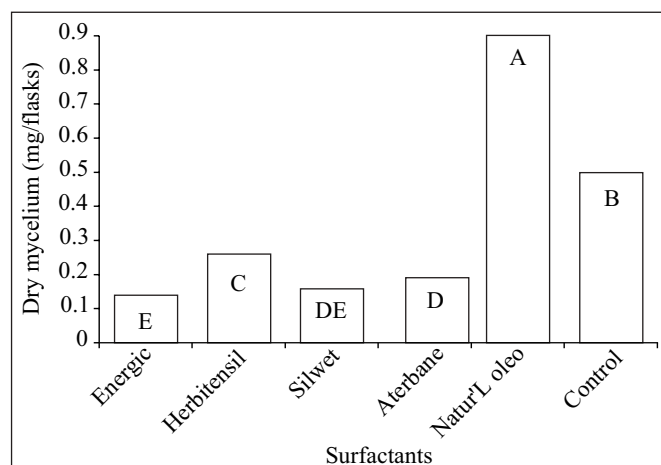


Figure 2. *B. euphorbiae* mycelium dry biomass from PD broth amended with surfactants. Average of 4 replications. Bars with the same letter do not differ by the Tukey Test.

pallescens and *Polyporus cinnabarinus* and, actually, stimulated growth (11).

Toffanelli (12) found that the surfactants Energic, Herbitensil, Triton X-100 and Silwet significantly inhibited *B. euphorbiae* radial growth. Herbitensil, at one half of the recommended dose, and Silwet, at the recommended dose, reduced the colony diameter by 78% on average.

Conidial germination

In general, most herbicides had an effect similar to the control (Fig. 3). Bentazon was the least inhibitory herbicide, allowing 50% of the conidia to germinate in 98 min. After 117 min incubation 50% of the conidia exposed to chlorimuron-ethyl, lactofen, oxasulfuron and the control (water) had germinated. This level of germination was reached on imazetaphyr treatment after 218 min and on glyphosate after 692 min incubation. Germination peaked after 205 min on chlorimuron-ethyl and lactofen at 87%; on oxasulfuron after 216 min at 85.8%; on bentazon after 113 min at 88.2%; on fomesafen after 285 min at 90.5%; on the control after 308 min at 93%; on imazetaphyr after 262 min at 85.9%. Glyphosate, at the end of the test (720 min) allowed 65% germination of *B. euphorbiae* conidia, thus, it can be considered, among the herbicides evaluated, the inhibitoriest to conidia germination. Imazetaphyr caused a delay in spore germination, which was observed only after 180 min incubation.

The germination of *B. euphorbiae* conidia in the presence of chemical herbicides generally is not affected (6). Tests demonstrated that spore germination, in Petri plates, in media amended with chlorimuron-ethyl (0.015 kg/ha), lactofen (0.15 kg/ha) and imazetaphyr (0.25 g/ha), reached 71%, 80% and 86%, respectively. Bentazon did not affect spore germination of *Colletotrichum coccodes*, biocontrol agent for *Abutilon*

theophrasti (veltleaf) (7). Chlamydospores of *Phytophthora palmivora* were not affected when applied in tank mixes with bromacil, diuron, glyphosate, paraquat or simazine (3). The triazines Goltrix and Igran induced diverse responses on several fungi (4). Goltrix stimulated spore germination of *Aspergillus fumigatus* and *Verticillium agaricinum* at all concentrations tested, while an opposite response was observed with *Fusarium oxysporum* and *Helminthosporium oryzae*. Igran was inhibitorier than goltrix to spore germination. All the concentrations tested affected negatively *H. oryzae* conidia germination (4). Spore germination of *Fusarium solani* f.sp. *cucurbitae* was significantly suppressed after incubation for 8 and 24 h in agar amended with different doses of trifluraline (14).

In general, most of the surfactants somehow affected *B. euphorbiae* conidia germination (Fig. 4). However, in all treatments, after 490 min incubation, maximum germination was observed (88.8%, on average). Spore germination on Silwet was similar to that of the control (water) during the course of the test (490 min), peaking at 84.6% after 218 min. Aterbane delayed the beginning of germination and peaked after 211 min at 85.9%. Although a similar trend was observed with Natur L'oleo with maximum germination of 82.9% after 241 min incubation, it was inferior to the previous treatments. Energic significantly retarded conidia germination. While 50% of the spores had germinated within 100 min of incubation with all previous surfactants, 256 min were required to reach this value under Energic, maximum spore germination was observed only at the end of the test. An even more drastic effect could be observed with Herbitensil.

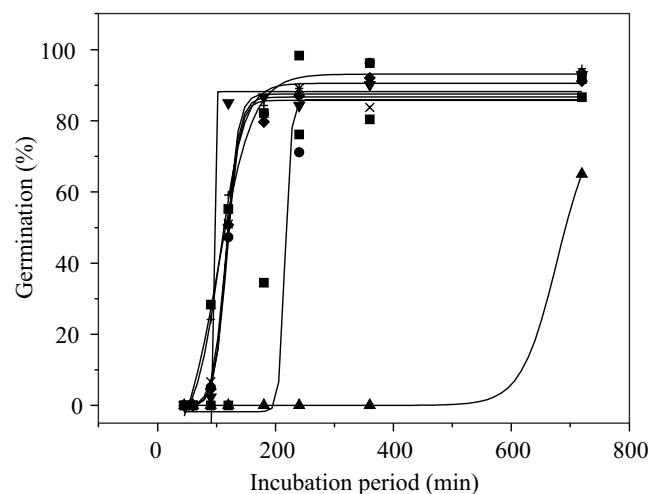


Figure 3. Germination of *B. euphorbiae* conidia on water-agar slides amended with herbicides. (■ water; ▼ bentazon; ■ imazetaphyr; ● oxasulfuron; ◆ fomesafen; + chlorimuron-ethyl; × lactofen, ▲ glyphosate).

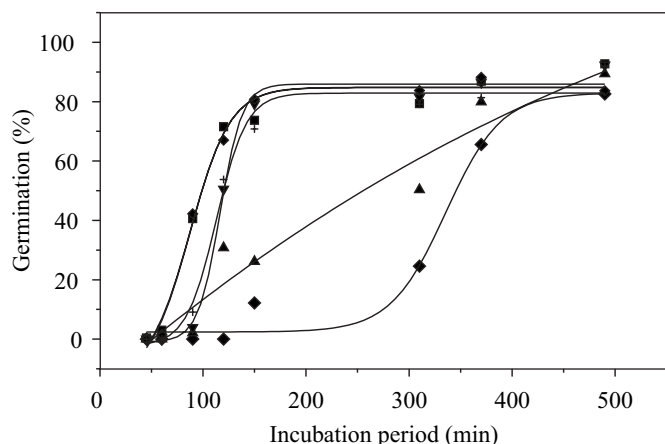


Figure 4. Germination of *B. euphorbiae* conidia on water-agar slides amended with surfactants. (■ Water; ▲ Energic; ◆ Herbitensil; ▼ Aterbane; ◆ Silwet; + Natur L'oleo).

Conidia started germinating after 120 min incubation and only reached 50% germination after 346 min, peaking at the end of the test.

Similar results of maximum germination after 12 h incubation were found by Toffanelli (12). The surfactants Energic, Herbitensil, Silwet and Triton X-100 did not affect *B. euphorbiae* germination.

Walker and Riley (13) mentioned that some surfactants could inhibit mycelial growth or spore germination of fungi. *Alternaria cassiae* conidia did not germinate normally in the presence of Tween-20 or Tween-80; however, non-ionic surfactants (nonoxynol) did not affect germination, and promoted a better leaf coverage, resulting in better control of *Senna obtusifolia* (sicklepod) (13). Mixtures of conidia with Natur L'óleo at 1.5% reduced by 90% *B. euphorbiae* spore germination in 3 hours, while Energic at 0.2% reduced 1% of the spore germination (5).

RESUMO

Germinação de conídios e crescimento micelial de *Bipolaris euphorbiae* Muchovej & Carvalho influenciados por herbicidas e surfatantes.

Bipolaris euphorbiae Muchovej & Carvalho é um forte candidato para o controle de *Euphorbia heterophylla* L. (amendoim bravo). Este fungo pode ser aplicado em combinação com herbicidas para controlar um maior espectro de espécies daninhas. Para tanto, experimentos laboratoriais foram realizados para verificar a possibilidade da utilização de mistura de tanque de esporos de *B. euphorbiae* e herbicidas ou surfatantes recomendados para a cultura da soja. Crescimento micelial e germinação de conídios foram avaliados em meio BDA acrescido

dos herbicidas, nas concentrações recomendadas dos produtos comerciais, oxasulfuron (80 g/ha), glifosato (4 L/ha), bentazon (1.5 L/ha), fomesafen (1 L/ha), chlorimuron-ethyl (80 g/ha), lactofen (1 L/ha) e imazetaphyr (1 L/ha) e dos surfatantes Energic (2 ml/L), Aterbane (2,5 ml/L), Silwet L-77Ag (1 ml/L), Herbitensil (2 ml/L) e Natur L'óleo (10 ml/L). Diluições dos herbicidas de 50% e 25% foram avaliadas com um consumo de calda equivalente a 300 L/ha. Os surfatantes foram somente utilizados nas concentrações recomendadas. O crescimento micelial não foi afetado por bentazon e fomesafen e apenas levemente por oxasulfuron. Porém, glifosato, chlorimuron-ethyl, lactofen, Energic, Herbitensil, Silwet, e Aterbane o reduziram drasticamente. A redução observada com imazetaphyr foi intermediária e Natur L'óleo promoveu o crescimento micelial. Na presença dos surfatantes, observou-se que todos permitiram uma porcentagem de germinação equivalente àquela alcançada na presença de água. Energic e Herbitensil causaram um retardamento expressivo. Com Herbitensil, o processo germinativo iniciou somente aos 120 minutos. Com herbicidas, foi observado que somente na presença de glifosato e imazetaphyr a germinação dos conídios não seguiu a tendência observada com água, como ocorreu com os outros produtos testados.

Palavras-chave: *Bipolaris euphorbiae*, crescimento micelial, germinação de esporos, herbicidas, surfatantes.

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