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ORGANIC ACIDS FROM THE ANAEROBIC DECOMPOSITION OF *AGROPYRON REPENS* RHIZOMES

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Abstract—Phytotoxicity originating from the anaerobic decomposition of couch grass rhizomes has been studied. Short chain aliphatic (acetic, propionic and butyric) acids appear to be mainly responsible but hexanoic, succinic, phenylacetic, cinnamic, *p*-coumaric, 4-hydroxyphenylpropionic and 3,4-dihydroxyphenylpropionic acids are also present in the phytotoxic solutions formed during the decomposition.

INTRODUCTION

After couch (quack) grass (*Agropyron repens*) has been treated with herbicide, poor plant establishment can sometimes result when a crop is drilled into the decomposing mats of rhizomes. This effect seems to result not from the presence of residual herbicide or nutrient deficiency, but from enzymic decay or microbial growth on the rhizomes leading to the production of phytotoxins or parasitic invasion (Lynch, J. M. and Penn, D. J., unpublished results). We now report measurements of the production of free organic acids during the decay of rhizomes. The possibility that combined acids might be released on hydrolysis has not been investigated as these would be less likely to cause phytotoxic effects.

RESULTS AND DISCUSSION

Analysis of the organic acids produced in solution during the anaerobic decay of rhizomes is shown in Table 1. The concentrations of total steam-volatile acids assessed by GLC and steam distillation were identical.

Table 1. Identification of organic acids from the anaerobic decomposition of couch grass

Acid	Relative retention time*	Concentration in solution (μM)	Characteristic *m/e* (relative intensity)
Steam-volatile fatty acids			
Acetic	1.000	16400	
Propionic	1.615	800	
Butyric	2.730	6700	
TMSi derivatives			
Hexanoic	0.490	9	M−15 173(56), 132(9), 131(8), 117(23), 75(99), 74(9), 73(100), 55(6), 45(8)
Phenylacetic	0.796	27	M−15 193(11), 165(4), 164(12), 117(4), 91(13), 75(38), 74(10), 73(100), 65(7), 45(8)
Succinic	0.824	10	M−15 247(18), 149(9), 148(15), 147(100), 75(39), 73(85), 55(11), 45(12)
Cinnamic	1.027	9†	M^+ 220(24), 206(21), 205(100), 161(55), 131(79), 103(48), 102(15), 77(36), 75(48), 73(24)
p-Hydroxyphenylpropionic	1.192	5	M^+ 310(26), 295(8), 193(15), 192(61), 180(17), 179(100), 147(16), 93(13), 75(52), 73(95)
p-Coumaric	1.309	30	M^+ 309(19), 308(69), 294(21), 293(82), 249(37), 220(15), 219(81), 179(14), 75(28), 73(100)
3,4-Dihydroxyphenyl-propionic	1.317	21	M−15 383(17), 281(10), 280(25), 268(17), 267(74), 180(21), 179(100), 147(8), 75(25), 73(90)

* Relative to malic acid internal standard for TMSi derivatives.

† Identified by 'spiking' peak on two stationary phases.

The germination of barley (*Hordeum vulgare* cv Proctor) seeds was only 22% in the presence of these solutions whereas all seeds germinated under control conditions. The root extension of the treated seedlings was reduced by 96%. When the rhizomes decomposed aerobically none of the acids accumulated, germination was not significantly affected and the 20% reduction in root extension was only just significant ($P = 0.05$).

The response of barley seeds and seedlings to acetic, propionic and butyric acids increases with carbon chain length and the toxicities of succinic and aromatic acids are in the range between that of propionic and butyric acids. In unbuffered solutions at a concentration of 5 mM, acetic, propionic and butyric acids inhibited root extension by 25, 50 and 71% respectively; all of the acids detected here only become toxic in the concentration range 1–5 mM (Lynch, J. M., unpublished results). Thus in our experiments acetic and butyric acids must have been mainly responsible for the observed phytotoxicity.

The steam volatile fatty acids probably originate from the fermentation of the cellulosic components of the plant tissue [1]. The phenolic components could originate from the breakdown of core lignin, but this is unlikely as such lignin is usually only broken down in the latter stages of decomposition. The presence of 4-hydroxy- and 3,4-dihydroxyphenylpropionic acids is noteworthy because only 2-hydroxy- and 2,3-dihydroxyphenylpropionic acids have been previously identified as products of soil micro-organisms [2, 3]. It is proposed that soil micro-organisms are capable of reducing the side chains of *p*-coumaric and ferulic (or caffeic) acid by mechanisms similar to those shown by bacteria of the human gastrointestinal tract [4]. The cinnamic acids have been shown to be present in most grasses, as esters linked to the lignin [5], and are easily liberated by mild hydrolysis.

EXPERIMENTAL

Decomposition of rhizomes. Freshly harvested couch (quack) grass rhizomes (25 g) and a Denchworth series clay soil (5 g) were added to distilled water (150 ml) contained in a 250 ml flask which was then sealed with a rubber bung. After 7 days at 25° the suspension was filtered and analysed immediately.

Assay of toxicity and analysis of steam volatile fatty acids. Toxicity of the extracts to germinating barley seeds on the surface of sand was assessed. The acids present were analysed by GLC before and after steam distillation. Details of these methods have been published previously [6].

Silylation and analysis of organic acids. Solns were acidified to pH 2 with HCl and extracted × 4 into Et_2O. After evaporating to dryness, the samples were left overnight *in vacuo*. Py (5 μl), containing malic acid as an int. standard, and Tri-Sil concentrate (10 μl; Pierce & Warriner (UK) Ltd., Chester), were added to the residue and the suspension was then centrifuged. Aliquots of the supernatant soln (1 μl) were analysed by GLC with a FID, 2.1 m × 6 mm glass column packed with 3% SE-30 stationary phase, N_2 carrier gas (15 ml/min), injector temp. 250° and temp. programmed from 5 min isothermal at 100° increasing to 300° at 12°/min; for GC–MS, Py (25 μl) containing malic acid, and BSTFA (50 μl) (*N,O*-bis(trimethylsilyl)-(trifluoroacetamide), were added to the residue. After centrifugation, aliquots were analysed by GLC with a FID detector, 2 m × 1.75 mm column packed with OV-1 stationary phase, N_2 carrier gas (15 ml/min), injector temp. 275° and temp. programmed from 6 min isothermal at 100° increasing to 250° at 10°/min. The GLC, a Pye 104, was linked to a VG MM 12B MS by a single stage glass jet separator, Helium was used as the carrier gas (30 ml/min). MS were obtained with electron impact ionization of 70 eV.

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