Degradation and Transformation of a Potential Natural Herbicide in Three Soils

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The methyl ester of fusaric acid (ME) is one of four toxins produced by the fungus *Fusarium nygamai*, which could be used as a natural herbicide against *Striga hermonthica*, a parasitic weed of sorghum and corn in a vast zone of West and Central Africa. A laboratory study was performed to measure the degradation of ME in three soil types and under different temperature and soil moisture conditions, so as to ascertain whether a single ME treatment would protect the crops against this weed during the critical phases of growth. The results show that the persistence in all soils and under all incubation conditions is long enough to protect the crops for the first week of growth, excluding the trial at 30 °C in the humic soil, where the half-life of 6 days would require more than one treatment. A degradation product of ME (butylpyridine, BP) was identified by gas chromatography/mass spectrometry and its degradation measured. The sum of ME and BP residues for the first 7 days was almost 100% of the applied compound in all soils and incubation conditions, thus indicating that BP may be the only transformation product of ME at this stage.

Keywords: *Fusaric acid; phytotoxins; natural herbicides; soil degradation*

INTRODUCTION

According to Watson (1989), bioherbicides are living entities (natural enemies) used deliberately to suppress the growth or reduce the population of a weed species. They act directly or indirectly through the production of toxins, which are termed natural herbicides and should not be included in the definition of a bioherbicide.

In the 1980s, mycoherbicides appeared with the release of several commercial products (e.g., Devine, Collego, Biomal). However, currently none of these products are commercially available for a number of reasons such as lack of marketability and sufficient research (Auld and Morin, 1995). In recent years the use of toxins produced by pathogens as natural herbicides has received increasing attention (Stierle et al., 1988; Cutler et al., 1988). These natural products are characterized by a high specific activity and high selectivity, and they are biodegradable. Their structures are extremely diverse and comprise many classes of compounds. The advantages of using natural herbicides rather than bioherbicides are evident: there is no diffusion of living organisms in the environment, thus avoiding any possible colonization of new plants, and there is no need to keep live microorganisms for application or storage. Furthermore, because a number of these toxins can be synthesized, harvesting microorganisms for their production would no longer be necessary.

The methyl ester of fusaric acid (ME) is one of four phytotoxins produced by *Fusarium nygamai*, a parasitic fungus of *Striga hermonthica* (Capasso et al., 1996), commonly called witch-weed, which causes severe losses in many staple cereal crops, that is, sorghum and corn, especially in West and Central Africa. The agronomical and chemical control of *Striga* spp. have not to date offered satisfactory results (Berner et al., 1995; Ransom, 1996; Kunjo, 1996). The direct use of fungus as a bioherbicide (Abbasher and Sauerborn, 1992) has its drawbacks as indicated above. The phytotoxicity of the four toxins produced by *F. nygamai* has been verified (Zonno et al., 1996), and the use of these toxins as natural herbicides could help in solving the problems related to *Striga* control. One of the disadvantages of using natural herbicides is their rapid dissipation in the environment, so that treatment would probably need to be repeated more than once. The present study is aimed at determining the persistence and degradation pathway of ME in three soil types and under different temperature and soil moisture conditions to ascertain whether a single treatment can protect the crops during the critical phases of growth. ME was chosen because it has proved to be more toxic against *Striga* seeds than the other three, even at low concentrations (Zonno et al., 1996).

MATERIALS AND METHODS

Soils and Analytical Standards. The three soils used were collected from the 0−30 cm layer of three different sites of South and Central Italy: a clay loam from Papiano, Perugia (CL); a sandy loam from Castiglione del Lago, Perugia (SL); and a humic sandy loam from Castellammare di Stabia, Napoli (H-SL); their properties are reported in Table 1; analyses were performed according to ASA−SSSA methods (Page, 1982; Klute, 1986). The analytical standard of ME was prepared by methylation of fusaric acid (produced by Sigma-Aldrich from *Gibberella fujikuroi*) according to the method of Vischetti et al. (1997). ME was identified by comparing the gas chromatography/mass spectrometry (GC/MS) spectrum of the methylated product with the ME spectrum of the instrument library (1992 NIST Library, Varian Associates Inc.). The analytical standard of butylpyridine (BP), 98% purity, was supplied by Aldrich Chemical Co., Milwaukee, WI.

Soil Sample Preparation, Contamination, and Recoveries. Triplicate soil samples (50 g) for each soil type, air-dried

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Table 1. Main Properties of the Three Soils

	PA	CM	CA
sand (%)	24.8	55.6	57.2
silt (%)	44.8	36.5	25.7
clay (%)	30.4	7.9	17.1
texture[a]	CL	H-SL	SL
pH_{H_2O}	8.1	7.5	8.2
organic matter (%)	1.6	6.0	1.3
CEC (mequiv·100 g^{-1})	15.5	24.5	8

[a] CL, clay loam; H-SL, humic sandy loam; SL, sandy loam; PA, Papiano; CM, Castellammare di Stabia; CA, Castiglione del Lago.

and 2-mm-sieved, were each contaminated with 1 mL of a methanolic solution containing 40 μg of ME, to obtain a concentration of 0.8 mg kg^{-1}. The samples were immediately extracted with 100 mL of a methanol/water solution (1:1), shaken in a mechanic shaker for 1 h, and centrifuged for 15 min at 6000 rpm. The extraction was repeated again. The extracts were filtered through an extra-rapid filter paper, partitioned twice against chloroform (40 mL × 2), evaporated to dryness, rinsed with 1 mL of methanol, and then analyzed. The procedure was repeated by doubling the concentration to 1.6 mg kg^{-1}. The entire experiment was repeated at the same concentrations to evaluate BP recoveries.

Degradation Studies. For each of the three soil types a 300 g portion of finely powdered soil sample was placed in a 1 L flask and 300 mL of acetone solution containing 12 mg of ME was added. After complete evaporation of acetone, 20 g of the powdered soil were used to fortify each of four 1 kg samples of 2-mm-sieved air-dried soil to obtain a final concentration of 0.8 mg kg^{-1}. Each of the containers was covered with perforated aluminum foil and incubated in the dark at different temperatures and soil moisture conditions [20 °C and 75% field capacity (fc); 20 °C and 33% fc; 10 °C and 75% fc, 30 °C and 75% fc]. Soil moisture contents were kept constant by daily additions of water. At different times after treatment, triplicate 50 g samples from each container were extracted and analyzed.

Analyses. The analytical apparatus consisted of a Fisons-Carlo Erba MFC 800 gas chromatograph, equipped with an NPD detector and a Mega SE 54 capillary column (0.25 mm i.d. × 25 m length). The column temperature was kept at 50 °C for 1 min and then raised at 45 °C min^{-1} to 260 °C; cleanup was at 270 °C for 5 min; carrier gas flow (helium) was 1.5 mL min^{-1}; and the on-column injector was set at 50 °C. Under these conditions retention time was 5.7 min for BP and 9.3 min for ME. The sensitivity of the method was 5 μg kg^{-1} for BP and 20 μg kg^{-1} for ME. The GC/MS analyses were performed with a Varian Star 3400 gas chromatograph equipped with an OV17 3% capillary column (0.25 mm i.d. × 25 m length) and a Varian Saturn II mass spectrometer, electron impact ionization at 70 eV. Injector temperature was 150 °C; column temperature was 50 °C isothermal for 1 min and then raised at 30 °C min^{-1} to 230 °C; cleanup was at 230 °C for 15 min; and carrier gas flow (helium) was 2 mL min^{-1}. Mass spectrometer conditions were as follows: transfer line, 260 °C; manifold, 270 °C; automatic gain control, on; electric current of emission, 10 mA. Under these conditions the retention time was 4.1 min for BP and 5.5 min for ME.

RESULTS AND DISCUSSION

The structures of the four toxins produced by *F. nygamai* are given in Figure 1. Figures 2 and 3 show the mass spectra of ME prepared in the laboratory and of a compound found in all treated soil samples. The identity of the two compounds was determined by comparing the mass spectra with those of the instrument library. The unknown compound was identified as BP.

In Table 2 the recoveries of BP and ME from the three soils are reported. They can be considered satisfactory,

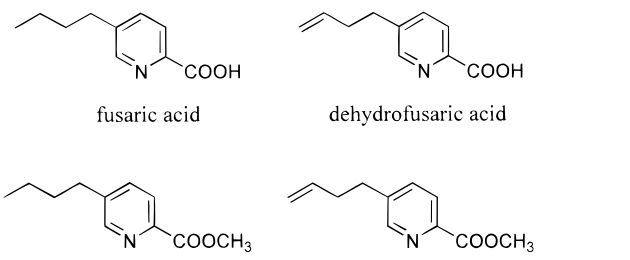

Figure 1. Structures of the four toxins produced by *F. nygamai*.

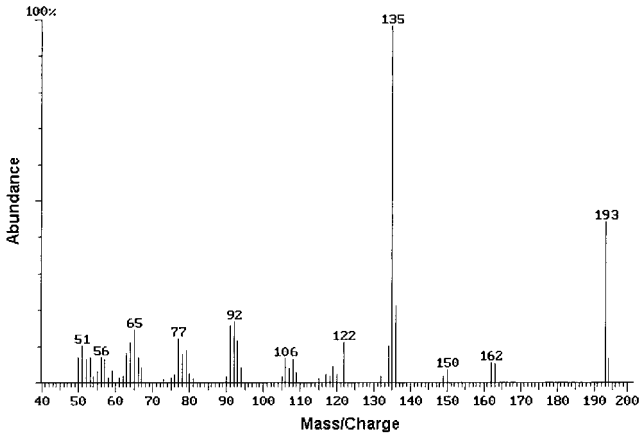

Figure 2. Mass spectrum of ME prepared in the laboratory.

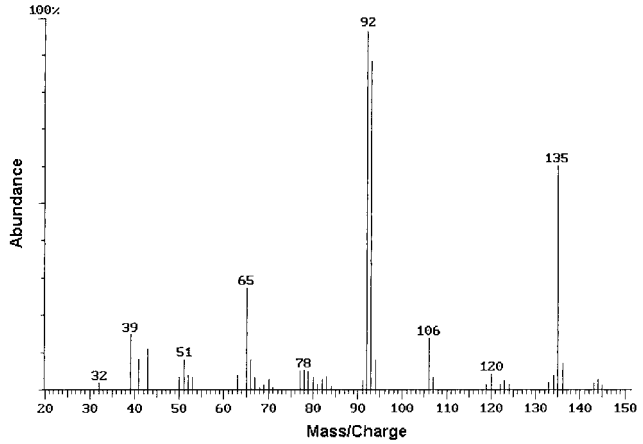

Figure 3. Mass spectrum of unknown compound found in all soil types.

varying from 83.4% for ME in the humic soil (H-SL) to 95.6% for ME in the sandy loam soil (SL).

The degradation kinetics for ME in the three soils under study are reported in Figure 4, whereas Table 3 shows the half-life values obtained when first-order kinetics is applied to degradation data ($p \leq 0.01$ for all cases) and the parameters for degradation dependence on temperature (E_a and Q_{10}) and soil moisture (B).

The effect of temperature on degradation was expressed using the Arrhenius equation

$$\log(H_1/H_2) = (E_a/19.145)(1/T_1 - 1/T_2)$$

where E_a is the activation energy (J mol^{-1}), H_1 and H_2 are the half-lives (days) at temperatures T_1 and T_2 (K), and 19.145 is a constant equal to R/(log e), where R is the universal gas constant (J K^{-1} mol^{-1}).

Table 2. Recoveries (Percent) of BP and ME from the Three Soils

	SL[a]		H-SL		CL	
concn (mg kg^{-1})	BP	ME	BP	ME	BP	ME
0.8	95.1 ± 2.0	94.6 ± 2.9	84.8 ± 2.3	83.4 ± 1.9	89.4 ± 5.7	89.4 ± 4.9
1.6	94.3 ± 4.1	95.6 ± 3.4	88.0 ± 5.3	87.8 ± 4.0	94.9 ± 3.2	89.4 ± 2.0

[a] CL, clay loam; H-SL, humic sandy loam; SL, sandy loam.

Table 3. Half-Life Values ($t_{1/2}$) and Parameters for Degradation Dependence on Temperature (E_a, Q_{10}) and Soil Moisture (B) in the Three Soils

conditions	SL[a]	H-SL	CL
	$t_{1/2}$ (Days)		
30 °C/75% fc	16.3	6.2	11.1
20 °C-75% fc	24.1	15.2	21.2
20 °C/33% fc	35.0	19.1	26.6
10 °C/75% fc	41.0	44.4	44.7
	Parameters		
E_a (J mol^{-1})	32500	68200	48300
Q_{10}	1.59	2.68	2.00
B	0.538	0.329	0.327

[a] CL, clay loam; H-SL, humic sandy loam; SL, sandy loam.

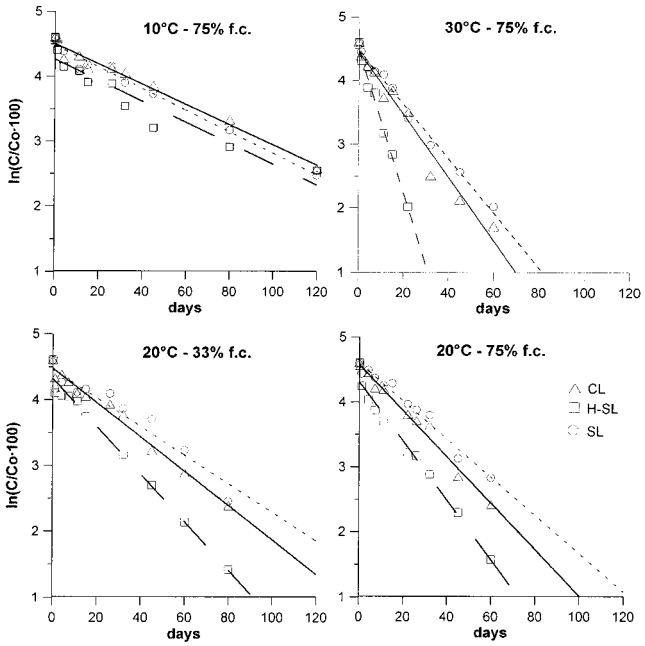

Figure 4. Degradation kinetics for the three soils under different incubation conditions. CL, clay loam; H-SL, humic sandy loam; SL, sandy loam.

The effect of temperature on degradation was also evaluated using a Q_{10} factor calculated as the ratio between the k rates at the two temperatures T_1 and T_2, which differ by 10 °C.

The effects of moisture were evaluated using an empirical equation developed by Walker (1978)

$$H = AM^{-B}$$

where H is the half-life (days) at soil moisture M (%, w/w), A is the half-life (days) at zero moisture content, and B is the sensitivity of herbicide degradation to soil water content. As can be seen in Figure 4, degradation was faster in the humic soil than in the other two soils, suggesting a direct effect of organic matter on degradation. The effect was weaker at 10 °C when the microbial activity was probably reduced somewhat; this reduction partially eliminated the difference in ME degradation

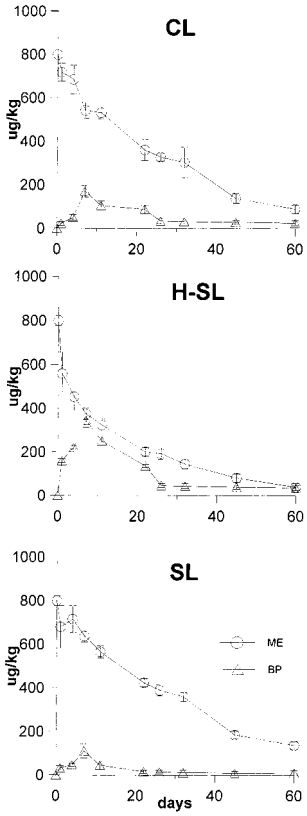

Figure 5. Formation and disappearance of BP compared to ME. CL, clay loam; H-SL, humic sandy loam; SL, sandy loam.

in the three soils and increased half-life values. Table 3 reports the mean of two values for Q_{10} and E_a, calculated at two temperature ranges (10−20 and 20−30 °C) and the B value, calculated for one soil moisture range (33−75% fc). The mean value of E_a shows a considerable effect of temperature on ME degradation compared to traditional herbicides in the humic soil (68200 J mol^{-1}) and a very slight effect in the sandy loam soil (32500 J mol^{-1}). Indeed, Boesten (1986) found a mean E_a of 55000 J mol^{-1} for 54 available measurements. Q_{10} values confirm this finding in that in the humic soil it was higher and in the sandy loam soil lower than the 2.2 found by Walker et al. (1996) as the mean Q_{10} value calculated for 140 pesticides.

To assess the influence of soil moisture on degradation, Ferris and Haigh (1992) have reviewed B values for a range of herbicides and reported a mean of 0.8 and a standard deviation of 0.53. They found that the relationship between soil water content and herbicide degradation was significant, with herbicide half-life increasing by 80% for any 50% decrease in soil water content. In the present experiment, the influence of soil moisture on degradation seemed very poor because the increase in half-life ranged from 25.5 to 45.8% (depending on soil type) for a 50% decrease in soil moisture.

The disappearance of ME from the soil coincided with the appearance of BP as early as the first few days of incubation. Figure 5 shows the trend of formation and

disappearance of BP in the three soils at 20 °C and 75% fc. The curves at other incubation conditions are not shown as they followed the same pattern. There was a typical trend for a pesticide metabolite in soil shown by the rise at the curve until it peaks at day 7 after treatment followed by a gradual decrease until day 60 after treatment. Our interest in tracing the fate of BP lay not only in its potential danger to the environment but also in determining whether fusaric acid is formed by ME in the soil, at least for the first 7 days after treatment. In fact, the sum of ME and BP residues up to day 7 after treatment was almost 100% of the ME applied, thus indicating that BP might be the sole transformation product of ME at this stage. The transformation of micrograms per kilogram of BP to micrograms per kilogram of ME was calculated by multiplying the BP concentration by the ratio between the molecular weights of ME and BP (1.43). Demethylation of the esteric group immediately followed by decarboxylation of the carboxylic group can be supposed as the reaction mechanism; in these conditions fusaric acid rapidly disappeared and could not be detected. Owing to BP degradation the sum of ME and BP residues was no longer 100% from day 7 on.

In conclusion, the persistence of ME in the three soils studied proved to be long enough to enable a single treatment during the critical phases of crop growth. The formation of fusaric acid did not occur during the first week following treatment; therefore, BP was the only transformation product found in the soil.

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