Phytotoxic metabolites produced by *Fusarium nygamai* from *Striga hermonthica*

M.C. ZONNO1, M. VURRO1, R. CAPASSO2, A. EVIDENTE2, A. CUTIGNANO2, J. SAUERBORN3 and H. THOMAS3

1 Istituto Tossine e Micotossine da Parasiti Vegetali, CNR, Bari, Italy
2 Dipartimento di Scienze Chimico-agrarie, Università di Napoli “Federico II”, Portici, Italy
3 University of Giessen, Tropical Crop Science, Giessen, Germany

Abstract. Fusaric and 9,10-dehydrofusaric acids and their corresponding methyl esters were shown to be the main phytotoxins produced by *Fusarium nygamai*, a potential control agent of *Striga hermonthica*. The phytotoxic properties of these metabolites were investigated using biological assays on striga plants, seedlings, leaves and seeds in order to test their possible use as natural herbicides. The application of very low amounts of toxins (10⁻⁶ M) caused a dramatic reduction of seed germination. The toxic effects of these metabolites also proved to be clearly expressed on punctured leaves, on which the toxins caused the appearance of large necrotic spots. The use of these metabolites against striga, possibly in combination with other cultural and biological methods, could be of assistance in control of this noxious weed.

Introduction

*Striga hermonthica* (Del.) Benth. (Scrophulariaceae) is a parasitic weed of cereal crops, mainly of: sorghum, *Sorghum bicolor* (L.) Moench.; millet, *Pennisetum glaucum* (L.) R.Br.; maize, *Zea mays* L.; and rice, *Oryza sativa* L., in semi-arid areas of Africa. The losses of grain-sorghum yields due to striga infestation may reach up to 70% (Basinski 1955; Last 1961; Doggett 1970; Bebawi and Farah 1981), and in case of severe infestation there may be no yield (Andrews 1945).

Striga is still very difficult to control, even using the control measures that have been proposed, including the use of herbicides and fertilizer, cultural methods and resistant crop varieties (Eplee et al. 1991; Awad et al. 1991; Olivier et al. 1992). Consequently, biological control is becoming important. Recently, Cotela et al. (1995) proposed the use of *Fusarium oxysporum* for striga biocountrol, and Abbasher and Sauerborn (1995) reported a survey of microorganisms pathogenic to *S. hermonthica* that are potentially useful for the biological control of this noxious weed. Among these, a strain of *Fusarium nygamai* Burgess and Trimbeli proved to be particularly promising (Abbasher and Sauerborn 1992) and causes large leaf- and stem-necrosis on the host, as well as inhibition of seed germination, without affecting sorghum plants.

Members of the genus *Fusarium* produce a range of phytotoxic compounds, such as fumonisin, enniatin, moniliformin and fusaric acid, that are chemically different and possess a broad range of biological activities and metabolic effects, e.g. growth inhibition, necrosis, chlorosis, wilting, inhibition of seed germination, effects on calli (Wakulinski 1989; Van Asch et al. 1990). Considering that phytotoxins could be used as bioherbicides, as alternatives to or in addition to the use of plant pathogens (Strobel et al. 1991), and considering the symptoms of damage caused by *F. nygamai*, it seemed to be of interest to investigate the production of toxins by a strain of *F. nygamai*, and to examine their biological effects on striga seeds and plants. Four phytotoxic compounds from *F. nygamai* were identified (Capasso et al. 1996). In this paper we describe the first results from using the main phytotoxins produced by *F. nygamai*, fusaric acid (FA) and 9,10-dehydrofusaric acid (DA) and their respective methyl esters (MFA and MDA) (Fig. 1), as natural herbicides for striga control.

Methods

Fungus

A strain of *Fusarium nygamai*, isolated from diseased *S. hermonthica* plants, harvested in Sudan in 1989, was used for the production of toxic metabolites and deposited in the fungal collection of the Istituto Tossine
Fig. 1. The structure of fusaric acid and of 9,10-dehydrofusaric acid and their respective methyl esters.

e Micottosine da Parassiti Vegetali, CNR, Bari, Italy (ITEM 2141).

Phytotoxin production, extraction and purification

Single-spore cultures of *F. nygamai* were maintained on potato-dextrose-agar medium, with subculturing at monthly intervals. For the production of toxic metabolites, 11 Erlenmeyer flasks (containing 200 ml of M-1D medium) were inoculated with 1 ml of an abundant conidial suspension. The flasks were incubated at 25°C, in static conditions, for four weeks.

The culture filtrate was exhaustively extracted with ethyl acetate at pH 2. The crude extract (7.67 g), which showed high phytotoxicity, was fractionated on a silica-gel column at medium pressure in 18 fraction groups. The residue of the fractions 3-4 and the most polar 17-18 showed considerable phytotoxic activity. TLC analysis of the latter showed two main metabolites, one having Rf identical to FA (also demonstrated by co-chromatography) and the other having similar properties. Further purification of this residue under low pressure on reverse phase column allowed us to obtain FA and DA (12.2 and 121.6 mg/l, respectively). The purification by semipreparative TLC steps of the less polar active fraction groups (3-4) yielded pure MFA and MDA (1.5 and 2.1 mg/ml).

The phytotoxic activity of the liquid culture filtrates and of chromatographic fractions was tested using a leaf-puncture assay on tomato. All the experimental data are described in detail by Capasso *et al.* (1996).

Mycoxins production, extraction and analysis

The ability of *F. nygamai* to produce mycoxins was determined by cultures on corn kernels. The experimental procedure for mycoxin production, extraction and analysis are described by Capasso *et al.* (1996).

Leaf-puncture assay

A puncture assay on *S. hermonthica* leaves was used to test the phytotoxic activity of pure compounds. The toxins were first dissolved in a small amount of methanol (0.4 μl) and then brought up to the final concentration of 8 μg/droplet with distilled water. Detached and fully-expanded young leaves (about 10 cm) were used, and 10 μl of the test solution was applied to previously needle-punctured sites on the leaves. The leaves were kept in a moist chamber to prevent the droplets from drying. The effects were observed two days after droplet application. Each experiment was repeated twice with at least four replications.

Seedling assay

FA, DA, MFA, MDA were assayed on *S. hermonthica* seedlings. The toxins were dissolved in a small amount of methanol and then brought up to the final concentration with distilled water. Each toxin was assayed at five different concentrations (10⁻⁴, 5 x 10⁻³, 10⁻⁵, 5 x 10⁻⁶, 10⁻⁶ M) and each experiment was repeated twice with two replications. Young plants (4-6 cm high) obtained in the greenhouse were cut and their stems were immersed in the toxin solutions for 48 h. They were then transferred to distilled water, and the symptoms were observed after a further 48 h.

Seed-germination assay

The following assay was performed to evaluate the effect of *F. nygamai* toxins on striga seeds. *Striga hermonthica* seeds were sterilized (5 min.) with sodium hypochloride (1%) and pre-conditioned for eight days in Petri plates on filter paper wetted with tap water, at 30°C in the dark. Four small pieces of filter paper, each containing at least 100 seeds were then transferred to a Petri plate, on larger filter paper, wetted with the test solutions (2 ml). The seeds were incubated for one day and the percentage germination was determined. The toxins were dissolved in a small amount of methanol (1% of final volume) and then brought up to the final concentration with the seed germination solution. Each toxin was assayed at five different concentrations (as above), and each experiment was repeated three times.

Seed-germination solution

Sorghum seeds were kept in the greenhouse on a thin layer of sand to germinate. After germination, the
sand was removed, and 25 seedlings were put in a 1 l Erlenmeyer flask, keeping the roots immersed in tap water at 25-35° C for three days. The seedlings were then removed and the solution was used to stimulate the germination of pre-conditioned striga seeds.

**Spray assay**

Solutions of toxins (10⁻⁴ M) were prepared by dissolving the toxin in MeOH (0.5 % of final volume) and then in distilled water to the final concentration. The solutions were sprayed, until run-off, onto young striga plants (4-5 cm high) which were kept in pots as previously described (Abbashe and Sauerborn 1992). Before spraying, small lesions were made on the leaves with a blade. The plants were then kept in a greenhouse at 25° C for one week, at high humidity, and observed daily for symptoms.

**Results**

**Production of toxins**

The main phytotoxins produced in vitro by *F. nygamae*, and characterized by chemical and spectroscopic methods, were identified as fusaric acid (FA), 9,10-dehydrofusaric acid (DA) and, in a lesser amount, their corresponding methyl esters (MFA and MDA) (Fig. 1) (Capasso et al. 1996). Strain ITEM 2141 was also assayed for its ability to produce other well known *F. nygamae* toxins, but it seemed unable to biosynthesize moniliformin, beauvericin, enniatin B, nivalenol, deoxynivalenol, 3-acetiledeoxynivalenol, T-2 and HT-2 toxins, neosolvinol, fusarone X, zearalenone and zearalenol, but it produced a very low amount of fumonisins B₁.

**Seed-germination assay.**

Both FA and DA, as well as their methyl esters were strongly active in this assay (Table 1). At higher concentrations (10⁻³ and 5x10⁻⁵ M) MDA and MFA caused total inhibition of seed germination (0% germination compared to 44% for the control). At lower concentrations (10⁻⁶ M) all the toxins were still active, reducing striga seed-germination by between 75% and 85%.

**Leaf-puncture assay.**

The application of droplets of toxin solutions caused the appearance, after one day, of necrotic spots, with a diameter of 5-7 mm. There were no clear differences between the effects of the different toxins.

**Seedling assay**

The immersion of young plants in the toxic solutions caused necrotic symptoms, mainly on the edges of the leaves, in the case of high concentration (10⁻⁴ M). At 5x10⁻⁵ M, only FA and MFA were shown to be slightly toxic, with symptoms that were similar to those caused by the fungus under natural- or artificial-infection conditions.

**Spray assay**

After four days on the sprayed plants it was possible to observe the appearance of necrotic symptoms similar to those obtained in the seedling assay, but these were less evident and more delayed. Also in this assay, FA and MFA were slightly more toxic than DA and MDA.

**Discussion**

The main toxins produced by *F. nygamae* (FA, DA, MFA and MDA) showed strong phytotoxic effects when assayed on leaves, seeds and seedlings of striga plants. The effects of these metabolites on germination of pre-conditioned striga seeds were particularly interesting, since the application of very low amounts of toxins (10⁻⁶ M) caused a dramatic reduction in seed germination. These results seem to be very promising from a practical point of view, considering that the production of large quantities of seeds in the field and the difficulty of observing seed germination are among the worst problems for striga control. Studies are in progress to investigate the possible pre-emergence application of these toxins to reduce germination in the field. Considering that at 10⁻⁴ M, FA and DA are not toxic to arthropods (Capasso 1996), they could be used without environmental risks.

The toxic effects of these metabolites were also clearly expressed on punctured leaves. The appearance of large necrotic spots on host leaves suggests an investigation should be undertaken on the possible practical use of these metabolites in post-emergence application. Moreover, the synthesis of derivatives may make it possible to obtain compounds that would be more active and, or, safer for the environment.

The use of these metabolites against striga, possibly in combination with other cultural and biological methods, could be of assistance in control of this noxious weed.
Table 1. The percentage germination of *Striga hermonthica* seeds that have been treated with toxins of *Fusarium nygamai*. FA-fusaric acid, DA - 9,10 dehydrofusaric acid and their respective methyl esters (MFA and MDA)

<table>
<thead>
<tr>
<th>Toxin</th>
<th>$10^{-4}$</th>
<th>$5 \times 10^{-5}$</th>
<th>$10^{-5}$</th>
<th>$5 \times 10^{-6}$</th>
<th>$10^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>6.2</td>
<td>3.0</td>
<td>8.8</td>
<td>7.3</td>
<td>6.8</td>
</tr>
<tr>
<td>DA</td>
<td>5.2</td>
<td>10.0</td>
<td>11.1</td>
<td>8.2</td>
<td>10.4</td>
</tr>
<tr>
<td>MFA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
<td>2.0</td>
<td>9.7</td>
</tr>
<tr>
<td>MDA</td>
<td>0.0</td>
<td>0.0</td>
<td>6.6</td>
<td>11.3</td>
<td>11.2</td>
</tr>
<tr>
<td>Untreated</td>
<td>44.7</td>
<td></td>
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References


