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Fungicidal Activity of Three Putrescine Analogues

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Abstract: The synthetic putrescine analogues (*E*)-*N*,*N*,*N*′,*N*′-tetraethyl-1,4-diaminobut-2-ene(E-TED), the (*Z*)-isomer (Z-TED) and (*E*)-*N*,*N*-dimethyl-1,4-diaminobut-2-ene(E-DMD) were prepared as their salts, and controlled five important crop pathogens (*Erysiphe graminis* DC f.sp. *hordei* Marchal, *Uromyces viciae-fabae* (Pers.) Schroet, *Botrytis fabae* Sardina, *Podosphaera leucotricha* (Ell. & Ev.) Salm. and *Phytophthora infestans* (Mont.) De Bary). E-TED was also effective *in vitro*, with growth of *P. infestans and Pyrenophora avenae* Ito & Kuribay completely inhibited at 1800 and 360 mg litre^{-1} respectively. When *P. avenae* was grown in the presence of 180 mg E-TED litre^{-1}, there were significant reductions in putrescine and spermidine concentrations (58% and 35% respectively). An apparent increase in ornithine decarboxylase activity and a decrease in *S*-adenosylmethionine decarboxylase activity in *P. avenae* exposed to E-TED at 36 mg litre^{-1} were not statistically significant. Diamine oxidase activity remained unchanged in fungal tissue exposed to E-TED.

1 INTRODUCTION

It has been shown previously that biotrophic fungal pathogens, e.g. rusts and powdery mildews, can be controlled effectively by inhibitors of polyamine biosynthesis.[1–3] Much of this early work used enzyme-activated, irreversible inhibitors, e.g. α-difluoromethylornithine (DFMO), which is an inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC).[4] DFMO was found to deplete intracellular concentrations of putrescine and spermidine in plant pathogenic fungi.[5] However, polyamine metabolism can also be disrupted by the use of polyamine analogues, with a concomitant effect on cell growth.[6] Keto-putrescine, a commercially available putrescine analogue, has been shown to possess fungicidal properties.[7]

As a result, a project was established to synthesize putrescine analogues and to assess their activity. This paper describes the synthesis, as their salts, and the fungicidal evaluation of three putrescine analogues, (*E*)-*N*,*N*,*N*′,*N*′-tetraethyl-1,4-diaminobut-2-ene (E-TED) and its (*Z*)-isomer (Z-TED), and (*E*)-*N*,*N*′-dimethyl-1,4-diaminobut-2-ene (E-DMD) (Fig. 1). The fungicidal properties of a range of salts of E-TED were also evaluated.

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2 EXPERIMENTAL METHODS

2.1 Chemical synthesis

2.1.1 (E)-N,N,N′,N′-*Tetraethyl-1,4-diaminobut-2-ene (E-TED) dihydrobromide* (***1a***)

Diethylamine (1·5 g, 20 mmol) in toluene (50 ml) was added to a stirred solution of (*E*)-1,4-dibromobut-2-ene (2·14 g, 10 mmol) in toluene (50 ml) at toom temperature, and the solution was stirred for 4 h. The white precipitate formed was filtered off, washed with diethyl ether (2 × 20 ml), and dissolved in hot aqueous ethanol. The solution was allowed to cool and acetone was added. The white precipitate formed was filtered off and washed with acetone to yield (*E*)-*N*,*N*,*N*′,*N*′-tetraethyl-1,4-

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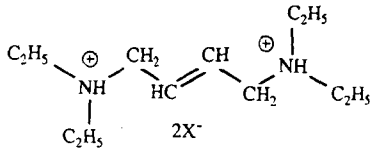
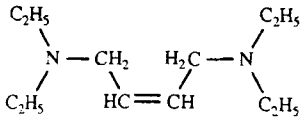


1 a. X= Br
b. X=C_6H_5COO
c. X=H_2PO_4
d. X=C_2H_5COO
e. X= fumarate



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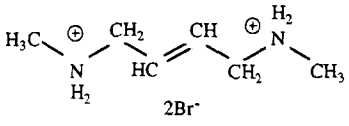

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Fig. 1. Structures of compounds studied.

diaminobut-2-ene (E-TED) dihydrobromide (21·6 g, 60%). [^{1}H]NMR (200 MHz, deuterium oxide) 1·15 (12H, t, J = 3·6 Hz) 3·08 (8H, q, J = 3·6 Hz), and 6·03 (2H, m) ppm. [^{13}C]NMR (50 MHz, deuterium oxide) 9·2 (CH_3), 48·3 (CH_2), 53·2 (CH_2), 129·6 (CH) ppm. Anal: calc. for $C_{12}H_{28}N_2Cl_2$: C 53·13%, H 10·40%, N 10·37%; found: C 53·26%, H 10·33%, N 10·17%.

2.1.2 *Synthesis of* (E)-N,N,N′,N′-*tetraethyl-1,4-diaminobut-2-ene* (*E-TED*) *dihydrobenzoate* (***1b***)

The synthesis of the free base was carried out by the general procedure of Roberts and Ross.[9] Diethylamine (3·285 g, 0·045 mol) was added during 15 min to a cool solution (0°C) of (*E*)-1,4-dibromobut-2-ene (1·07 g, 0·005 mol) in benzene (5 ml). The product was diluted with chloroform (25 ml) and the organic layer was washed with water (4 × 25 ml). The chloroform layer was dried, filtered and concentrated under vacuum to given (*E*)-*N,N,N′,N′*-tetraethyl-1,4-diaminobut-2-ene as an oil (0·82 g, 82%). [^{1}H]NMR (90 MHz, deuterochloroform) 1·10 (12H, t), 2·60 (8H, q), 3·20 (4H, m), and 5·75 (2H, m) ppm.

(*E*)-*N,N,N′,N′*-tetraethyl-1,4-diaminobut-2-ene (0·72 g, 3·6 mmol) was stirred with benzoic acid (0·87 g, 7·13 mmol) in benzene (5 ml) for 1 h. The precipitate was filtered and washed with diethyl ether to afford (*E*)-*N,N,N′,N′*-tetraethyl-1,4-diaminobut-2-ene (E-TED) dihydrobenzoate as a white solid (1·11 g, 69%). [^{1}H]NMR (200 MHz, deuterium oxide) 1·05 (12H, t), 2·94 (8H, q), 3·58 (4H, m), 5·88 (2H, m), and 7·34 (10H, m) ppm. [^{13}C]NMR (50 MHz, deuterium oxide) 9·1, 48·0, 53·0, 129·1, 129·5, 129·6, 132·1, 176·4 ppm. IR: 3443, 2986, 1637, 1599, 1379, 972 cm^{-1}. MS (*m/z*) 198, 122, 105, 86, 77, 72, 51, 28, 18. Anal: calc. for $C_{26}H_{38}N_2O_4$: C 70·58%, H 8·59%, N 6·29%; found: C 70·71%, H 8·84%, N 6·33%.

Other compounds were prepared by this method:

E-TED phosphate (**1c**) (0·56 g, 72%). [^{1}H]NMR (200 MHz, deuterium oxide) 1·07 (12H, t), 2·98 (8H, q), 3·64 (4H, m), and 5·93 (2H, m) ppm. [^{13}C]NMR (50 MHz, deuterium oxide) 9·1, 48·1, 53·0, 129·5 ppm. IR: 3440, 2970, 2820, 2470, 1625, 1480, 1390, 950 cm^{-1}. MS (*m/z*) 126, 86, 73, 58, 44, 30.

E-TED fumarate (**1e**) (1·21 g, 68%). [^{1}H]NMR (200 MHz, deuterium oxide) 1·09 (12H, t), 2·99 (8H, q), 3·66 (4H, m) 5·95 (2H, m) and 6·51 (2H, s) ppm. [^{13}C]NMR (50 MHz, deuterium oxide) 9·1, 48·1, 53·1, 129·5, 135·4, 171·7. IR: 3493, 3429, 2932, 2652, 2496, 1686, 1458, 1431, 1296 cm^{-1}. MS (*m/z*) 126, 98, 86, 72, 55, 45, 28.

E-TED propionate (**1d**) (0·72 g, 58%). [^{1}H]NMR (200 MHz, deuterium oxide) 0·82 (6H, t), 1·06 (12H, t), 1·97 (4H, q), 2·98 (8H, q), 3·63 (4H, m), and 5·92 (2H, m) ppm. [^{13}C]NMR (50 MHz, deuterium oxide) 9·1, 1·07, 30·9, 48·1, 53·0, 129·5 ppm. IR: 3420, 2980, 1940, 2500, 1720, 1575, 1460, 1290, 820 cm^{-1}. MS (*m/z*) 199, 126, 86, 72, 56, 42, 28.

2.1.3 (Z)-N,N,N′,N′-*Tetraethyl-1,4-diaminobut-2-ene* (*Z-TED*) (***2***)

The first step was carried out by modifying the general procedure of Biel and DiPierro.[9] 1,4-Dichlorobut-2-yne (2 ml, 20 mmol) was cooled to 0°C and diethylamine (7·48 ml, 80 mmol) was added dropwise with stirring. The mixture was allowed to warm to room temperature and stirred for 1 h, then left to stand for 1 h. Water (10 ml) was added and this aqueous solution was then saturated with potassium hydroxide solution. The mixture was extracted with ether (5 × 20 ml) and the organic extracts were dried, filtered and concentrated under vacuum to yield *N,N,N′,N′*-tetraethyl-1,4-diaminobut-2-yne (2·81 g, 72%). [^{1}H]NMR (90 MHz, deuterochloroform) 1·05 (4H, s), 2·55 (8H, q), 3·40 (12H, t) ppm.

The second stage in this synthesis was adapted from the procedure of Aerssens.[10] The reduction was accomplished using activated zinc: In a 250-ml round-bottomed, three-necked flask, equipped with a gas inlet, a mechanical stirred and a reflux condenser, were placed Analar grade zinc powder (17·5 g) and 100% ethanol (35 ml). Under nitrogen, 1,2-dibromoethane (1 ml) was added. The stirrer mixture was heated until a vigorous reaction started. When the intensity of the reaction subsided, an additional portion of 1,2-dibromoethane (2 ml) was added. After the exothermic reaction had ceased, the suspension was refluxed for 10 min under nitrogen. After cooling the stirred suspension to about 50°C, a solution of copper(I) bromide (4 g) and anhydrous lithium

bromide (6 g) in tetrahydrofuran (THF; 20 ml) was added over 2 min with efficient stirring. *N,N,N',N'*-tetraethyl-1,4-diaminobut-2-yne (2·81 g, 14 mmol) was added to the activated zinc and this stirred suspension was heated under reflux for 4 h. During this period a slow stream of nitrogen was passed through the flask. After cooling to room temperature, the suspension was poured into an aqueous solution (100 ml) of ammonium chloride (25 g). The suspension was then basified with potassium hydroxide solution and extracted with dichloromethane (7 × 100 ml). The combined extracts were dried, filtered and concentrated under vacuum to yield (*Z*)-*N,N,N',N'*-tetraethyl-1,4-but-2-ene (0·22 g, 8%). [^{1}H]NMR (200 MHz, deuterochloroform) 1·04 (12H, t) 2·52 (8H, q), 3·08 (4H, m), 5·64 (2H, m) ppm. [^{13}C]NMR (50 MHz, deuterochloroform) 11·6, 46·5, 54·8, 130·4. IR: 2972, 2936, 2501, 1456, 981, 659 cm^{-1}, MS (*m/z*) 198(M^+), 126, 110, 86, 72, 58, 30.

2.1.4 (E)-N,N'-*Dimethyl-1,4-diaminobut-2-ene dihydrobromide* (*E-DMD*) (*3*)

The synthesis of the free base was carried out by modifying the general procedure of Roberts and Ross.[8] (*E*)-1,4-dibromobut-2-ene (2·14 g, 10 mmol) in benzene (50 ml) was added dropwise to a stirred solution of methylamine (300 g litre^{-1} in methanol, 21 ml, 200 mmol) in benzene (50 ml) at room temperature. The resulting solution was washed with water (3 × 100 ml). The organic layer was concentrated under vacuum in an oily residue which was partitioned between chloroform (50 ml) and hydrochloric acid (2 M, 50 ml). The aqueous layer was decanted, washed with chloroform (2 × 25 ml) and concentrated to dryness under vacuum to afford (*E*)-*N,N'*-dimethyl-1,4-diaminobut-2-ene dihydrobromide (2·26 g, 42%). [^{1}H]NMR (200 MHz, deuterium oxide) 2·63 (6H, s), 3·68 (4H, d, J = 3 Hz), and 5·95 (2H, m) ppm. [^{13}C]NMR (50 MHz, deuterium oxide) 127·5 (CH), 50·2 (CH_2), 33·2 (CH_3) ppm. Anal: calc. for $C_6H_{16}N_2Cl_2$: C 38·51%, H 8·62%, N 14·97%; found: C 38·62%, H 8·57%, N 15·03%.

2.2 Determination of the fungicidal activity of E-TED

The analogues were applied to plants and their fungicidal activity determined as described previously.[11] Briefly, the protectant and curative action of E-TED was studied using barley infected with the powdery mildew fungus, *Erysiphe graminis* DC f.sp. *hordei* Marchal and broad bean infected with rust, *Uromyces viciae-fabae* (Pers.) Schroet or chocolate spot, *Botrytis fabae* Sardina. The curative action of E-TED was also examined using potato leaf discs infected with the blight fungus, *Phytophthora infestans* (Mont) De Bary and apple seedlings infected with the powdery mildew fungus, *Podosphaera leucotricha* (Ell. & Ev.) Salm. The effects of the timing of E-TED application, and a comparison of E-TED with the commercial fungicide propiconazole, were studied using the barley/powdery mildew system.

The fungicidal effects of E-DMD and Z-TED were tested on the barley/powdery mildew and broad bean/rust systems. Experiments on the effects of E-TED on polyamine concentration and the activity polyamine of enzymes of biosynthesis or catabolism were performed on *Pyrenophora avenae* Ito & Kuribay in liquid culture.

Inhibitors were applied to plants as aqueous solutions containing 0·1 ml litre^{-1} 'Tween' 20, except in the comparison with propiconazole, where E-TED was applied in 0·1 m litre^{-1} 'Agral' 90.

2.3 Effects of E-TED on growth, enzyme activities and polyamine concentrations in *Pyrenophora avenae*

The effect of E-TED on mycelial growth, polyamine concentrations and the activity of ornithine decarboxylase (ODC; EC 4.1.1.17) and *S*-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50) was determined as described previously.[5] The activity of diamine oxidase (DAO; EC 1.4.3.6) was determined using a modification of the method of Okayama and Kobayashi.[12] Briefly, the fungus was macerated in potassium phosphate buffer (100 mM, pH 8·0) containing dithiothreitol (2 mM, 300 mg in 2 ml of buffer). The fungus was then centrifuged at 20 000*g* for 20 min at 4°C and the supernatant (0·5 ml) added to potassium phosphate buffer (0·5 ml, pH 8·0) containing spermidine (1 mM) and catalase (30 μg). [1,4-^{14}C]Spermidine (3 μl; 3·76 GBq mmol^{-1}; Amersham) was then added to the reaction vessel and incubated at 37°C for 30 min. The reaction was stopped by adding aqueous potassium hydroxide (4 M; 1 ml) and shaking. Enzyme products were extracted by addition of toluene (2 ml) and vortexing for 10 s. An aliquot of the toluene phase (2 ml) was then added to a scintillation vial containing 10 ml Emulsifier Safe scintillation fluid (Packard) and counted on an LKB 1215 Rackbeta liquid-scintillation counter. Protein was asssayed by the Lowry method using BSA as a standard. Activity was expressed as nmol product (mg protein)$^{-1}$ h^{-1}

3 RESULTS

3.1 Fungicidal activity of E-TED

E-TED applied as a post-inoculation spray gave substantial control of powdery mildew (*E. graminis* f.sp. *hordei*) on barley, rust (*U. viciae-fabae*) on broad bean, chocolate spot (*B. fabae*) on broad bean and powdery mildew (*P. leucotricha*) on apple (Table 1). Infection of potato leaf discs by the late blight fungus, *P. infestans*, was also reduced by 360 mg E-TED litre^{-1} (Table 1). Although application of E-TED 5 days before or after inoculation controlled mildew infection on barley seedlings (35% and 71% respectively), best control was achieved when E-TED was applied 2 days after inoculation (83%; Table 2).

TABLE 1
Fungicidal Activity of E-TED[a]

A Pathogen–plant interaction	Leaf area infected (%)		Disease control (%)
	Control	E-TED	
Erysiphe graminis/barley	9·3 (±0·82)	1·8 (±0·20)[b]	80
Uromyces viciae-fabae/broad bean	9·6 (±1·58)	3·8 (±0·93)[c]	60
Botrytis fabae/broad bean	26·9 (±5·11)	15·0 (±1·63)[c]	45

B	Mean disease score[d]	
	Control	E-TED
Phytophthora infestans/potato	5·0	2·5
Podosphaera leucotricha/apple	2·0	1·0

[a] E-TED was used as the dihydrobromide at 360 mg litre^{-1} (1 mM) and applied as a post-inoculation spray on all interactions except the *P. infestans*/potato system.
[b,c] Significantly different from the control at $P \leqslant 0{\cdot}001$ and $P \leqslant 0{\cdot}01$, respectively.
[d] Potato leaf blight was assessed using the following key: 0 = No growth, up to 5 = Leaf disc entirely covered; Apple powdery mildew was assessed using the following key: 0 = No infection, 1 = A few isolated spores, 2 = <50% infection, 3 = >50% infection.

TABLE 2
Effect of Timing of E-TED Dihydrobromide Application, at 360 mg litre^{-1} (1 mM), on Infection of Barley with the Powdery Mildew Fungus, *Erysiphe graminis* f.sp. *hordei*

	Leaf area infected (%)			
		Pre-inoculation treatment		
A	Control	5 days	2 days	1 day
	15·1 (±0·83)	9·9 (±1·02)[b]	8·07 (±0·60)[b]	11·1 (±1·06)[a]
	Leaf area infected (%)			
		Post-inoculation treatment		
B	Control	1 day	2 days	5 days
	24·1 (±1·80)	14·7 (±2·25)[a]	4·2 (±1·14)[b]	7·1 (±0·86)[b]

[a,b] Significantly different from the control at $p \leqslant 0{\cdot}01$ and $P \leqslant 0{\cdot}001$, respectively.

Various salts of E-TED also reduced powdery mildew infection on barley seedlings when used as post-inoculation sprays. Greatest control was obtained using 400 or 438 mg litre^{-1} of the phosphate or furmarate salts of E-TED, respectively (Table 3). The derivatives of E-TED, E-DMD and Z-TED also provided substantial control of powdery mildew on barley and rust on broad bean, with best control obtained using a post-inoculation spray of 226 mg litre^{-1} E-DMD against barley mildew (80%; Table 4). In a glasshouse evaluation, E-TED reduced mildew infection on barley by an amount similar to that achieved with the commercial fungicide, propiconazole (Table 5).

TABLE 3
Comparison of the Effects of Different Salts of E-TED on Infection of Barley with the Powdery Mildew Fungus, *Erysiphe graminis* f.sp. *hordei*

Treatment[a]	Leaf area infected (%)	Disease control (%)
Control	15·7 (±0·82)	
E-TED benzoate	7·3 (±0·40)[b]	54
E-TED phosphate	5·1 (±0·40)[b]	68
E-TED fumarate	5·5 (±0·49)[b]	65
E-TED propionate	8·3 (±0·47)[b]	48

[a] Salts were used at 1 mM, i.e. 326, 400, 438 and 350 mg litre^{-1}, respectively, and were applied as post-inoculation treatments.
[b] Significantly different from the control at $P \leqslant 0{\cdot}001$.

TABLE 4
Fungicidal Effects of E-TED Derivatives

	Leaf area infected (%)		
	Erysiphe graminis/barley		*Uromyces viciae-fabae*/broad bean
Treatment[a]	Pre-inoculation	Post-inoculation	Post-inoculation[b]
Control	15·1 (±0·83)	9·3 (±0·83)	36·7 (±2·89)
E-DMD	11·9 (±0·78)[c]	1·9 (±0·20)[d]	17·9 (±1·79)[d]
Disease control (%)	22	80	52
Control	6·3 (±0·51)	6·3 (±0·49)	6·4 (±1·52)
Z-TED	3·8 (±0·44)[d]	2·2 (±0·29)[d]	1·9 (±0·35)[c]
Disease control (%)	40	66	71

[a] E-DMD and Z-TED were used as the dihydrobromide salt and free base at 1 mM, i.e. 226 and 360 mg litre^{-1}, respectively.
[b] E-DMD was used as the dihydrochloride salt at 5 mM, i.e. 1130 mg litre^{-1}.
[c,d] Significantly different from control at $P \leqslant 0{\cdot}05$, and $P \leqslant 0{\cdot}001$, respectively.

TABLE 5
Comparison of the Effect of E-TED and Propiconazole on Infection of Barley with the Powdery Mildew Fungus, *Erysiphe graminis* f.sp. *hordei*

Treatment	*Leaf area infected (%)*	*Disease control (%)*
Control	13·9 (±1·57)	
E-TED[a]	1·4 (±0·23)[c]	90
Propiconazole[b]	0·9 (±0·13)[c]	94

[a] E-TED was used as the dihydrobromide salt at 1800 mg litre^{-1} (5 mM).
[b] Propiconazole was used at 2500 mg litre^{-1}.
[c] Significantly different from the control at $P \leqslant 0{\cdot}001$.

3.2 Effects of E-TED on enzyme activities and polyamine concentrations in *Pyrenophora avenae*

E-TED reduced growth of the oat stripe pathogen, *P. avenae*, although significantly so only at 180 mg litre^{-1} (Table 6). Putrescine and spermidine concentrations were reduced, by 58% and 35%, respectively, in the presence of 180 mg litre^{-1} E-TED, although the spermine concentration was not significantly altered (Table 7). In all experiments to determine the effects of E-TED on enzyme activities, insufficient fungal material was obtained when E-TED was supplied at 180 mg litre^{-1}. Therefore, these studies were performed using E-TED at 36 mg litre^{-1}. Growth of *P. avenae* in the presence of E-TED (36 mg litre^{-1}) increased ODC activity (81%) and substantially decreased AdoMetDC activity (63%; Table 8), although neither of these changes was statistically significant. Similarly, although DAO activity was reduced by exposure to 36 mg E-TED litre^{-1}, this was not significant (Table 8).

TABLE 6
Effect of E-TED Dihydrobromide on the Growth of *Pyrenophora avenae* in Liquid Culture

Treatment with E-TED	*Mean weight (g)*
Control	3·2 (±0·5)
0·01 mM (3·6 mg litre^{-1})	2·7 (±0·11)
0·1 mM (36 mg litre^{-1})	2·6 (±0·21)
0·5 mM (180 mg litre^{-1})	1·3 (±0·15)[a]

[a] Significantly different from the control at $P \leqslant 0{\cdot}01$.

TABLE 7
Effect of E-TED Dihydrobromide on Polyamine Concentrations in *Pyrenophora avenae*

Treatment with E-TED	*Polyamine concentration (μmol g^{-1} fresh weight)*		
	Putrescine	*Spermidine*	*Spermine*
Control	8·3 (±0·94)	16·5 (±0·64)	41·9 (±9·37)
180 mg litre^{-1}	3·5 (±0·34)[a]	10·7 (±0·78)[b]	38·4 (±1·80)
36 mg litre^{-1}	4·2 (±0·27)[a]	13·4 (±0·53)[a]	40·0 (±1·53)

[a,b] Significantly different from the control at $P \leqslant 0{\cdot}05$ and $P \leqslant 0{\cdot}01$, respectively.

TABLE 8
Effect of E-TED Dihydrobromide on Enzyme Activities in *Pyrenophora avenae*

A *Treatment with E-TED*	*Enzyme activity (pmol CO_2 mg protein^{-1} h^{-1})* *ODC*	*AdoMetDC*
Control	8·9 (±2·72)	31·8 (±15·36)
36 mg litre^{-1}	16·1 (±3·17)	12·0 (±2·41)

B *Treatment with E-TED*	*Diamine oxidase activity (nmol product mg protein^{-1} h^{-1})*
Control	0·6 (±0·28)
36 mg litre^{-1} E-TED)	0·2 (±0·03)

4 DISCUSSION

E-TED, a putrescine analogue, was shown to possess substantial fungicidal activity. Similar fungicidal activity was obtained with the commercially available putrescine analogue, keto-putrescine[7] and with a synthetic putrescine analogue, (*E*)-1,4-diaminobut-2-ene.[11]

Greatest control of *E. graminis* was achieved when the compound was applied 2 days after inoculation. This could be because of a perturbation of polyamine biosynthesis in the germinating conidia on the leaf surface. The ODC inhibitor DFMO has already been shown to inhibit the germination of rust uredospores,[13] and a range of polyamine biosynthesis inhibitors reduced conidial germination, germ tube growth and haustorial development in *E. graminis* (Hannif & Walters, unpublished results).

E-TED was initially synthesized as the dihydrobromide salt. Another four salts gave reasonable control of *E. graminis*, although none was superior to the dihydrobromide salt. The other stereoisomer, Z-TED, controlled *E. graminis* on barley and *U. viciae-fabae* on broad bean, and a derivative of E-TED, (*E*)-*N*,*N*′-dimethyl-1,4-diaminobut-2-ene (E-DMD), was also fungicidal.

In the glasshouse, E-TED compared favourably to the commercial fungicide propiconazole; E-TED at 180 mg litre^{-1} reduced mildew infection by 90% compared to 94% obtained with propiconazole at 2500 mg litre^{-1}.

E-TED at 360 mg litre^{-1} did not reduce growth of *P. avenae* on solid media (data not shown). This contrasts sharply with growth of *P. avenae* in liquid culture, where 360 mg litre^{-1} E-TED completely inhibited fungal growth (data not shown), and 180 mg litre^{-1} E-TED reduced fungal growth by 60% (Table 6). Exposure of *P. avenae* to E-TED (180 mg litre^{-1}) significantly reduced putrescine and spermidine concentrations, but not the spermine concentration, while exposure to 36 mg litre^{-1} E-TED increased ODC and decreased AdoMetDC activity. Thus, although the reduced spermidine concentration could be accounted for by the lowered AdoMetDC activity, the greatly increased ODC activity should have led to elevated putrescine levels. Since the activity of DAO was not significantly altered by treatment with E-TED, the reduction in putrescine cannot be due to enhanced catabolism. It is possible that there is a greater efflux of putrescine from *P. avenae* grown in the presence of E-TED, or that the putrescine is acetylated to form *N*-acetylputrescine, thus reducing the pool of free putrescine in E-TED treated cells. Interestingly, increases in ODC activity have also been observed in *P. avenae* and *Aspergillus nidulans* (Eidam) Winter grown in the presence of keto-putrescine.[7,14] It is thought that this increase in ODC could be a result of enzyme stabilization.

The reduction in spermidine could be responsible for the reduced growth of *P. avenae* in the presence of E-TED, and, more generally for the fungicidal effects of E-TED, especially since spermidine is known to be important for fungal growth; in some fungi, e.g. *Neurospora crassa* Shear & Dodge, there is an absolute requirement for spermidine.[15] However, *P. avenae* grown in the presence of E-TED still contained 10·8 μmol spermidine g^{-1} fresh weight and substantial amounts of putrescine and spermine. It seems likely, therefore, that the effects of E-TED on *P. avenae* can only partly be attributed to perturbation of polyamine biosynthesis and catabolism, and that E-TED possesses an additional mode of action. Whether this is also true for effects of E-TED on rust and powdery mildew fungi is not known, since powdery mildew cannot be grown axenically and rusts can only be cultured *in vitro* with difficulty. In conclusion, these results, together with earlier work on keto-putrescine and the synthetic putrescine analogue (*E*)-1,4-diaminobut-2-ene, show that perturbation of polyamine biosynthesis using polyamine analogues provides a possible alternative mode of action for the development of new fungicides.[7,11,16,17]

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