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CONTROL OF FUNGAL PLANT DISEASE USING  
INHIBITORS OF POLYAMINE BIOSYNTHESIS

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HELEN WEST  
H.N.D., C.Biol., M.I.Biol.

A THESIS SUBMITTED FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY AT THE UNIVERSITY OF GLASGOW

DECEMBER 1989

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## ADDENDUM

### Materials and Methods

Page 60, line 21 : the tips of the leaves were embedded in the agar and held in place with cover slips placed obliquely into the agar, overlying the leaf tips.

Page 65 : the use of the term septae is incorrect. Substitute cell.

### Tables

10 and 11 : values for  $V_{\max}$  should be expressed as  $\text{mM g}^{-1} \text{h}^{-1}$

Text and appendix : the significant differences presented in the Tables in the text and the appendix, represent differences from the control only and not differences between treatments.

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WEST, H.M. and WALTERS, D.R. (1988). The effects of polyamine biosynthesis inhibitors on infection of Hordeum vulgare L. by Erysiphe graminis f.sp. hordei Marchal. New Phytologist 110, 193-200.

WEST, H.M. and WALTERS, D.R. (1989). Effects of polyamine biosynthesis inhibitors on growth of Pyrenophora teres, Gaeumannomyces graminis, Fusarium culmorum and Septoria nodorum in vitro. Mycological Research 92, 453-457.

## SUMMARY

The effects of a number of inhibitors of polyamine biosynthesis on the growth of two species of biotrophic fungi, five species of necrotrophic fungi and one hemibiotroph were studied. The fungi used were:

1. Erysiphe graminis DC. ex Merat f.sp. hordei Marchal
2. Ustilago maydis (DC.) Corda
3. Pythium ultimum Trow
4. Septoria nodorum (Berk.) Berk. apud and Br.
5. Pyrenophora teres Drechsler
6. Gaeumannomyces graminis (Sacc.) v. Arx and Olivier f.sp. tritici  
Walker
7. Fusarium culmorum (W.G.Sm.) Sacc.
8. Phytophthora infestans (Montague) de Bary.

The work carried out involved the following inhibitors:

1. 2-difluoromethylornithine (DFMO)
2. (E)-2-(fluoromethyl) dehydroornithine ( $\Delta$ -MFMO)
3. (E)-2-(fluoromethyl) dehydroornithine methyl ester ( $\Delta$ -MFMO.Me)
4. (2R, 5R)-6-heptyne-2, 5-diamine (RR-MAP)
5. 2-hydrazinoornithine
6. 2-difluoromethylarginine (DFMA)
7. Methylglyoxal-bis (guanylhydrazone) (MGBG)
8. Cyclohexylamine (CHA)

Compounds 1-5 are inhibitors of ornithine decarboxylase, 6 inhibits arginine decarboxylase, 7 is an inhibitor of S-adenosylmethionine decarboxylase and 8 inhibits spermidine synthase.

The effects of each of these inhibitors on infection of barley leaves by mildew were examined. Leaves of barley cv. Golden Promise were sprayed with various concentrations of the inhibitors as post-inoculation treatments. DFMO, MGBG and a combination of the two were also applied as pre-inoculation treatments. All of the inhibitors significantly reduced mildew infection. With the exception of the DFMA treatments, post-inoculation sprays were more effective than pre-inoculation ones. When DFMO, MGBG and DFMA were sprayed onto leaves at different time intervals, DFMO and MGBG most effectively controlled mildew when sprayed on the third day after inoculation. DFMA was more efficient as a pre-inoculation treatment. Addition of polyamines to DFMO sprays increased mildew infection compared to that resulting from the DFMO treatment alone, although infection was less than in the control. Whereas pre-inoculation sprays of putrescine and spermidine resulted in an increase in mildew infection by the final day of measuring, the post-inoculation treatments of polyamines reduced mildew growth.

Several of the inhibitors (1-4 and 6-8) were used to examine their effects on growth of the remaining fungi in vitro. This was achieved by supplementing growth media with the inhibitors and also with polyamines, both alone and in combination with DFMO. Species-dependent responses to the inhibitors were observed and although growth of some fungi (notably G. graminis) was reduced by various inhibitors, some increases in growth were noted after treatment. This was possibly a result of secondary enzyme production or overproduction of the target enzyme, leading to an initial surge of polyamine synthesis and enhanced growth. The effects of DFMO were reversed by the addition of polyamines to the growth medium.

Once it had been established that the inhibitors did have an effect on fungal growth, an attempt was made to understand the processes involved in uptake of polyamines, precursors and DFMO by the fungi. F. culmorum and G. graminis were selected for this work because of their different responses to the inhibitors. Uptake of the polyamines, ornithine and arginine appeared to be biphasic, with one system operating at low substrate concentrations and another at high substrate concentrations. DFMO was taken up linearly and was non-saturable over the concentrations studied. Uptake of the amino acids and polyamines by F. culmorum was pH dependent and competition work suggests that putrescine, spermidine and ornithine have different uptake systems. However, uptake of DFMO was inhibited in the presence of putrescine, spermidine and ornithine suggesting that its uptake may not be limited to one uptake system. The reduction in uptake of putrescine and DFMO by F. culmorum when sodium azide (a respiratory inhibitor) was added suggests a partial energy requirement. This was not the case for spermidine uptake.

The fungi, when grown in the presence of DFMO did not generally show enhanced uptake of polyamines as may have been expected, perhaps because the inhibitor can be rendered relatively ineffective in long term studies by intracellular decarboxylation. Exposure to MGBG, however, resulted in a decrease in uptake, possibly due to mitochondrial damage or non-specific effects of this inhibitor.

Cations present within the growth and assay media affected uptake of polyamines by both F. culmorum and G. graminis, with magnesium ions causing the greatest inhibitory effects.

SECTION 1

INTRODUCTION

FIGURE 1. Crystals of spermine phosphate in human semen. (Taken from Smith, 1972).





## 1.1 POLYAMINES AND THEIR BIOSYNTHESIS

Research into polyamines (PAs) in plants, microorganisms and animals is rapidly expanding. The importance of these compounds came to light in the 1970's when Merrell-Dow Pharmaceuticals synthesized specific inhibitors of polyamine biosynthesis called 2-difluoromethylornithine (DFMO) and 2-difluoromethylarginine (DFMA) (Metcalf et al., 1978; Kallio, McCann and Bey, 1981). The use of these and more recently synthesized inhibitors is helping researchers to understand the complexities of polyamine metabolism in various organisms, and is proving to be a very exciting new weapon in the continuing fight against animal and plant diseases.

### 1.1.1 Polyamines

Polyamines were first discovered in 1678 by van Leeuwenhoek who observed crystals of spermine within samples of human semen (Figure 1). However, in spite of this early discovery, it is only relatively recently that research into polyamines has developed.

Polyamines are simple organic compounds which have low molecular weights, are soluble in water and are cationic at cellular pH. Although the term polyamine is widely used, it is strictly a misnomer, as the compounds are actually aliphatic diamines, triamines and tetraamines. Average positive charges at cellular pH are two, two point five and three for putrescine (Put), spermidine (Spd) and spermine (Spm) respectively (Stevens and Winther, 1979).

A number of polyamines occur naturally but the most widely distributed ones are the diamine putrescine (1,4-diaminobutane), the triamine spermidine (4-aza-octane-1, 8-diamine) and the tetraamine spermine (4,9-diazadodecane-1, 12-diamine) (Table 1). These are the major polyamines in eukaryotes, including fungi. Studies so far carried out on bacteria indicate that synthesis of spermine is non-existent or rare (Tabor and Tabor, 1972). Spermidine appears to be the predominant polyamine in most fungi, with spermine and putrescine also being present in yeasts and many filamentous fungi (Stevens and Winther, 1979; Nickerson, Dunkle and van Etten, 1977; Bushnell and Bull, 1974).

All living cells contain at least one of these three amines. Other polyamines have also been identified, but these are less widely distributed than putrescine, spermidine and spermine. Examples of such polyamines are 1,3-diaminopropane (Smith, 1970; North and Turner, 1977), 1,5-diaminopentane and 2-hydroxyputrescine (Dion and Cohen, 1972).

Putrescine, spermidine and spermine occur either as free bases or they may be conjugated. In microorganisms it is more usual for the amines to be free, although Tabor and Dobbs (1970) and Tabor and Tabor (1975) report the existence of amines conjugated as acetyl or glutathionyl derivatives in Escherichia coli. In higher plants, however, conjugated forms of polyamines are generally found and may be important in regulating levels of free polyamines (Slocum and Galston, 1985a).

TABLE 1  
CHEMICAL STRUCTURES OF POLYAMINES  
(Pegg and McCann, 1988)

POLYAMINE	STRUCTURE
Putrescine	$\text{H}_2\text{N} (\text{CH}_2)_4 \text{NH}_2$
Spermidine	$\text{H}_2\text{N} (\text{CH}_2)_3 \text{NH} (\text{CH}_2)_4 \text{NH}_2$
Spermine	$\text{H}_2\text{N} (\text{CH}_2)_3 \text{NH} (\text{CH}_2)_4 \text{NH} (\text{CH}_2)_3 \text{NH}_2$

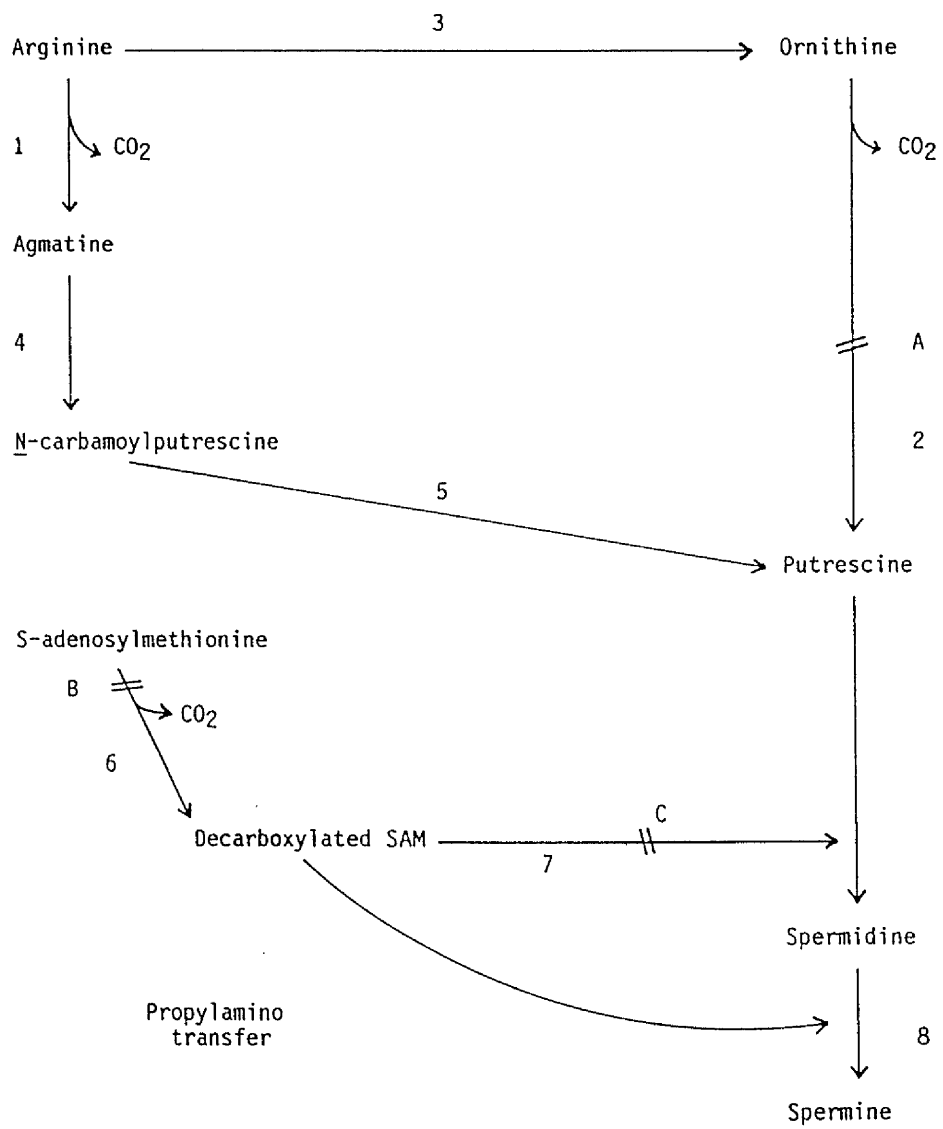
Polyamines have also been found in alkaloids (Smith, 1971) and nucleotides (Kelln and Warren, 1973). There are a number of problems in determining the distribution of polyamines within a cell. As a direct result of their solubility and charge, polyamines are prone to becoming re-distributed within the cell when organelles are isolated (Stevens and Winther, 1979).

The cellular concentrations of polyamines range between  $10^{-6}$  and  $10^{-2}$  molar (M) depending on the species studied. More specifically, concentrations vary between particular strains of organisms, with culture conditions and state of growth (Cohn, Tabor and Tabor, 1978; Stevens and Winther, 1979). The polyamine content is highly regulated by several biosynthetic enzymes which rapidly respond to changes in concentrations of the amines.

#### 1.1.2 Polyamine Biosynthesis

The synthesis of polyamines occurs directly by one pathway in mammalian cells, fungal cells and possibly most protozoa, whereas in plant and bacterial cells, two routes may be employed (Figure 2). In mammalian cells, the first step in the biogenesis of polyamines is decarboxylation of ornithine to form putrescine. This reaction is catalyzed by the enzyme ornithine decarboxylase (ODC; EC 4.1.1.17). Higher plants and bacteria can form putrescine in a similar manner. In addition, putrescine can be produced from agmatine which itself is formed by the decarboxylation of arginine by the enzyme arginine decarboxylase (ADC; EC 4.1.1.19).

FIGURE 2. Biosynthetic pathway of putrescine, spermidine and spermine and points at which inhibitors act. 1 = arginine decarboxylase, 2 = ornithine decarboxylase, 3 = arginase, 4 = agmatine iminohydrolase, 5 = N-carbamoylputrescine aminohydrolase, 6 = S-adenosylmethionine decarboxylase, 7 = spermidine synthase, 8 = spermine synthase. Points at which inhibitors act: A = DFMO,  $\Delta$ -MFMO,  $\Delta$ -MFMO.Me, RR-MAP; B = MGBG; C = CHA.



Spermidine and spermine are formed by the subsequent addition of aminopropyl groups to putrescine. These aminopropyl groups are formed by the decarboxylation of S-adenosylmethionine by the enzyme S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50). Spermidine is then formed by the addition of an aminopropyl group to putrescine in a reaction catalyzed by the enzyme spermidine synthase (EC 2.5.1.16), while spermine is formed by the addition of an aminopropyl group to spermidine, in a reaction catalyzed by spermine synthase (EC 2.5.1.22) (Stevens and Winther, 1979; Pegg and McCann, 1982; Smith, 1985; Walters, 1989; Tabor and Tabor, 1985). S-adenosylmethionine is required not only by the polyamine biosynthetic machinery, but also for ethylene biosynthesis. Thus, an increase in ethylene biosynthesis, depleting S-adenosylmethionine concentrations, will often lead to a reduction in polyamine synthesis and vice versa (Bakanashvili et al., 1987). This interaction between polyamines and ethylene has important implications for the regulation of senescence in plants.

The main rate limiting enzymes in these reactions are ornithine decarboxylase and S-adenosylmethionine decarboxylase. It has been shown that in a number of organisms there is a dramatic increase in ornithine decarboxylase activity at the onset of cell proliferation. This has been observed in fungi during germination of conidia of Aspergillus nidulans (Stevens, McKinnon and Winther, 1976) and Neurospora crassa (Sikora and McDougall, 1978). The way in which ornithine decarboxylase is controlled is not fully understood but the enzyme has a rapid

turnover rate which may be important in controlling its activity (Tabor and Tabor, 1976).

Morris and Fillingham (1974) suggest that there are two forms of ornithine decarboxylase - a biodegradative type which regulates the pH of the medium and a biosynthetic form which is involved in putrescine synthesis. It has been found that Physarum polycephalum possesses two forms of the enzyme which differ in their affinity for pyridoxal phosphate (Mitchell and Carter, 1977; Mitchell and Kottas, 1979; Mitchell, Carter and Rybski, 1978). However, Stevens and Stevens (1981) examined regulation of ornithine decarboxylase activity during conidial germination of A. nidulans and found no evidence for multiple forms of the enzyme. They were unable to conclude whether ornithine decarboxylase is degraded or inactivated because of difficulties involved in purifying the enzyme. Nevertheless, several theories regarding regulation of ornithine decarboxylase have been proposed, including an interconversion between the enzyme forms (Mitchell and Carter, 1977) and the production of a specific inhibitor (Canellakis et al., 1979). It is probable that ornithine decarboxylase activity is controlled both by its synthesis and breakdown. Indeed, Stevens and Winther (1979) report that activity of a non-specific proteinase has been detected in conidia of A. nidulans after germination had occurred.

More recently, Holm, Persson and Stjernborg (1989) showed that the synthesis of ornithine decarboxylase was controlled by polyamines in Ehrlich ascites tumour cells, mainly at the level of translation efficiency. Thus, polyamine deprivation results in



enhanced ornithine decarboxylase synthesis and enzyme activity, while elevation of intracellular polyamine levels leads to greatly reduced ornithine decarboxylase synthesis and activity.

S-adenosylmethionine decarboxylase has been purified from Saccharomyces cerevisiae (Poso, Sinervirta and Janne, 1975), E. coli, yeast, mammalian cells, plants and P. polycephalum (Williams-Ashman and Pegg, 1981; Pegg, 1984; Tabor and Tabor, 1984; Yamanoha and Cohen, 1985). The enzymes isolated from the different organisms vary in structure and cation requirement but they all contain pyruvate as a covalently bound prosthetic group. The enzyme obtained from Sacch. cerevisiae is activated by putrescine and recent work has shown that polyamines also exert a feedback control on the synthesis of S-adenosylmethionine decarboxylase in Ehrlich ascites tumour cells (Persson, Khomutov and Khomutov, 1989).

Very little information has been obtained about spermidine and spermine synthases. These are also termed putrescine aminopropyltransferase and spermidine aminopropyltransferase respectively. Spermidine synthase has been isolated from E. coli (Bowman, Tabor and Tabor, 1973), bovine brain and rat prostate (Raina et al., 1984) and it has been partially separated from Sacch. cerevisiae (Janne, Williams-Ashman and Schenone, 1971). The two synthases are not considered to be rate limiting enzymes and are regulated by substrate availability. The reactions brought about by spermidine and spermine synthases are considered to be irreversible. However, an interconversion does occur between spermidine and spermine into putrescine which has

been established in mammalian cells. The conversion is carried out by the enzymes spermidine-N'-acetyltransferase and polyamine oxidase (PAO; EC 1.4.3.4) (Pegg et al., 1981). However, the reactions involving polyamine oxidase in mammalian cells occur in the presence of aldehyde activators at levels which would not be present in vivo. Polyamine oxidase activity has also been found in the Gramineae (Smith, 1985) and in species of Aspergillus, Penicillium, Mucor, Rhizopus, Absidia, Pullularia, Fusarium, Gibberella, Phytophthora, Cylindrocarpon and Verticillium (Yamada, Isobe and Tani, 1980). Fungal polyamine oxidase is thought to act in a similar manner as the mammalian enzyme, although it has not been confirmed whether it is fundamental in polyamine regulation or turnover.

## 1.2 FUNCTIONS OF POLYAMINES

Polyamines are very important in the functioning of all cells. This is largely because cationic polyamines interact with nucleic acids, negatively charged functional groups on cell membranes, and proteins within the cell. The positive charge of a polyamine is distributed over the whole molecule as opposed to being on a specific point as is the case for inorganic ions. The determination of the many and varied functions of polyamines has been made possible by the synthesis of specific inhibitors of polyamine biogenesis. However, in spite of the progress which has been made, there are still many grey areas in the understanding of the complex roles which polyamines play. A greater comprehension of the cellular compartmentalization of polyamines is necessary to fully elucidate the functions.

In this section, the fundamental functions of polyamines will be discussed. Since arguably the most important function of polyamines is in influencing growth, probably via effects on membranes and nucleic acids, these specific effects will be discussed before considering growth itself. The use of inhibitors to study and manipulate cell growth will be discussed in sections 1.4 to 1.7.

### 1.2.1 Regulation of Cell Membrane Properties

The stability and characteristics of cell membranes can be altered by the binding of polyamines onto phospholipid and other anionic groups on membranes. It has been shown that in this way, polyamines can stabilize chloroplast thylakoid membranes and reduce loss of chlorophyll in senescing barley leaf tissue (Cohen, Popovic and Zalik, 1979).

Altman, Kaur-Sawhney and Galston (1977) have shown that spermidine and spermine have a stabilizing influence on oat protoplasts in relation to lysis. Also, Altman (1982) and Naik and Srivastava (1978) demonstrated a reduction in leakage of betacyanin from beet storage tissue when spermidine and spermine were applied.

In addition to influencing stability, polyamines have been implicated in membrane fluidity changes. It is thought that this affects enzymes which are associated with the cell membrane. For example, it has been shown that during senescence of apple tissue the membrane viscosity is reduced with associated effects on the ethylene-synthesizing complex associated with the

plasma membrane. Addition of spermine inhibits ethylene production in a manner related to decreasing membrane viscosity which induces conformational changes in the ethylene-synthesizing complex (Ben-Arie, Lurie and Mattoo, 1982).

Polyamines have also been found to stabilize bacterial protoplasts (Tabor, 1962; Mager, Benedict and Artman, 1962). Tabor (1962) showed that low concentrations of spermine were adequate in preventing protoplast lysis. As this concentration (1.0 mM) is smaller than the necessary concentration of sucrose (0.5 M) they proposed that spermine stabilizes the membrane by forming complexes with acidic groups and these reduce repulsive forces. Mammalian membranes are also stabilized by polyamines. Little (1962) showed that spermine can partially inhibit reticulocyte haemolysis brought about by treatment with saline and Chun et al. (1976) working on humans suffering from sickle cell anaemia, observed increased polyamine levels in the blood and also that electrokinetic properties of the membrane can be changed by polyamines. More recently, Schindler, Koppel and Sheetz (1980) found that mobility of glycoproteins within the erythrocyte membrane is reduced by spermine. In addition, Ballas et al. (1983) demonstrated that polyamines on the cell membrane could not be replaced by calcium or magnesium ions and therefore, the charge was not the only factor in reducing deformability of the cells. Since increased polyamine levels and decreased deformability in red blood cells have been associated with several haematological diseases, there is scope for much exciting and beneficial research in this area.

### 1.2.2 Associations with Nucleic Acids

There is much evidence to indicate that polyamines are associated with nucleic acids in viruses, bacteria and mammalian cells. However, information regarding the exact character of the association is lacking. The connection may be specific or non-specific and the polyamines function at a number of organizational levels.

Work carried out indicates that specific transfer RNAs (tRNAs) have different stoichiometry and nature of binding of polyamines (Marton and Morris, 1987). It was shown in 1983 by Tropp and Redfield that the secondary and tertiary structure of tRNA was stabilized by spermidine and in 1985 Nothig-Laslo et al. (cited by Marton and Morris, 1987), found that spermine enhances the binding of manganese to tRNA. In addition to the mammalian work carried out, it has been observed that spermidine is a component of plant viral RNA cores (Cohen and Greenberg, 1981).

Polyamines also bind to DNA. It was suggested by Bloomfield and Wilson (1981) that polyamines bind to DNA as a function of salt concentration but without a specific site. They describe DNA as being negatively charged in a linear fashion with polyamines positively charged at point concentrations. This is termed counterion condensation theory.

Spermidine and spermine have an effect on the transition of DNA (B-DNA  $\longrightarrow$  A-DNA; A-DNA is the form containing 11 residues per turn of the helix, while the B-DNA is the form containing 10

residues per turn; see Stryer, 1981), and also on conformational changes in methylated synthetic polynucleotides (Behe and Felsenfeld, 1981; cited by Slocum, Kaur-Sawhney and Galston, 1984).

In a similar vein, Hung et al. (1983) applied the polyamine biosynthetic inhibitor 2-difluoromethylornithine to rat brain tumour cells in order to deplete the cells of polyamines. They found that DNA conformation was altered by the treatment with DFMO, an effect which was reversed by the addition of exogenous putrescine. The manipulation of DNA conformation using polyamines could be of importance to patients who have undergone chemotherapy where polyamine biosynthetic inhibitors have been used as the remedial drug. However, most work has been carried out on isolated DNA and the relationship between polyamines and the nucleic acid within the intact cell must be clarified.

### 1.2.3 Effect on Protein Synthesis

Many studies have been carried out which point to an enhancement of protein synthesis when exogenous polyamines are added to a system. For example, spermidine increases the rate of peptide chain initiation and elongation in wheat germ in vitro (Takemoto, Nagamatsu and Oka, 1983). Cocucci and Bagni (1968) (cited by Smith, 1972) broke dormancy of Helianthus using auxin and reported a relationship between the synthesis of polyamines and proteins. Here the presence of spermidine and spermine bound to ribosomes from the active tissue was greater than that from the dormant tissue. Morris and Jorstad (1973) studied

growth of polyamine-deficient mutants of E. coli and found protein synthesis and growth to be reduced when not supplied with exogenous polyamines.

Polyamines would appear to have dual effects in their association with protein synthesis. That is, they function on the one hand, as a simple non-specific result of their cationic charge and, on the other hand, also influence ribosomal functioning or structure in a definite manner yet to be clarified. It would seem that polyamines preferentially stimulate the genesis of larger polypeptides than low-molecular-weight proteins. This has been observed in mammalian and plant systems (Atkins et al., 1975; Takemoto, Nagamatsu and Oka, 1983).

#### 1.2.4 Differentiation, Growth and Development

Polyamines are thought to influence the activity of cells in a number of ways, but perhaps the most startling results have arisen from work carried out on their effects on growth and development. In 1948, Herbst and Snell discovered that putrescine was essential for the growth of Haemophilus parainfluenzae, and since then many experiments have been performed in order to determine the effects polyamines have on growth and differentiation. Overall, it has been found that active growth and cell division are associated with substantial polyamine biosynthesis, in plants, animals and microorganisms. Thus, Palavan and Galston (1982) found enhanced polyamine biosynthesis and increased polyamine concentrations in growing buds and leaves of Phaseolus vulgaris, and Bagni, Malucelli and Torrigiani (1980)

reported a similar trend in buds of Jerusalem artichoke tubers during the breaking of dormancy. In the tomato plant, fruit formation is associated with increased polyamine biosynthesis and ornithine decarboxylase activity. Indeed, Cohen et al. (1982) found that DFMO inhibited fruit development and that putrescine reversed the inhibitory effects, thus suggesting a connection between fruit formation and ornithine decarboxylase activity. There is also evidence of a relationship between ornithine decarboxylase activity and growth in mammalian cells. Thus, ornithine decarboxylase levels and polyamine biosynthesis increase during embryogenesis in rodents (Guha and Janne, 1976; Fozard, et al., 1980) and embryonic development can be retarded by the addition of DFMO.

In relation to microbes, Stevens, McKinnon and Winther (1976) found that both ornithine decarboxylase and S-adenosylmethionine decarboxylase activities were increased over a hundred-fold during germ-tube emergence of Aspergillus nidulans. Here cyclo-**heximide** applications, intended to delay germination, produced a similar postponement of the increase in enzyme activity.

The evidence that polyamines play an important role in growth and differentiation is convincing, but it is often difficult to determine separate effects on cell proliferation and differentiation. This aspect will be discussed more fully in sections 1.4 to 1.7 inclusive.

Finally, polyamines also play other less well established roles within cells such as metabolic buffering of pH in plants exposed



to aerial pollution (Priebe, Klein and Jager, 1978) or to acid stress (Young and Galston, 1983). Polyamines have also been implicated in plant responses to osmotic stress (Slocum, Kaur-Sawhney and Galston, 1984), chilling stress (Guye, Vigh and Wilson, 1986) and in fungal-infected plants (see Walters, 1989), when it is likely that they interact with cell membranes. A role for polyamines in the regulation of plant senescence has been proposed (Kaur-Sawhney and Galston, 1979), since it is well known that elevated polyamine levels normally accompany tissue juvenility and the exogenous addition of polyamines to excised tissue delays senescing. The senescence-delaying effects of polyamines appear to be due, in fact, to inhibition of protease and RNase activity and to decreased ethylene biosynthesis (Kaur-Sawhney and Galston, 1979).

The roles of polyamines within cells are numerous and not as yet fully determined. It does appear, however, that polyamines are a prerequisite for the normal functioning of all living cells.

### 1.3 INHIBITORS OF POLYAMINE BIOSYNTHESIS

The determination of the roles of polyamines within living cells has been made possible by the synthesis of specific inhibitors of polyamine biogenesis. Most of the work carried out to date refers to inhibitor effects within mammalian systems. This is because the products were originally designed as anti-cancer drugs. As a result, comparatively little is known about the mechanisms of action of the inhibitors within plant and fungal systems. It is relatively recently that interest has grown in the potential of

using the drugs as anti-fungal agents, particularly in relation to agriculturally important pathogens.

Inhibitors of polyamine biosynthesis generally fall into one of three categories:

- a) analogues of ornithine or putrescine which are competitive inhibitors;
- b) antagonists of pyridoxal phosphate - molecules which interact or combine with the cofactor;
- c) compounds which act against specific enzymes within the pathway - for example, amino acid decarboxylases, S-adenosylmethionine decarboxylase and spermidine and spermine synthases.

Only inhibitors within group c) are irreversible.

### 1.3.1 Inhibitors of Ornithine Decarboxylase

Ornithine decarboxylase is an important rate limiting enzyme within the polyamine biosynthetic pathway. It is, thus, an obvious target for attack and many of the inhibitors are designed to restrain production of this enzyme. Competitive inhibitors in the form of ornithine and putrescine analogues have been studied. Thus, several diamines suppress ornithine decarboxylase activity by inducing the production of an antizyme. The antizyme, which is a protein, reacts with the ornithine decarboxylase protein thus forming an inactive ornithine decarboxylase-antizyme complex. Examples of such diamines are 1,3-diaminopropane, 1,5-diaminopentane and 1,6-diaminohexane (Danzin and Mamont, 1987).

Other competitive, reversible inhibitors of ornithine decarboxylase include DL- $\alpha$ -hydrazino- $\beta$ -aminovaleric acid (DL-HAVA) and diaminobutene (Inoue et al., 1975; Kameji, Murakami and Hayashi, 1979; cited by Danzin and Mamont, 1987). These inhibitors are not without drawbacks. Diaminobutene is metabolized to a spermidine analogue and may act as an endogenous polyamine and DL-HAVA reacts with other enzymes which also require pyridoxal phosphate. The ornithine analogue 2-hydrazinoornithine competes with ornithine decarboxylase and the inhibitory effects produced can be reversed by adding pyridoxal phosphate. This fact infers that the compound does not interact with the phosphate, unlike some ornithine analogues such as canaline. Rather, this oxamino analogue functions by forming a Schiff base product with pyridoxal phosphate leading to an inhibitory effect. It can also react with other phosphate requiring enzymes (Rahiala et al., 1971). 2-Hydrazinoornithine is disappointing because it is not especially potent in mammalian systems, is not selective and increases the half-life of ornithine decarboxylase hence creating an accumulation of the enzyme.

Generally speaking, analogues produced to date are not fulfilling their initial potential, mainly because they are not specific and show signs of toxicity. They are also rapidly metabolized and may behave in a similar manner to endogenous polyamines (Holttta, Korpela and Hovi, 1981).

Perhaps the most promising results have been obtained from the third category of inhibitors. That is, those which act against specific enzymes and are irreversible. These include the orni-

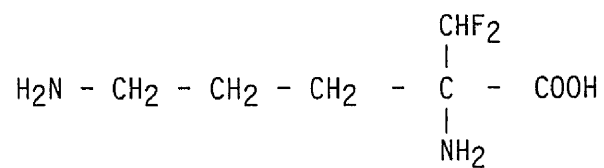
thine analogues 2-difluoromethylornithine (DFMO), (E)-2-(fluoromethyl) dehydroornithine ( $\Delta$ -MFMO) and (E)-2-(fluoromethyl) dehydroornithine methyl ester ( $\Delta$ -MFMO.Me), the putrescine analogue (2R, 5R)-6-heptyne-2,5-diamine (also termed methyl acetylenic putrescine, or RR-MAP) and analogues of spermidine and spermine termed bis (benzyl) polyamine analogues.

DFMO was one of the first to be studied (Figure 3). It was synthesized by Merrell-Dow Pharmaceuticals (Cincinnati) in the early 1970's with the intention of carrying out research into its anti-cancer properties. It has been widely used ever since and has been invaluable in elucidating the metabolic functions of polyamines. This enzyme-activated irreversible inhibitor binds to ornithine decarboxylase and in so doing forms an aldimine between the aldehyde of the pyridoxal phosphate and the  $\alpha$ -amino group of the DFMO. The DFMO then undergoes decarboxylation and the enzyme is inactivated due to alkylation of a nucleophilic residue at the active site. The Merrell-Dow team concluded that DFMO is an irreversible inhibitor as they could not detect any enzyme activity even after extensive dialysis (Metcalf et al., 1978).

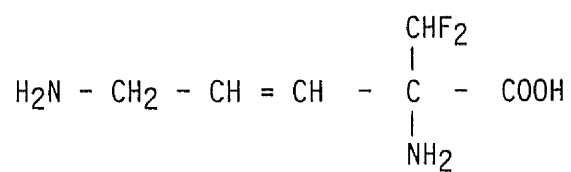
Although DFMO depletes the cell of polyamines, the process is incomplete because spermine is not eliminated. This leads to cytostatic rather than cytotoxic effects in mammalian and trypanosomal systems. However, in cells where the drug can remove spermine the effects are cytotoxic (Sunkara et al., 1983). Thus, DFMO is particularly effective against trypanosomes where ornithine decarboxylase activity is inhibited, as is DNA and RNA

FIGURE 3. Chemical structures of 5 inhibitors of polyamine biosynthesis  
(Pera et al., 1986; Pegg and McCann, 1988).

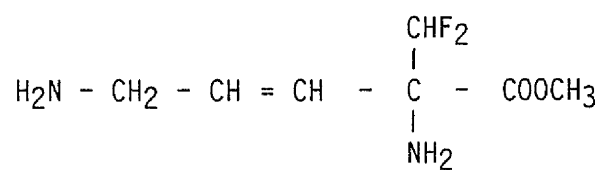
DFMO



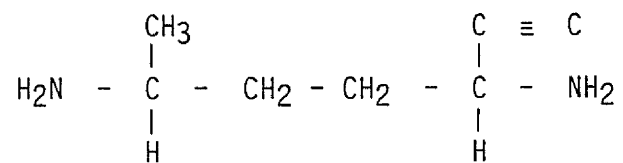
$\Delta$ -MFMO



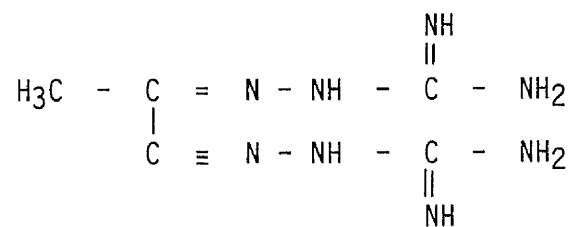
$\Delta$ -MFMO.Me



RR-MAP



MGBG



synthesis and cell proliferation (Bacchi et al., 1987). Another limitation in the usefulness of the compound is that it enters mammalian cells passively. This fact dictates that high extracellular concentrations must be used in order to reach a reasonable intracellular dose (Erwin and Pegg, 1982).

DFMO has been used as the prototype by Merrell-Dow for other more recently synthesized inhibitors. These include the ornithine analogues  $\Delta$ -MFMO and  $\Delta$ -MFMO.Me (Figure 3).  $\Delta$ -MFMO is more efficient than DFMO, having a  $K_i$  of 2.7  $\mu$ M compared to that of 40.0  $\mu$ M for DFMO.  $\Delta$ -MFMO.Me is more efficient again, although the  $K_i$  is assumed to be the same as that of the non-esterified form of the inhibitor. However, cellular uptake of  $\Delta$ -MFMO.Me is greater than for  $\Delta$ -MFMO, but once inside the cell it is de-esterified. These inhibitors appear to have some potential in controlling tumours and trypanosomes (Pera et al., 1986).

The putrescine analogue RR-MAP has been synthesized as the racemic mixture and as individual stereoisomers (Casara et al., 1985; cited by Danzin and Mamont, 1987), although the inhibitory effects are associated mainly with the (2R, 5R) stereoisomer. This has a high affinity for ODC with a  $K_i$  value of 3.0  $\mu$ M. The drug appears to be taken up by cells more readily than the ornithine analogues, and is ten times more potent than DFMO, possibly because it is able to deplete cells of spermidine (Danzin et al., 1983). Other putrescine analogues which act as irreversible inhibitors of ornithine decarboxylase are  $\alpha$ -mono-(fluoromethyl)-putrescine and  $\alpha$ -di-(fluoromethyl)-putrescine. However, these have a lower efficacy than RR-MAP.

The most recent work to be carried out in this area is on bis-ethyl derivatives of spermidine and spermine. These compounds have been found to be effective in inhibiting polyamine biosynthesis in Plasmodium falciparum and rat hepatoma cells (Bitonti et al., 1989; Bitonti, Bush and McCann, 1989).

Recent research has shown that these analogues cause a rapid decrease in the activities of both ornithine decarboxylase and S-adenosylmethionine decarboxylase and deplete rat hepatoma cells of putrescine and spermidine (Bitonti, Bush and McCann, 1989). This work also showed that the bis (benzyl) polyamine analogues were acted upon by intracellular polyamine oxidase and converted to a free-amine analogue. This free-amine analogue was then responsible for the inhibition of polyamine synthesis.

Most of the information regarding the mode of action of these inhibitors of ornithine decarboxylase synthesis relates to animal systems. There is little or no information about the mechanism of action within plant and fungal cells. There is thus, a great deal of exciting research to be carried out in this field, with the potential for being able to utilize inhibitors which may not be suitable in mammalian cells and even for use of different stereoisomers of the products.

### 1.3.2 Inhibitors of S-Adenosylmethionine Decarboxylase

After ornithine decarboxylase, S-adenosylmethionine decarboxylase is perhaps the next most obvious enzyme to target an attack upon. Inhibitors of this enzyme may be classed as either diguanidines or analogues of S-adenosylmethionine.



A number of analogues of S-adenosylmethionine have been produced but only two to date have any real effect against the decarboxylase. These are S-adenosyl-3-methylthiopropylamine and 5'-(dimethylsulfonio)-5'-deoxyadenosine. The  $K_i$  values obtained from S-adenosylmethionine decarboxylase isolated from Sacch. cerevisiae and E. coli range from 7.0 - 13.0  $\mu\text{M}$ . No work has been carried out on these compounds in vivo (Pegg and Jacobs, 1983).

The group of inhibitors classed as diguanidines include methylglyoxal bis (guanylhydrazone) (MGBG), ethylglyoxal bis (guanylhydrazone) (EGBG), and ethylmethylglyoxal bis (guanylhydrazone) sulphate (EMGBG). MGBG (Figure 3) was one of the first inhibitors of polyamines to be recognised. It competes with S-adenosylmethionine and its inhibition is reversible (Corti et al., 1974; Holttä, Hanonnen, Pispä and Janne, 1973). Although S-adenosylmethionine decarboxylase is a variable enzyme depending on which species it is isolated from, the response of each type to the inhibitor is the same. However, S-adenosylmethionine decarboxylase from E. coli is less sensitive to MGBG than the mammalian form (Williams-Ashman and Schenone, 1972) and therefore, a greater concentration of MGBG is required to effect a response. Although MGBG inhibits polyamine biosynthesis and reduces the cellular content of spermidine and spermine, it is regarded as a non-specific inhibitor. This is because the anti-proliferative action of the drug may be due to effects on processes other than polyamine depletion. Thus, there is evidence that MGBG causes morphological damage to mitochondria and inhibits DNA synthesis within the organelle (Feverstein, Porter and Dave, 1979). Moreover, Nikula et al. (1984) suggest that MGBG could affect lipid

metabolism by inhibiting fatty acid oxidation. These workers found that carnitine reverses this inhibition, providing low concentrations of MGBG are used. The carnitine is effective in this reversal because it is likely that MGBG inhibits oxidation of fatty acids - a reaction dependent on carnitine. It is also thought that the depletion of spermine by MGBG has an effect on mitochondrial metabolism.

In addition to the above, MGBG is also known to inhibit the diamine oxidase enzyme (DAO; EC 1.4.3.6) causing levels of putrescine to rise. This is a problem in patients being treated with the inhibitor (Holttä et al., 1973). The non-specific effects caused by MGBG lead to problems of toxicity in patients when it is used clinically in the treatment of leukaemias. To combat toxicity effects, cells have been treated with DFMO prior to adding MGBG. This pre-treatment reduces polyamine content within cells and at the same time there is a stimulation of uptake of spermidine to compensate. However, since MGBG can compete with spermidine for uptake, the inhibitor is rapidly taken up into cells (Sunkara et al., 1983).

Theoretically, lower concentrations of MGBG can be used to initiate good responses by such pre-treatment with DFMO, although it is likely that uptake into the tumour is not selective and toxicity will still be a problem.

EGBG, the ethyl analogue of MGBG, is a superior inhibitor of S-adenosylmethionine decarboxylase. However, there are some discrepancies in the literature regarding this compound. Accor-

ding to Porter and Sufrin (1986), EGBG is a potent inhibitor of mitochondrial activity, whereas Elo et al. (1986) state that this is not the case.

Another analogue of MGBG, EMGBG, is also a competitive inhibitor of S-adenosylmethionine decarboxylase. Elo et al. (1986) state that this compound differs from MGBG in that it does not inhibit proliferation of mouse leukaemia cells in vitro and hence resembles EGBG more closely. In spite of this, they declare the product to be the most vigorous inhibitor of S-adenosylmethionine decarboxylase.

### 1.3.3 Inhibitors of Spermidine Synthase

S-adenosyl-1,8-diamino-3-thiooctane inhibits spermidine synthase. It is based on the transition-state structure of the synthase reaction and is, therefore, specific to this enzyme (Tang, Pegg and Coward, 1980). The compound is very effective in vitro but its efficacy has not been established in vivo. Although it depletes spermidine, it causes an increase in putrescine and spermine and thus, will only be truly effective if used in conjunction with other inhibitors (Porter and Sufrin, 1986).

Cyclohexylamine is an inhibitor of spermidine synthase which acts by competing with putrescine in the synthase reaction. Cyclohexylamine has previously been referred to as dicyclohexylamine but this term is incorrect (Batchelor, Smith and Watson, 1985). The actual mechanism of action has yet to be discerned,

as have selectivity and toxicity of the inhibitor. However, in animal work, the life span of mice with leukaemia was increased, although some doubts were expressed as to whether this was a result of inhibiting spermidine synthase. Doubts arose because concentrations which affected tumour growth were lower than those required to upset the balance of polyamines (Ito et al., 1982).

#### 1.3.4 Cellular Compensatory Reactions Resulting from Polyamine Deprivation

Polyamines are extremely important compounds and no living cell can function without them. Due to this, polyamine biosynthesis is very tightly regulated (see Janne et al., 1981). It is not surprising, therefore, that cells will compensate for any interference in polyamine metabolism. The inhibitor treated cells respond in ways designed to conserve intracellular polyamine levels. These include enhanced uptake of extracellular polyamines, the manufacture of secondary enzymes, increased production of the target enzyme resulting from gene amplification and formation of polyamine analogues not normally found within the cell. The research so far carried out has been mostly on carcinoma cells in culture. However, it is likely that similar reactions could take place in plant and fungal cells.

One of the difficulties encountered when attempting to inhibit ornithine decarboxylase and S-adenosylmethionine decarboxylase, is that of rapid turnover of the enzymes due to their short half-lives. For example, ornithine decarboxylase activity in cells treated with the ornithine analogue 2-hydrazinoornithine

is reduced in vitro but enzyme activity increased in vivo. This latter effect is a result of a diminution of enzyme degradation but may also be the result of increased ornithine decarboxylase synthesis (Holm, Persson and Stjernborg, 1989). MGBG has the same effect on S-adenosylmethionine decarboxylase and results in a build-up of enzyme (Alhonen-Hongisto, Poso and Janne, 1980; Pegg, 1979). In fact S-adenosylmethionine decarboxylase can also be produced when cells are treated with ornithine decarboxylase inhibitors, although reasons for this are unknown.

Studying these compensatory mechanisms in detail Alhonen-Hongisto (1980), found that the depletion of spermidine and spermine caused an increase in S-adenosylmethionine decarboxylase but the same effect did not occur when diamine concentrations were reduced. It is probable that the quantity of S-adenosylmethionine decarboxylase is controlled at the level of transcription by spermidine and/or spermine. Indeed, recent work on Ehrlich ascites tumour cells has shown that polyamines exert a feedback control on S-adenosylmethionine decarboxylase synthesis, although further work is needed to pin-point the particular polyamines involved (Persson, Khomutov and Khomutov, 1989).

Cells treated with ornithine decarboxylase inhibitors often possess high levels of spermine. The compensatory mechanisms discussed possibly account for this, as the increased amounts of S-adenosylmethionine will be available for use in the production of decarboxylated S-adenosylmethionine. This in turn will be used in spermine synthesis. Also, reduced levels of putrescine brought about by the action of the ornithine decarboxylase inhi-

bitor will encourage spermine production as, the diamine acts as a natural inhibitor of spermine synthase (Hannonen, Janne and Raina, 1972).

Polyamine conservation and enhanced uptake are also practiced in order to counterbalance any reductions in the vital amines. Thus, Melvin and Keir (1978) observed that BHK-21 cells treated with DFMO and MGBG did not expel as great concentrations into the medium as did untreated cells. However, putrescine analogues acting as ornithine decarboxylase inhibitors caused an increase in excretion into the medium. This is possibly because the diamines competed with the polyamines for binding sites and hence dislodged amines would be expelled. The mechanism of increased uptake when polyamine deprivation occurs is unknown. Nevertheless, cultured carcinoma cells, after being treated with DFMO, took up polyamines very efficiently (Janne *et al.*, 1981). This observation could be used to our advantage by encouraging enhanced uptake of MGBG into tumour cells after DFMO treatment. However, toxicity effects do have to be taken into account.

Alhonen-Hongisto and Janne (1980), report that cadaverine is produced in response to polyamine reductions. Increased levels of decarboxylated S-adenosylmethionine lead to conversion of cadaverine to analogues of spermidine and spermine called aminopropylcadaverine and bis (aminopropyl) cadaverine. These function as polyamines within the cell. Cadaverine production may be a result of decarboxylation of lysine by ornithine decarboxylase or by a specific lysine decarboxylase, although according

to Pegg and McGill (1979), ornithine decarboxylase does not decarboxylate lysine very efficiently.

The ability of the carcinoma cells to modify their metabolism to counteract the effects of the biosynthetic inhibitors could indicate that if applied individually, their use will be limited. This view is enhanced by the observations that DFMO-resistant tumour cells develop if grown in the presence of the inhibitor for substantial periods. The resistance is inferred by over production of the target enzyme which maintains the polyamine levels (Alhonen-Hongisto et al., 1982). The anti-proliferative effect of the DFMO is thus reduced. It has been concluded that this effect is due to amplification of the ornithine decarboxylase gene (Alhonen-Hongisto et al., 1985).

#### 1.4 CANCER RESEARCH

It is important to remember that the early compounds, for example DFMO, were originally synthesized as anti-cancer drugs. Thus, before the use of these inhibitors against plant pathogenic fungi is examined, some of the more important and useful work on inhibition of polyamine biosynthesis and the growth of tumours will be considered. This is especially important since there are many lessons to be learnt from the innovative research carried out in this area and in the field of protozoal chemotherapy over the past fifteen years. It has been observed that ornithine decarboxylase activity within tumours is highly increased and inhibitors of the enzyme are capable of leading to a reduction in tumour development. Thus, Scalabrino et al. (1978) when studying diaminoazoben-

zene-induced liver carcinogenesis found that ornithine decarboxylase and S-adenosylmethionine decarboxylase levels were heightened and remained so until the appearance of tumours. Williams-Ashman, Coppoc and Weber (1972) attempted to correlate polyamine concentrations with the growth rate of hepatomas in rat liver. They concluded that putrescine and ornithine decarboxylase levels were elevated in cancerous liver with putrescine being excessively enhanced in the most vigorously growing hepatoma. Pegg and McCann (1988) report that Luk and Baylin (1984) found elevated ornithine decarboxylase levels in the colonic mucosa of patients suffering from familial polyposis. Interestingly, the enzyme level of immediate family of the individuals was greater than that of unrelated people. From this, Luk and Baylin suggested that it may be possible to identify asymptomatic relatives who carry the genotype by assessing ornithine decarboxylase levels.

This idea of Luk and Baylin (1984) to assess ornithine decarboxylase levels in an attempt to identify risk groups, was in essence examined by Russell et al. (1971) and Russell (1977) when they reported the presence of polyamines in urine. It is logical that if high concentrations of polyamines are being produced in response to tumours then they should be excreted in body fluids. Russell (1977) concluded that utilizing this information will not be useful in diagnosing cancer, but may be helpful in assessing reduction in tumour size after chemotherapy.

The main objective of chemotherapy is one of selectivity. DFMO fulfils this aim as it is enzyme specific. DFMO applied to a variety of cancerous growths has given some encouraging results,



but there are also limitations to its use. Prakash et al. (1978) analyzed the effect of the inhibitor on L1210 leukaemia in mice and showed that frequent treatment gave rise to an increased life span of twenty-three per cent compared to untreated controls. Interesting results have been obtained from DFMO applications to mice with Lewis lung tumours. Approximately three weeks after initial tumour transplantation the tumour metastasizes (migrates) to the lung. Treatment with DFMO led to a forty-three per cent inhibition of primary tumour growth, with a corresponding decrease in putrescine and spermidine levels. The metastases in the lungs were reduced by seventy-nine per cent and a quarter of the animals appeared to be free of metastases. This effect could be reversed by addition of putrescine. The way in which DFMO inhibits the secondary tumours is not known.

DFMO would seem to be cytostatic rather than cytotoxic, resulting in incomplete inhibition of cancerous growths. In 1979, clinical tests on humans were carried out, but these did not prove to be very successful, possibly because the purpose of the trials was to determine human tolerance to the drug rather than its efficacy. Also, in rodent trials the time limit between tumour development and treatment was minimal in comparison to that of the human tests. DFMO may, therefore, be exerting a prophylactic instead of a curative effect in rodents (Sjoerdsma and Schechter, 1984).

It has been proposed that DFMO cannot fully prevent rapid cell proliferation because it is not able to deplete spermine levels. In order to cause total polyamine deficiency, DFMO could be used in conjunction with other inhibitors. Drug combination strategies

could include other inhibitors of polyamine biosynthesis, or possibly immunomodulators. MGBG has been used in conjunction with DFMO, but the toxic effects of this diguanidine must be determined more fully. A promising synergistic combination is DFMO and the immunomodulator interferon. The biochemistry of the interaction is unknown but Sunkara and Prakash (1984) found a synergistic effect between the two compounds when used against Lewis lung carcinoma. Although the use of DFMO has drawbacks, it also has the advantage of being well tolerated by humans, with only relatively minor reversible side effects being noted.

Studies on other ornithine decarboxylase inhibitors have been undertaken with some success. Mamont et al. (1986) showed that  $\Delta$ -MFMO.Me can be used at ten times lower concentrations than  $\Delta$ -MFMO or DFMO, to achieve the same reduction of spermidine and putrescine in rat hepatoma cells. The most promising of the more recent ornithine decarboxylase inhibitors appears to be RR-MAP, as shown by Pera et al. (1986), who found that in murine lymphocytic leukaemia cells, the IC<sub>50</sub> (concentration at which fifty per cent inhibition of growth occurs) for this compound was more than fifty times lower than that for DFMO. The advantage of RR-MAP is that it can decrease spermine in addition to putrescine and spermidine. Using rat hepatoma cells, Mamont et al. (1984) demonstrated that with a depletion of the three main polyamines, there was a corresponding reduction in cell replication, resulting in cell death.

## 1.5 PROTOZOAL RESEARCH

Although DFMO was synthesized originally as an anti-cancer drug, perhaps the most promising use of this compound is as an anti-protozoal agent. Indeed, exciting developments regarding the control of sleeping sickness and malaria by DFMO have occurred during the last decade.

Trypanosomes appear to be particularly sensitive to the effects of DFMO, although cytostatic rather than cytotoxic effects have been reported (Bacchi et al., 1983; de Gee et al., 1984; Giffin et al., 1986). Bacchi et al. (1980) cured mice of a lethal dose of Trypanosoma brucei brucei by feeding them with a one per cent solution of DFMO. The complete mode of action is not yet fully understood but ideas proposed suggest that the reduction in polyamines could affect glycolysis (Giffin, McCann and Bacchi, 1986), and/or that trypanosomes may contain a unique spermidine-containing glutathione reductase cofactor (Fairlamb et al., 1985). Giffin et al. (1986) found that DFMO causes a morphological alteration of the trypanosome into a stunted non-replicating form. It is clear that the mode of action has not as yet been clarified. However, because polyamine biosynthesis is also a necessary function of the host cells, DFMO must be particularly selective towards the parasites. Bacchi et al. (1983) state that the trypanosomes are able to take up DFMO more rapidly than the host cells. Also, as the trypanosomes proliferate rapidly, they would synthesize polyamines at a faster rate than the host. The effects of the inhibitor would thus be more dramatic in the parasites. Sjoerdsma and Schechter (1984) suggest that the immune system of the host can

be encouraged by DFMO treatment to respond against protozoal antigens. Nevertheless, whatever the mode of action, DFMO has proved to be successful in controlling T. brucei brucei in mice and also T. brucei gambiense, the cause of African sleeping sickness in humans (McCann et al., 1986). Thus, within one to four days after treatment, the T. brucei gambiense had been eliminated from the body tissues and fluids and it seems possible that DFMO could be the first drug for over thirty years to become available for the control of sleeping sickness. DFMO is also showing promise with regard to controlling Pneumocystis carinii, a protozoan which causes disease in immunocompromised patients. This parasite is usually restrained by treating patients with trimethoprim-sulfamethoxazole (TMP-SMX) or pentamidine. However, people suffering from acquired immunodeficiency syndrome (AIDS) find these drugs toxic or inadequate, but do respond well to DFMO treatment (Golden, Sjoerdsma and Santi, 1984). In addition, recent experimental evidence suggests that the parasite responsible for malaria, Plasmodium falciparum, should be effectively controlled by DFMO (Whaun and Brown, 1985), provided that a suitable application programme can be developed. More recently still, Bitonti et al. (1989) have shown that the use of bis (benzyl) polyamine analogues could also prove to be effective against P. falciparum.

Research into trypanosome control using DFMO would appear to have produced some important and exciting findings. However, Phillips and Wang (1987), report that they have isolated a strain of T. brucei brucei resistant to DFMO. Resistance was not reduced even after numerous serial passages in the absence of DFMO. As the

mutant possesses the same amount of ornithine decarboxylase as the wild type, and the enzyme is independently inhibited by DFMO, it is thought that resistance is conferred by a reduced uptake level into the trypanosome. This problem could be overcome by using polyamine biosynthesis inhibitors which are more effectively taken up by the parasites. Bacchi et al. (1987) found  $\Delta$ -MFMO.Me to be an efficient trypanocide, and reports that it is accumulated by T. brucei brucei up to eight times faster than DFMO. This suggests that  $\Delta$ -MFMO.Me could be effective in a shorter course of treatment and at lower doses.

## 1.6 HIGHER PLANT RESEARCH

Polyamines play an important role in the physiology of higher plants, especially in plant responses to stress (see page 15). However, since this thesis is concerned with the use of polyamine biosynthesis inhibitors in controlling growth, in this section the emphasis will be on polyamine metabolism in plant growth and the use of inhibitors in such research. This is important, since in studies of plant disease control using inhibitors of polyamine biosynthesis, we must know the effects of this inhibition on plant growth.

### 1.6.1 Growth and Development

A great deal of work has been carried out which correlates increased rates of polyamine biosynthesis with cell division and active growth. Polyamine content and activities of arginine decarboxylase and ornithine decarboxylase of Phaseolus vulgaris

were studied by Palavan and Galston (1982) during vegetative growth, before and after anthesis and throughout fruit development. They found the highest levels of polyamines and enzymes in rapidly growing tissues such as root apices, hypocotyls, young internodes, young leaves, flower buds, young pods and pericarps. Mature tissues had relatively low titres. These results appear to show a relationship between polyamine levels and cell division. However, where the main growth process is cell elongation, particular polyamine gradients may be observed. Dumortier et al. (1983) found putrescine to be present in high concentrations at the base of the maize coleoptile, with the amine decreasing acropetally. In contrast, spermidine was uniformly present. Spermine was not detected within the non-dividing coleoptile but was found in the apical meristematic zone of the root. In similar research Jarvis, Yasmin and Coleman (1985) indicated that polyamines appear to be essential for root initiation and early growth of P. aureus. Application of MGBG reduced endogenous polyamine levels and inhibited root induction and growth both in the presence and absence of indolebutyric acid.

Slocum and Galston (1985c) looked at post fertilization growth of tobacco ovaries and found that ornithine decarboxylase represented more than ninety-nine per cent of the total decarboxylase. Treatment with DFMO caused a reduction in the content of polyamines, fresh weight and protein content. DFMA treatment also inhibited polyamine production. As arginine decarboxylase content was not very great, the inhibitory effect was due to an

arginase-mediated hydrolysis of DFMA to urea and DFMO. The DFMO thus inactivated ornithine decarboxylase.

In contrast to studies on the role of polyamines in growth, research on polyamines in embryogenesis is very complex. This is because in plants, unlike animals, growth and differentiation occur simultaneously. As a result, it is difficult to separate events specific to differentiation from those specific to growth. Nevertheless, in elegant experiments Fienberg et al. (1984) determined polyamine titre during somatic embryogenesis of carrot cell cultures and found increased levels of the amines in embryonic cultures as compared to non-differentiating controls. A mutant line which did not form embryos, but grew at the same rate as the wild type, had lower polyamine titres than the line forming embryos. They thus concluded that embryo development was associated with polyamine content. This conclusion was corroborated by the fact that inhibition of polyamine synthesis in the cultures using DFMA led to a reduction in embryogenesis which was induced again by addition of spermidine. Previous work carried out in 1978 by Montague, Koppenbrink and Jaworski showed that embryogenic cells of carrot incorporated ( $^{14}\text{C}$ ) arginine into putrescine at twice the rate of control cells, thus indicating a link between polyamines and cellular differentiation during embryogenesis. In this system it appears that arginine decarboxylase activity is responsible for polyamine synthesis linked to embryogenesis (Fienberg et al., 1984). Active polyamine metabolism also occurs during the development of soybean seeds (Lin et al., 1984), seedlings of Phaseolus spp. (Bagni, 1970) and Lathyrus (Ramakrishna and Adiga, 1975).

Several authors report increased polyamine biosynthesis in plant tissues after growth regulators have been applied. For example, Bagni, Torrigiani and Barbieri (1981) applied auxin to Helianthus tuberosus, Dai, Kaur-Sawhney and Galston (1982) promoted polyamine synthesis in dwarf peas using gibberellin and Suresh, Ramakrishna and Adiga (1978) studied cytokinins in relation to arginine decarboxylase and putrescine levels in cucumber. The increase in polyamine titre associated with such growth regulator applications has led to the hypothesis that polyamines may be intracellular second messengers. Galston (1983) uses the analogy of the second messenger c-AMP after Atmar, Daniels and Kuehn (1978) reported a polyamine-activated nuclear protein kinase that phosphorylates non-histone protein in Physarum polycephalum. Some workers argue that polyamines (and perhaps polyamine conjugates) are a class of plant growth regulator. This argument has gained considerable impetus since the recent report of long distance transport in a variety of plant species, especially in response to stress (Friedman, Levin and Altman, 1986).

Work considered so far is very positive in concluding that polyamines are essential for growth and cell division. However, a definite causal relationship between cell growth and polyamines in higher plants cannot be formed with absolute certainty. Thus, Meijer and Simmonds (1988) concluded from work on alfalfa that the need for polyamines in somatic embryogenesis may not be the same for different varieties. Two lines were studied which both reflected an auxin-induced build up of putrescine. Polyamine biosynthetic inhibitors caused a decrease in polyamine titres which resulted in inhibited embryogenesis in one of the



genotypes but not the other. Similarly no relationship was detected between polyamine concentrations in rapidly dividing and irradiation-blocked tissues of artichoke explants. Nor was there a correlation between cell division and polyamine titre (Phillips *et al.*, 1988). Cell division was not affected by DFMA treatment, but xylogenesis did appear to be sensitive. It was found that exogenous spermidine inhibited differentiation of the xylem at a concentration which did not affect cell division. This may be related to the findings that DFMO and MGBG treatment led to elevated spermidine levels and apparent inhibition of xylogenesis but not cell division. It may, therefore, be possible that spermidine accumulation resulting from growth conditions may inhibit cytodifferentiation or xylogenesis.

It should be noted however, that in this work, no indication was given of possible changes in polyamine conjugates. Changes in polyamine conjugates could be important in plant growth responses (see Slocum and Galston, 1985a). Equally, given that polyamines are compartmentalized within cells (see Torrigiani *et al.*, 1986), very small changes in a pool of free cytosolic polyamines may be able to cause changes in growth.

#### 1.6.2 Fruit Development

Considering the role polyamines play in cell division and growth in many plant species, it is not surprising that they perform a function in fruit development. This may have practical implications in terms of in vitro fruit production as fruit cultured in vitro tends to be smaller than equivalent in vivo fruit and

often does not produce viable seed. It has been shown by Cohen et al. (1982) that during cell proliferation in tomato fruits ornithine decarboxylase activity increases and that putrescine is necessary for fruit development. Teitel et al. (1985), also working on tomato fruit, correlated enzyme activity with cell division and the polyamine titre with DNA content, cell size and fresh weight. These findings have been corroborated by others. For example, Nathan, Altman and Monselise (1984) related arginine decarboxylase and ornithine decarboxylase activities to fruit set and development of 'Murcott' mandarins and Biasi, Bagni and Costa (1988) found that levels of free polyamines were high immediately after full bloom of Golden Delicious apples but decreased thereafter. The latter workers analyzed the levels of bound polyamines and found these to be higher than the free titres. It was noted that the decline of polyamine levels was related to abscission peaks. Previous to this study, Costa and Bagni (1983) found that fruit set and yield of apples could be increased by spraying polyamines onto flowers nine days after full bloom. This treatment possibly enhanced the rate of growth by affecting cell division. However, there is some variation in results obtained when polyamines are applied with the intention of increasing fruit yield. Thus, Volz and Knight (1986) found cultivar variations when putrescine was applied to apple. Treatment apparently enhanced fruit set in one cultivar but not in two others. Teitel et al. (1985) did not find any stimulation of in vitro tomato fruits when exogenous putrescine was added to the system. This may simply be because the fruit contained sufficient quantities of polyamines for its metabolic requirements. Thus, an additional supply would be ineffective.

Although in most of the systems used for studying fruit development, ornithine decarboxylase activity is correlated with plant growth, avocado fruit has been found to possess arginine decarboxylase in both the seed coat and the mesocarp. Winer and Apelbaum (1986) detected high arginine decarboxylase levels in tissues during a period of rapid cell division and growth. After the fruit was harvested, putrescine levels decreased and were depleted totally at the climacteric peak. This is also related to ethylene production within the fruit at this stage, where enhanced ethylene biosynthesis would lead to a reduction in polyamine biogenesis.

## 1.7 FUNGAL RESEARCH

Inhibitors of polyamine biosynthesis have great potential as anti-fungal agents. Recently, Pfaller, Gerarden and Riley (1987) studied the effects of DFMO on the growth of Candida albicans, C. tropicalis and C. parapsilosis. These yeasts are opportunistic and are becoming increasingly important, particularly in immunocompromised patients. The authors report substantial inhibition of growth after DFMO treatment, with C. tropicalis being particularly affected. Rather high levels of DFMO were required to inhibit Candida growth, but the potential is there and further work should be carried out on this and related inhibitors. Numerous pathogenic fungi affecting humans and other animals could possibly be controlled using such inhibitors.

Plant pathogens have, to date, received more attention than animal-infecting fungi. The key factor involved in the potential

control of fungal pathogens is that fungi can synthesize polyamines via ornithine decarboxylase, whereas the biogenesis of putrescine can occur via both ornithine and arginine decarboxylases in plant systems. Theoretically, this makes it possible to eradicate a fungal pathogen on a crop plant without reducing the growth and yield potential of the plant itself. It has actually been reported that as a result of DFMO application to oat leaves, arginine decarboxylase activity increases by about twenty per cent (Flores and Galston, 1982). Rajam, Weinstein and Galston (1986) noted a rise in putrescine and spermidine levels, explained by the elevation of arginine decarboxylase activity, but Walters (1986) found no effects on endogenous polyamine concentrations after DFMO treatment of broad bean.

Several studies have been made on fungal responses to DFMO treatment, both in vitro and in vivo. It is apparent that differences occur in the level of control achieved by the inhibitor, with different fungi responding in varying ways. In vivo experiments have mainly involved rusts, although Weinstein et al. (1987) briefly looked at powdery mildew on wheat. They concluded that Erysiphe graminis was more tolerant to DFMO than Puccinia recondita (leaf rust) or P. graminis f.sp. tritici (stem rust), although the inhibitor gave good control of all the fungi. Rajam, Weinstein and Galston (1985 and 1986) studied the effects of applying DFMO to Uromyces phaseoli (bean rust) on Pinto beans. These papers report that the inhibitor was more effective as a post-inoculatory treatment and treatment up to three days after inoculation inhibits fungal growth totally. The authors also show that DFMO is capable of some form of systemic action. They specu-

late that the protection of unsprayed leaves could be a result of translocation of DFMO, of a DFMO metabolite or due to an induced antifungal compound, for example, a phytoalexin. Work carried out by Walters (1986) agrees with these findings. This author examined the effects of DFMO on U. viciae-fabae infecting broad bean. The translocatory effect of the inhibitor was observed as treatment of one half of a leaf led to a protective effect on the untreated half (as separated by the mid-rib). It seems reasonable to assume that for effective systemic action, DFMO should be applied before inoculation in order to allow time for translocation to occur.

In vitro studies have been carried out on a variety of fungal species. Botrytis spp., B. cinerea, Rhizoctonia solani and Monilinia fructicola were all inhibited by DFMO and DFMA treatments. Each species showed individual response patterns and DFMO was more effective than DFMA. The inhibitors had the effect of altering mycelial morphology and reducing cell lengths of Botrytis spp. and M. fructicola. These two species were treated with putrescine and spermidine and a promotion of growth was observed, thus reversing the effects of the biosynthetic inhibitors (Rajam and Galston, 1985). Birecka et al. (1986) reported that DFMO inhibited mycelial growth and sporulation of Helminthosporium maydis (corn leaf blight) with DFMA showing a weak inhibitory effect which was not concentration dependent. DFMO and DFMA also retarded mycelial growth of Verticillium dahliae, the cause of Verticillium wilt of tomato.

The ability of DFMA to inhibit growth is surprising as fungi have not been shown to possess arginine decarboxylase. With the exception of H. maydis, this effect is likely to be a result of an arginase-mediated conversion of DFMA to DFMO. Birecka et al. (1986) suggest that as the inhibitory effect of DFMA on H. maydis was not concentration dependent, this reaction probably does not occur within this particular fungus. As no arginine decarboxylase was detected, the authors describe the inhibition as a non-specific effect. Mussell et al. (1987) found that inhibition of V. dahliae by DFMA occurred at about half that of DFMO. A trace of arginine decarboxylase activity was observed but the authors are doubtful regarding this and suggest that it was a result of other aspects of arginine metabolism. However, arginase activity was observed and it was suggested (after Slocum and Galston, 1985b) that this enzyme converts DFMA to urea and DFMO. When the inhibitory effects of both inhibitors were tested together, no additive response was noted, thus inferring that both compounds exert their effects at the same metabolic site.

Results so far obtained indicate that inhibitors of polyamine biosynthesis could be useful fungicides. Rajam, Weinstein and Galston (1985) calculated that sixty-two grams per hectare of DFMO would be required to protect a bean crop from rust infection, assuming a spray rate of nine hundred and forty litres per hectare. It must be remembered that in the work performed so far, none of the inhibitors has been 'formulated' and it is well known that formulation is important in aiding the biological activity of pesticides. Proper formulation will no doubt enhance the fungicidal performance of these inhibitors. Also, other inhibitors

have been produced by Merrell-Dow Pharmaceutical Company. Work to date has not been published recording the effects of these 'improved' compounds on fungal pathogens, nor on the effects of inhibitors of S-adenosylmethionine decarboxylase or spermidine synthase on fungal growth.

## 1.8 UPTAKE OF POLYAMINES

If analogues and homologues of polyamines are to be utilized to their full potential as inhibitors of polyamine biogenesis, then the mechanisms of amine uptake must be understood. Relatively little is known about polyamine or inhibitor uptake by cells although it is clear that such information is important for effective chemotherapy.

### 1.8.1 Uptake into Bacteria

Tabor and Tabor (1966) characterized transport systems for polyamines into E. coli. They concluded that (<sup>14</sup>C) polyamines were accumulated both by adsorption mainly to the negatively charged cell walls and by an active metabolic process which was time-dependent. The adsorption was rapid and occurred at 0°C and 37°C. Adsorbed amines were easily removed when the bacteria were washed in unlabelled polyamine, whereas amines accumulated by the active mechanism were not readily removed on washing. The rate of uptake by the metabolic route was faster for putrescine than for spermidine or spermine. The higher polyamines thus possess a greater affinity for the system.

Further work carried out by Kashiwagi, Kobayashi and Igarashi (1986) on a polyamine-deficient mutant of E. coli suggests that polyamine transport is accomplished using a proton motive force. Amine uptake was inhibited by protonophores but enhanced when D-lactate was added as a source of energy and when an artificial membrane potential was created by adding valinomycin to cells containing potassium. Radioactive spermidine was not readily removed by washing intact cells but was excreted from membrane vesicles. The authors indicate that the unidirectional uptake was only apparent and did not occur as a result of the system itself. Rather, it was a consequence of limited amounts of free polyamines within intact cells due to rapid binding of the amines to nucleic acids.

The mechanism of putrescine uptake into the cyanobacteria Anacystis nidulans is possibly that of ion trapping. According to Guarino and Cohen (1979), uptake was not actively regulated by the cell because accumulation was proportional to the exogenous concentration in the medium.

### 1.8.2 Uptake into Plants

Putrescine uptake into single petals of Saintpaulia ionantha occurred against a concentration gradient at low concentrations but at higher levels the uptake was proportional to the concentration. The addition of the uncouplers 2, 4-dinitrophenol and carbonylcyanide-m-chlorophenylhydrazone (CCCP) made no difference to uptake, although spermidine transport was inhibited at zero degrees centigrade. Bagni and Pistocchi (1985) thus con-



cluded that uptake was possibly carried out by a passive ATP-independent carrier-mediated system. Pistocchi, Bagni and Creus (1986) followed this by looking at uptake of spermidine and spermine, in addition to putrescine, by Saintpaulia petals. They report a dependency of uptake on the external pH. Putrescine and spermidine showed no signs of competition for uptake sites, nor was their absorption inhibited by inorganic ions, except for spermidine which was inhibited by calcium and enhanced by potassium. The overall trend indicates that the polyamines and inorganic ions are transported via different channels. Putrescine appeared to be accumulated in the vacuole with little being recovered from cell walls. The authors concluded that the mechanism involved is complex, is partially energy dependent and may be related to membrane potential.

Pistocchi, Bagni and Creus (1987) found the transport of putrescine and spermidine to be more rapid in carrot cells than in Saintpaulia petals. A biphasic system operational at low and high external polyamine concentrations was evident, as was the case with the violet petals. The addition of calcium enhanced spermidine uptake, but only had a slight effect on that of putrescine at low concentrations and inhibited accumulation of the diamine at high concentrations. It is suggested that the transport mechanism for spermidine is more specific than the system for putrescine. Not unrelated to this are the differences in compartmentalization observed between putrescine and spermidine. Putrescine was found in greater quantities in the cytoplasmic fraction whilst spermidine was associated mainly with cell walls. It was found in a later study of transport of

polyamines into carrot protoplasts and vacuoles that exogenously applied spermidine could be located within the vacuole (Pistocchi et al., 1988). The authors thus proposed the hypothesis that polyamines diffuse across the plasmalemma whence a carrier-mediated system transports the amines out of the cytosol and into organelles and the vacuole.

Little work has been carried out on the whole plant with regard to uptake or transport. However, Young and Galston (1983) injected (<sup>14</sup>C) polyamines and amino acids into cotyledons of pea seedlings. They found that the polyamines were not readily transported but the amino acids were, as diamines, which had been produced from the radiolabelled precursors, were recovered from the plant axis. However, more recent work by Bagni and Pistocchi (1988) suggests that putrescine is taken up by plant roots and is transported in tomato, maize and pine seedlings. They showed that putrescine uptake and translocation was greatly dependent on transpiration rate although an energy-dependent component was detected. This agrees with work by Friedman, Levin and Altman (1986) who detected polyamines in the xylem sap of a variety of plants.

### 1.8.3 Uptake into Fungi

Fungi growing on complex media will have an exogenous source of polyamines available to them. This will possibly affect the rates of amine synthesis and size of polyamine pools. However, it could be argued that because fungi are capable of synthesiz-

ing their own polyamines to requirement, then a specific amine uptake system would not be necessary.

Most of the work carried out on true fungi has been on A. nidulans and Neurospora crassa. Two uptake systems have been proposed for A. nidulans, one for putrescine and one for spermidine. Both systems appear to be inhibited by azide indicating the existence of an active component. Putrescine is taken up more rapidly than spermidine and therefore, has a greater affinity for the system. The presence of two systems is assumed as uptake of spermidine is inhibited by putrescine and spermine, but putrescine uptake is not affected by the other polyamines (Spathas, Pateman and Clutterbuck, 1982).

Like that of A. nidulans, transport in N. crassa is energy dependent (Davis and Ristow, 1988). Uptake into the fungus was inhibited by the polyamines themselves and also by inorganic cations. Transport into N. crassa is concentrative, possibly as a result of intracellular binding to nucleic acids and phospholipids.

By using a mutant strain of N. crassa which lacked ornithine decarboxylase, Davis and Ristow (1989) showed that spermidine uptake was just sufficient to saturate internal binding sites. They suggested that normally, the rate of spermidine synthesis probably saturated spermidine binding sites with little excess spermidine left over. They further suggested that because this excess (diffusible pool) is probably very small, it would be

highly responsive to the rate of spermidine synthesis and would thus be well suited to a role as a regulatory signal.

#### 1.8.4 Uptake into Animal Cells

The human platelet, an anucleate blood cell which is differentiated and non-proliferative, is still able to take up putrescine. The process itself is energy dependent since it is inhibited by 2,4-dinitrophenol. It seems probable that pH gradient and membrane potential are important factors in the transport of putrescine. It was proposed by Nadler and Takahashi (1985) that the uptake may take place via the serotonin system. This hypothesis was arrived at due to the fact that membrane proteins which have been implicated in the uptake and subsequently identified, are similar to those involved in serotonin transport. The proteins were identified as Mr 55 000 and Mr 65 000.

Porter, Bergeron and Stolowich (1982) looked at uptake of spermidine derivatives into L1210 leukaemia cells. The specificity of the uptake system was related to primary amine availability and to aliphatic chain length separating the amines. A more recent study of uptake into L1210 cells by Porter, Miller and Bergeron (1984), indicates that the system is more specific to homologues which are most like the higher polyamines but not putrescine. A competition experiment carried out by these workers suggests that in spite of the previous finding regarding chain lengths, putrescine, spermidine and spermine probably utilize the same transport system. However, the system is essentially energy dependent and carrier-mediated.

#### 1.8.5 Uptake of DFMO

Active transport systems do exist for polyamines and also for amino acids like ornithine. Theoretically, since DFMO is an analogue of ornithine, it should also be transported actively. However, work carried out with mouse fibroblasts (Erwin and Pegg, 1982) and T. brucei brucei (Bitonti et al., 1986) suggests that DFMO enters cells via passive diffusion. In both animal systems it was found that uptake was non-saturable and not antagonized by the presence of polyamines or amino acids. Erwin and Pegg (1982) draw attention to the fact that reversal effects observed when polyamines are applied after DFMO treatment are, therefore, not brought about by interference with DFMO uptake.

On the other hand, recent work by Walters and Kingham (1989) has shown that DFMO uptake into roots of intact barley plants was similar to uptake of amino acids in several systems. Thus, uptake was biphasic with respect to external concentrations and was saturable. Furthermore, these authors claim that since DFMO uptake was substantially reduced by ornithine in the medium, it is likely that DFMO is taken up by an amino acid transport system, as suggested by Slocum and Galston (1987).

#### 1.9 PATHOGENS USED

Two biotrophic pathogens, E. graminis and Ustilago maydis were studied, in addition to one hemibiotroph (P. infestans) and five necrotrophs. Brief introductions to the fungi are given below.

### 1.9.1 Pythium ultimum Trow

#### Disease:

P. ultimum causes damping-off of young seedlings. Most plants are subject to infection, particularly if damp conditions prevail. The fungus can attack the seed at the pre-emergence stage or after emergence has taken place. The disease may be recognised by brownish watery lesions.

#### Economic importance:

The losses incurred by P. ultimum tend to vary from one year to the next. An outbreak will usually ruin a crop totally. Sugar-beet and vegetable crops seem to be most at risk, although forest trees may also be affected.

#### Control:

Control is effected by ensuring that soil is well drained and by chemical treatment of the soil and seed. Fenaminosulf and pro-pamocarb may be used as soil treatments (Bouhot and Smith, 1988).

### 1.9.2 Phytophthora infestans (Montague) de Bary

#### Disease:

Infection by this pathogen leads to late blight of potato and tomato. The fungus also attacks other members of the Solanaceae. All aerial plant parts are affected with brown or black necrotic lesions appearing on the leaf tips and margins and also on the stems. At harvest, potato tubers may become infected by

zoospores washed down from the infected haulms or when lifted, which leads to the development of a brown granular rot beneath the skin. Secondary rotting may occur as a result of bacterial invasion.

Sporangia are readily disseminated by wind and rain splash and therefore, other crops may become infected. The fungus can overwinter in tubers, whether seed potatoes or those left in clamps. Volunteer plants are a source of infection.

**Economic importance:**

Late developing crops are most likely to suffer economic losses as a result of defoliation which affects tuber yield. The actual relationship between yield potential and loss in relation to infection by P. infestans has yet to be clarified (Rotem, Bashi and Kranz, 1983). When blight is prevalent on potato crops, tomatoes tend to also be affected. The disease is of world wide distribution and is present wherever potatoes are produced.

**Control:**

Resistant varieties of potato are available, although a particular cultivar may only be resistant to some races of P. infestans and susceptible to others. Cultural practices include destroying potato clamps, reducing growth of volunteer plants and never planting infected seed potatoes. Integrated control methods are most efficient, where polygenic resistance is utilized along with fungicide sprays, applied at times when infection periods

are predicted. Suitable fungicides include maneb, zineb and captafol (Rich, 1983; Shattock, 1988; CMI, No. 838, 1985).

### 1.9.3 Septoria nodorum (Berk.) Berk. apud and Br.

#### Disease:

Symptoms of infection appear both on the leaf and the ear. When the ear is infected, it is termed glume blotch and appears as brown lesions. Lesions occurring on the leaf begin as water-soaked green areas which develop into elongated pale brown areas surrounded by a lighter border which eventually darkens. The disease is principally seed-borne but may survive on wheat stubble. Once the crop is harvested, the fungus becomes saprophytic.

#### Economic importance:

The importance of the disease varies from season to season and depends on the stage of plant development at which infection occurs. For example, seedling infection will reduce tiller production and cause a greater yield loss than would be the case if infection occurred after flowering.

#### Control:

Some varieties of cereal are less susceptible than others, but it would be fair to say that resistant cultivars are only moderately successful. Cultural control includes practices such as using healthy seed and varieties which mature early, in addition to crop rotations. Several fungicides are useful if applied before establishment of the disease on upper leaves, for



example, captafol, carbendazin plus mancozeb and prochloraz (Sanderson and Scott, 1988; Shipton et al., 1971; ADAS, No. 2257, 1984; CMI, No. 86, 1966).

#### 1.9.4 Pyrenophora teres (Drechsler)

##### Disease:

The fungus causes net blotch of barley, although other cereals may occasionally show signs of infection. Seed-borne inoculum causes a primary infection which develops as pale lesions on the first leaves. Secondary infection on older leaves is identified by brown net-like lesions. Two forms of the pathogen exist, one forming net-like lesions and the other, brown spots. The differences in symptom expression are genetically determined.

##### Economic importance:

The importance of the pathogen is rapidly increasing, possibly as a result of continual barley cropping and the increase in early sown autumn crops.

##### Control:

Seed dressings are able to reduce the primary source of inoculum but secondary sources such as debris are still prevalent. Crop rotation practices, the destruction of stubble and the use of resistant varieties are all useful in controlling the pathogen. A number of fungicides are effective control agents, for example, prochloraz, triadimefon and propiconazole (Smedegaard-Petersen, 1988; ADAS, No. 2257, 1984; CMI, No. 390, 1973).

1.9.5 Gaeumannomyces graminis (Sacc.) v. Arx and Olivier

**Disease:**

The disease caused by this fungus is known as take-all. It is a soil-borne pathogen which attacks the roots of cereals. Wheat is more susceptible than barley. Seedlings may be killed and older plants stunted with bleached inflorescences termed white-heads.

**Economic importance:**

Where cereals are intensively grown, take-all is an important disease on a world-wide basis. However, if susceptible crops are part of a rotation then it causes minimal concern.

**Control:**

The only method of control available at the moment is rotational cropping or utilization of take-all decline. Research into potential use of various chemicals as control agents has been carried out but with limited success. However, Ballinger and Kollmorgen (1986) have looked at the potential of using triazole and benzimidazole fungicides on take-all of wheat. The compounds were applied with seed and it was found that good control was obtained. There is, therefore, the potential for chemical control (Lemaire, 1988; CMI, No. 383, 1973).

1.9.6 Fusarium culmorum (W.G.Sm.) Sacc.

**Disease:**

F. culmorum causes root rots of cereals and is associated with other fungi in forming a 'foot-rot complex'. The fungus is also

responsible for cob and stem rot of maize, rots of asparagus and of peas. Pinkish mycelium can often be observed at stem bases of infected plants. The pathogen may also cause storage rots of apples, potato and sugar-beet. Brown-patch of turf-grass results from infection as F. culmorum is often prevalent in grassland. The fungus inhabits the soil and is very competitive. It is able to overwinter both as conidia and mycelium.

**Economic importance:**

Yield losses very much depend on environmental factors and few quantitative results are available. Many seedlings may be killed around the time of emergence and often the crop cannot compensate for the reduction in plant numbers.

**Control:**

Seed treatments have been shown to partially control the disease. Examples of such fungicides are organomercury, carboxin plus thiabendazole and triadimenol plus fuberidazole. Ploughing in of stubble and the use of healthy seed is advised. It is difficult to fully prevent outbreaks of this disease due to its competitive nature and persistence (Fehrmann, 1988; ADAS, No. 854, 1983; ADAS, No. 2257, 1984; CMI, No. 26, 1964).

1.9.7 Erysiphe graminis DC. ex Merat

**Disease:**

The disease caused by this pathogen is powdery mildew of cereals and grasses. The symptoms are visible on aerial parts and consist of white fluffy pustules mainly on leaves and sheaths. The

pustules become brown with age. Necrotic spotting may be seen on varieties of cereal which are resistant to powdery mildew. Straw, stubble and overwintering plants may all harbour conidia or mycelium and act as a source of infection.

**Economic importance:**

Yield losses in cereals can be severe, with barley being subjected to the highest incidence of disease. Mildew is the most widespread of the cereal leaf diseases.

**Control:**

Control is carried out via the use of resistant varieties, fungicides and cultural practices. Cultivars belonging to different diversification groups will reduce incidence of the disease especially if integrated with the use of fungicides. A host of fungicides is available both as seed treatments and sprays. Examples are ethirimol, prochloraz, tridemorph and nuarimol. Cultural practices include avoiding high nitrogen levels and sowing spring crops near to diseased autumn crops (Schwarzbach and Smith, 1988; ADAS, No. 579, 1982; ADAS, No. 2257, 1984; CMI, No. 153, 1967).

#### 1.9.8 Ustilago maydis (DC.) Corda

**Disease:**

U. maydis is a gall-forming smut which affects maize. Spores are formed within irregular swellings which vary in size from one to ten centimetres on inflorescences, leaves and stems. Initially, the galls are white but later rupture to release

black spores. Spores may survive for a number of years in the soil.

**Economic importance:**

The disease can be observed on a worldwide basis wherever maize is grown, although it is absent from Australia and New Zealand. Seedlings can be killed and surviving infected plants give rise to small cobs. Losses in Europe tend to be small.

**Control:**

No control method presently exists. Rotational practices are of no use due to the longevity of the spores and fungicides have not proved to be particularly efficient. The vast number of biotypes of U. maydis causes problems when attempting to develop resistant varieties (Cassini and Smith, 1988; CMI, No. 79, 1965).

## 1.10 AIMS OF THE PROJECT

It is clear that polyamines are important in the functioning of all cells and that these compounds are synthesized via ornithine and arginine decarboxylases in plants, but only by way of ornithine decarboxylase in fungi. Given this information, it should be possible to control plant diseases using inhibitors of polyamine biosynthesis. The aims of this project are, therefore, to:

- a) assess the fungicidal properties of a number of specific inhibitors of polyamine biosynthesis;
- b) determine whether necrotrophic and biotrophic fungi have similar response patterns to treatment with the inhibitors;

- c) determine the mechanisms of uptake of polyamines, amino acid precursors and a biosynthetic inhibitor into selected fungi.

If inhibitors of polyamine biosynthesis are to be utilized as fungicides, then this information is important. An understanding of the mechanisms involved will be useful in the rational improvement and design of specific inhibitors.

SECTION 2

MATERIALS AND METHODS

## 2.1 EFFECT OF THE INHIBITORS ON INFECTION OF BARLEY BY ERYSIPHE GRAMINIS

### 2.1.1 Maintenance of the Pathogen

E. graminis f.sp. hordei was maintained on barley plants (Hordeum vulgare L., cv. Golden Promise) which were kept in a growth chamber. The temperature was  $20 \pm 5^{\circ}\text{C}$  and artificial lighting was provided by fluorescent tubes for 16h per day. Barley plants were sown and sequentially inoculated on a weekly basis at about the fourth leaf stage.

### 2.1.2 Plant Material

Healthy plant material was obtained by sowing seeds of barley (cv. Golden Promise) in Fison's Levington compost in 36 cm seed trays. The plants were grown in a glasshouse under natural daylight supplemented for 16h daily by 400W mercury vapour lamps. The maximum temperature was  $24^{\circ}\text{C}$  during the day and fell to a minimum of  $9^{\circ}\text{C}$  at night.

Plants at growth stage 12 (second leaf unfolded, Zadok's scale) were used for experiments. First leaves were removed from the plants, cut to a length of 7 cm and placed into 9 cm diameter, single vent, plastic Petri dishes containing Oxoid No. 3 agar supplemented with 0.02% (w/v) benzimidazole. The tips of the leaves were embedded in the agar and held in place with coverslips. The leaves were treated with solutions of inhibitors and/or polyamines and were inoculated with E. graminis f.sp.



hordei by the transfer of conidia from stock plants using a camel hair brush.

### 2.1.3 Treatment with Inhibitors and/or Polyamines

Solutions of the inhibitors and/or polyamines were made up in 0.01% (v/v) Tween 20 (BDH Ltd, Poole, Dorset) and adjusted to pH 7.0 using sodium hydroxide. Concentrations ranged from 0.05 to 2.0 mM depending on the inhibitor or polyamine used. The concentrations used for each treatment were as follows:

1. DFMO; 0.5, 1.0 and 2.0 mM; pre- and post-inoculation treatments.
2.  $\Delta$ -MFMO; 0.5 and 1.0 mM; post-inoculation treatments.
3.  $\Delta$ -MFMO.Me; 0.5 and 1.0 mM; post-inoculation treatments.
4. RR-MAP; 0.05, 0.1 and 0.5 mM; post-inoculation treatments.
5. 2-Hydrazinoornithine; 0.5 mM; post-inoculation treatment.
6. CHA; 0.5 and 1.0 mM; post-inoculation treatments.
7. MGBG; 0.05, 0.5, 1.0 and 2.0 mM; pre- and post-inoculation treatments.
8. DFMO + MGBG; 0.05, 0.1 and 0.5 mM MGBG + 0.5 mM DFMO; 0.05, 0.1 and 0.5 mM MGBG + 1.0 mM DFMO; post-inoculation treatments.
9. DFMO + putrescine; 0.1 and 0.5 mM putrescine + 1.0 mM DFMO; pre- and post-inoculation treatments.
10. DFMO + spermidine; 0.1 and 0.5 mM spermidine + 1.0 mM DFMO; pre- and post-inoculation treatments.
11. Polyamines; 0.5 mM putrescine or 0.5 mM spermidine; pre- and post-inoculation treatments.

## 12. Water; pre- and post-inoculation treatments.

Controls consisted of leaves which had been; a) inoculated only and b) sprayed with inhibitor solutions in the absence of inoculum. Type a) controls are shown in all figures and type b) controls were visually assessed to determine possible phytotoxic effects of the inhibitors.

A Shandon spray unit was used to apply inhibitor and/or polyamine solutions to all leaves except for control type a), and sprays were applied until run-off. In pre-inoculation treatments, the leaves within the Petri dishes were sprayed with solutions of the inhibitors and/or polyamines and left for 2h before inoculation.

### 2.1.4 Timing of Inhibitor Applications

Leaves were treated with DFMO (1.0 mM), MGBG (1.0 mM), MGBG + DFMO (0.5 + 0.5 mM) and DFMA (1.0 mM) at times from 0 days (a pre-inoculation treatment) to 5 days after inoculation.

After treatment and inoculation, the Petri dishes were placed randomly in a controlled environment. The temperature was 18.5°C, falling to 16.5°C at night, and artificial light was provided by fluorescent tubes for 16h per day to give a mean irradiance of 251  $\mu\text{mol}/\text{m}^2/\text{s}$ .

Visual assessment of infection was carried out using a standard area diagram 6, 9 and 12 days after inoculation and recorded as

the per cent of leaf area infected. Petri dishes were coded and randomly assessed to prevent personal bias.

## 2.2 EFFECT OF THE INHIBITORS ON GROWTH OF FUNGI GROWN IN VITRO

### 2.2.1 Maintenance of the Pathogens

Stock cultures of P. teres, G. graminis, F. culmorum, U. maydis, P. infestans and P. ultimum were grown on Potato Dextrose Agar (PDA) (Oxoid). S. nodorum was grown on Czapek Dox Agar (Oxoid) plus V-8 juice using the following recipe (per litre): 60 ml V-8 juice, 13.62g Czapek Dox Agar (Oxoid), 0.9g calcium carbonate, 3g Oxoid No. 3 Agar, 240 ml distilled water. All media were autoclaved at 115°C for 20 minutes.

The cultures were kept in the dark at a temperature of 24°C. Fresh culture plates were initiated on a 7-21 day rota depending on the growth of each fungus.

### 2.2.2 Treatment with Inhibitors and/or Polyamines

For the experimental work, all cultures were grown on Czapek Dox medium containing 0.5% mycological peptone (Oxoid) except for P. ultimum and P. infestans which remained on PDA. The Czapek Dox medium was made as described in the Oxoid Manual and supplemented with 0.5% mycological peptone. The formula used (per litre) was as follows: 2.0g sodium nitrate, 0.5g potassium chloride, 0.5g magnesium glycerophosphate, 30g sucrose, 5.0g mycological peptone. Prior to carrying out experimental work, it was established that each fungus would grow satisfactorily on this medium.

During the course of routine sub-culturing some cultures of P. teres (grey) spontaneously developed tufts of orange mycelium. Subsequent culturing of these orange tufts produced stable orange variants. Such variation is well documented for P. teres (see McDonald, 1967). The grey and orange forms of P. teres were studied in detail in all subsequent experiments.

Czapek Dox + peptone medium was made up, autoclaved (115°C for 20 minutes) and allowed to cool to 40-42°C. Inhibitors, polyamines and polyamines plus inhibitor solutions were filter-sterilized using Ministart blue (0.2 µm) filters (Sartorius, Surrey, England) and added to the cooled medium. Twenty ml of the amended media were transferred to plastic 90 mm, single vent Petri dishes. Control plates consisted only of the Czapek Dox medium with 0.5% mycological peptone. The concentrations used for each treatment were as follows:

1. DFMO; 1.0, 2.0 and 4.0 mM.
2. Δ-MFMO; 0.5 and 1.0 mM.
3. Δ-MFMO.Me; 0.5 and 1.0 mM.
4. RR-MAP; 0.5 and 1.0 mM
5. DFMA; 1.0 mM.
6. DFMO + DFMA; 1.0 + 1.0 mM.
7. MGBG; 0.5, 1.0 and 2.0 mM.
8. DFMO + MGBG; 0.5 + 0.5 mM and 1.0 + 1.0 mM.
9. CHA; 0.5, 1.0, 2.0 and 4.0 mM.
10. Putrescine; 0.5 and 1.0 mM.
11. Spermidine; 0.5 and 1.0 mM.
12. DFMO + putrescine; 4.0 + 1.0 mM.
13. DFMO + spermidine; 4.0 + 1.0 mM.

Only P. infestans and P. ultimum were treated with DFMA and DFMO + DFMA.

Mycelial bores, 1.0 cm diameter, from Petri dishes containing stock cultures of the fungi, were placed upside down on the centre of each experimental plate, which was then incubated in the dark at 24°C. Replicate numbers ranged from 5 to 7 depending upon the availability of the particular inhibitors. Growth was monitored by measuring the distance between the new growth and the edge of the original mycelial plug. Three measurements were taken, the mean of which represents the growth of the colony in mm. Measurements were taken at different times following inoculation, the interval being dependent on the growth of the fungi. These measurements were converted to percentages of the control value and expressed as such in the results section (3.2).

### 2.2.3 Cell Lengths and Diameters

The lengths and diameters of septae were measured using a calibrated micrometer, on day 6 after inoculation. For ease, the septae will be referred to as cells. Mycelium was scraped from the Petri dishes, placed onto microscope slides and stained with lactophenol cotton blue. It was not possible to measure cell size for every inhibitor as mycelial growth was rather sparse for some treatments. Measurements were replicated 10 times. The measurements for the grey and orange forms of P. teres were pooled and thus the replicate number was 20.

### 2.3 UPTAKE OF POLYAMINES, PRECURSORS AND DFMO BY F. CULMORUM AND G. GRAMINIS

F. culmorum and G. graminis were chosen for experiments in which uptake of polyamines, precursors and DFMO were to be studied. The differences in growth effects observed with different treatments of the inhibitors, suggested it would be useful to compare the two species. However, the most detailed work was carried out using F. culmorum.

#### 2.3.1 General Methods

Mycelial suspensions of F. culmorum and G. graminis were grown in Czapek Dox + peptone liquid medium and incubated in a Denley orbital shaker at 144 rpm for 5 to 7 days under controlled conditions. The pH of the medium varied depending on the particular experiment. The temperature was 18.5°C falling to 16.5°C at night, and artificial light was provided by fluorescent tubes for 16h per day to give an irradiance of 251  $\mu\text{mol}/\text{M}^2/\text{s}$ .

After 5 to 7 days, mycelium was harvested by centrifugation (Europa 24M, MSE Scientific Instruments) for 3 minutes at 14000 rpm and at 18-20°C. The mycelium was washed once by re-suspending in 10-15 ml of fresh Czapek Dox + peptone medium corresponding to the pH at which it was grown, and centrifuging again. The pellet of wet mycelium was removed from the centrifuge tubes and 0.5g quantities were weighed and added to flasks containing 10 ml Czapek Dox + peptone medium, also of the pH corresponding to that of the original growth medium. Three replicates of each

treatment were used. The flasks were incubated at room temperature and under natural daylight for 40 minutes in a Denley reciprocal shaker at 136 rpm. Standard solutions of varying substrate concentrations were made and 2.5 ml of the unlabelled substrate were added to the mycelial suspensions after 40 minutes. In addition, 12.5  $\mu$ l (23 KBq) of labelled substrate obtained from Amersham International was added and the flasks were incubated for a determined time in the reciprocal shaker at 136 rpm.

Following incubation, mycelia were removed by centrifugation for 3-5 minutes at 14000 rpm. The pellet was washed twice with 10 ml distilled water by re-suspending the mycelium and centrifuging. After the final wash and centrifugation, the pellet was deposited in a glass scintillation vial containing 2 ml Soluene-100 (United Technologies, Packard). The vials were shaken in a rotamixer and incubated for 3h at 60°C, then left overnight at room temperature. Ten ml Hionic-Fluor scintillation fluid (United Technologies, Packard) was then added to each vial, and radioactivity counted using an LKB 1215 Rackbeta liquid scintillation counter.

The control treatments contained everything except the radio-labelled substrate. These methods were developed from work carried out by de Waard and van Nistlerooy, 1979 and Sheard and Farrar, 1987.

### 2.3.2 Outline of Methods used to Determine the Effect of pH on Uptake

Mycelium of *F. culmorum* was prepared as described in section 2.3.1. The pH values looked at were pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The mycelium was grown at each specified pH and uptake was studied at that pH, the values of which were adjusted using sodium hydroxide. The cold substrates used at each pH value were ornithine, arginine, putrescine, spermidine, spermine and DFMO at a concentration of 1.0 mM. Labelled substrate was added as described in section 2.3.1.

### 2.3.3 Outline of Methods used to Determine Effect of Time on Uptake

Mycelium of *F. culmorum* was prepared as described in section 2.3.1. The pH of the growth and uptake medium was determined by the experiment described in section 2.3.2 and varied depending on the substrate used. The pH values at which the experiment was carried out corresponded to the optimum uptake of the substrate at that value and were: arginine, pH 5.0; ornithine and putrescine, pH 6.0; DFMO, pH 7.0; spermidine and spermine, pH 8.0. These values were adhered to throughout the remaining uptake experiments, except for the study of competition between cations and polyamines for uptake by the fungi (see section 2.3.8). The pH values of the media were adjusted using sodium hydroxide. The incubation times studied were, 10, 20, 30, 40, 50, 60 and 120 minutes and 1.0 mM concentration of each unlabelled substrate was used. Labelled substrate was added as described in section 2.3.1.



#### 2.3.4 Outline of Methods Used to Study Kinetics of Uptake by *F. culmorum* and *G. graminis*

Mycelium was prepared as detailed in section 2.3.1 with the pH of growth and uptake medium as described in section 2.3.2.

The substrates used were arginine, ornithine, putrescine, spermidine, spermine and DFMO. In experiments using *F. culmorum* the substrate concentrations used were 0.006, 0.013, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 10, 25 and 50 mM. However, 0.1 mM concentration was not used for analysis of spermidine uptake. In experiments using *G. graminis*, the substrate concentrations used were 0.1, 0.25, 0.5, 1.0, 10 and 25 mM for arginine, putrescine, spermine and DFMO. In the experiment looking at ornithine uptake, 0.25 mM treatment was omitted and the concentrations of spermidine studied were 0.4, 0.6, 2.0, 8.5 and 21 mM. Radio-labelled substrate was added as described in section 2.3.1.

From these experiments, the optimum substrate concentrations were determined by plotting the data and calculating the  $K_m$  (affinity constant) from an Eadie-Hofstee equation. In subsequent experiments, in order to avoid substrate limitation during uptake, a value of 3 times the overall  $K_m$  for each substrate was used (Tables 10 and 11, Text, detail the  $K_m$  values calculated) with the exception of the experiment described in section 2.3.6. The  $K_m$  of DFMO uptake could not be calculated, so a value relating to the  $K_m$  of ornithine was used (DFMO being an ornithine analogue).

### 2.3.5 Outline of Methods Used to Study the Effect of a Respiratory Inhibitor on Uptake

Mycelium of F. culmorum was prepared as described in section 2.3.1 with the pH of growth and uptake medium as detailed in section 2.3.2.

The substrates employed were putrescine, spermidine and DFMO and were used at quantities corresponding to 3 times the  $K_m$  of each polyamine and 3 times the  $K_m$  of ornithine for the DFMO treatments. Sodium azide (1.0 mM) (Sigma Chemical Co., Poole, England) was added to the uptake medium with the unlabelled substrates. The control treatments did not contain sodium azide. Radioactive substrate was added as described in section 2.3.1.

### 2.3.6 Outline of Methods Used to Study Antagonistic Effects of Polyamines, Ornithine and DFMO on Uptake of Polyamines and DFMO

Mycelium of F. culmorum was prepared as described in section 2.3.1. The experimental treatments used were as follows:

$^{14}C$ putrescine	+	cold putrescine
"	"	+ cold spermidine
"	"	+ cold ornithine
"	"	+ cold DFMO
$^{14}C$ spermidine	+	cold spermidine
"	"	+ cold putrescine
"	"	+ cold ornithine
"	"	+ cold DFMO

<sup>14</sup> C DFMO	+	cold DFMO
" "	+	cold putrescine
" "	+	cold spermidine
" "	+	cold ornithine

One mM concentration of each unlabelled substrate was used and labelled substrate was added as described in section 2.3.1. The Km values previously obtained were not useful for this study as the pH of the medium corresponded to the optimum pH of the radiolabelled substrate and not necessarily to that of the cold substrate. The pH values used were pH 6.0 for putrescine, 7.0 for DFMO and 8.0 for spermidine uptake.

### 2.3.7 Outline of Methods Used to Study the Effect on Uptake of Polyamines of Growing Fungi in Inhibitor Amended Medium

Mycelium was prepared as described in section 2.3.1 with the amendment that inhibitor solutions were added to the growth medium.

Filter sterilized inhibitor solutions were added to autoclaved Czapek Dox + peptone medium which had been cooled to 40-42°C. Ministart blue (0.2 µm) filters (Sartorius, Surrey, England) were used. The inhibitor solutions used in the amended media for growth of F. culmorum were as follows:

1. DFMO; 1.0, 2.0 and 4.0 mM.
2. MGBG; 0.5, 1.0 and 2.0 mM.
3. DFMO + MGBG; 0.5 + 0.5 mM and 1.0 + 1.0 mM.

G. graminis was grown only in the presence of DFMO, 1.0 and 4.0 mM and MGBG, 0.5 mM. This fungus would not grow well in liquid culture in the presence of greater concentrations of inhibitor. Inhibitors were not present in the uptake medium. Labelled substrate was added as described in section 2.3.1.

### 2.3.8 Outline of Methods Used to Study Competition Between Cations and Polyamines During Uptake

Mycelium was prepared as described in section 2.3.1. The fungus was grown at pH 7.0.

The uptake medium consisted of MOPS free acid buffer (3-(N-Morpholino) propanesulfonic acid) (Sigma Chemical Co., Poole, England) adjusted to pH 7.0 using sodium hydroxide. Uptake by both F. culmorum and G. graminis was studied. Two mM quantities of compounds containing individual cations were added to the MOPS buffer at the same time as the unlabelled substrate. The compounds used were, sodium nitrate, potassium chloride and magnesium glycerophosphate, and were chosen because of their presence within the Czapek Dox medium used in previous experiments. Radioactive substrate was added as described in section 2.3.1.

Uptake of putrescine, spermidine and spermine by F. culmorum and G. graminis was studied both in the presence and absence of each cation.

### 2.3.9 Details of Compounds Used

Throughout the treatise of the work undertaken, chemicals have been referred to without giving reference to the actual formulation of the compounds. The following list indicates more specifically the products used:

- Tween 20; polyoxethylene (20) sorbitan monolaurate.
- MGBG; methylglyoxal bis - (guanylhydrazone) dihydrochloride.
- Putrescine; putrescine dihydrochloride.
- Spermidine; dispermidine triphosphate.
- Spermine; spermine tetrahydrochloride.
- Arginine; L-arginine hydrochloride.
- Ornithine; L-ornithine hydrochloride.

All purchased from Sigma Chemical Co., Poole, England.

- 2-Hydrazinoornithine; 2-hydrazinoornithine monohydrochloride (Calbiochem-Behring Corp., California).
- CHA; sold as dicyclohexylamine (C<sub>6</sub>H<sub>11</sub>)<sub>2</sub> NH, (Adrich Chemical Co., Dorset, England).

Radioactive Chemicals:

- PUTRESCINE; (1, 4-C<sup>14</sup>) putrescine dihydrochloride  
S.A. = 118 mCi/mM
- SPERMIDINE; (C<sup>14</sup>) spermidine trihydrochloride  
S.A. = 117 mCi/mM
- SPERMINE; (C<sup>14</sup>) spermine tetrahydrochloride  
S.A. = 111 mCi/mM
- ORNITHINE; L-(U - C<sup>14</sup>) ornithine hydrochloride  
S.A. = 285 mCi/mM

- ARGININE; L-(U - C14) arginine monohydrochloride  
S.A. = 348 mCi/mM
- DFMO; D L-alpha-difluoromethyl (5 - C14) ornithine  
S.A. = 60 mCi/mM

All purchased from Amersham International PLC, Buckinghamshire, England.

The inhibitors of polyamine biosynthesis, DFMO,  $\Delta$ -MFMO,  $\Delta$ -MFMO.Me, RR-MAP and DFMA were gifts from Merrell-Dow Pharmaceutical Co., Cincinnati, USA and were described in the Introduction.

SECTION 3

RESULTS

### 3.1 EFFECT OF THE INHIBITORS ON INFECTION OF BARLEY BY ERYSIPHE GRAMINIS

The percentage of leaf area infected was established by visual assessment using a standard area diagram 6, 9 and 12 days after inoculation. The control treatment consisted of leaves which had been inoculated only.

The effect of application of water as both a pre- and post-inoculation spray was examined (Figure 4, Text). The pre-inoculation spray reduced infection levels on day 6 after inoculation but increased per cent infection on day 9. It is fair to say, considering the variability of the actual infection, that this treatment had little effect. However, the post-inoculation application of water significantly reduced infection, although not to the extent of the inhibitors of polyamine biosynthesis.

#### 3.1.1 Statistical Analysis

The standard error of the mean was calculated and significances were determined using a t-test. As results are expressed in the form of percentages, it was assumed that population variances were not equal. Therefore, the following formulae were used to calculate differences (taken from Parker, 1983):



$$\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S_1^2/N_1 + S_2^2/N_2}} = t \text{ with } f \text{ degrees of freedom}$$

$$1/f = u^2/N_1 - 1 + (1-u)^2/N_2 - 1$$

$$u = \frac{S_1^2/N_1}{S_1^2/N_1 + S_2^2/N_2}$$

$\bar{X}_1$  = mean of sample 1 (control)

$\bar{X}_2$  = mean of sample 2

$S_1^2$  = variance of sample 1

$S_2^2$  = variance of sample 2

$N_1$  = number of replicates in sample 1

$N_2$  = number of replicates in sample 2

### 3.1.2 Effect of ODC Inhibitors on Mildew Growth

Treatment with the ODC inhibitors DFMO,  $\Delta$ -MFMO,  $\Delta$ -MFMO.Me, RR-MAP and 2-hydrazinoornithine significantly reduced percentage of leaf area infected with mildew, when compared to the control (Figures 5, 6, 7 and 8, Text). A general trend observed with each inhibitor was that of greater mildew control with increasing concentration.

DFMO was applied as both pre-and post-inoculation treatments, the latter having a greater efficacy (Figure 5, Text). Indeed a 2.0 mM post-inoculation application of DFMO almost totally controlled mildew infection.

Due to their limited supply  $\Delta$ -MFMO and  $\Delta$ -MFMO.Me were only applied as post-inoculation treatments (Figure 6, Text). Both

inhibitors controlled mildew infection, with the 1.0 mM application of  $\Delta$ -MFMO.Me being the more efficient. However, at the lower concentration (0.5 mM), there appeared to be little difference in the efficacy of these two inhibitors.

RR-MAP significantly reduced pathogen infection (Figure 7, Text) although not as markedly as the other ODC inhibitors.

2-Hydrazinoornithine gave the greatest control if compared to the other ODC inhibitors of similar application concentrations (Figure 8, Text).

### 3.1.3 Effect of CHA on Mildew Growth

Both concentrations of CHA used significantly reduced mildew growth (Figure 8, Text). The inhibitor appeared to be more potent than RR-MAP,  $\Delta$ -MFMO,  $\Delta$ -MFMO.Me but less efficient than 2-hydrazinoornithine and MGBG and on a par with DFMO.

FIGURE 4. Effect of pre- or post-inoculation treatment with water on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.02$  for pre-inoculation treatment day 9, and  $P = 0.001$  for others.

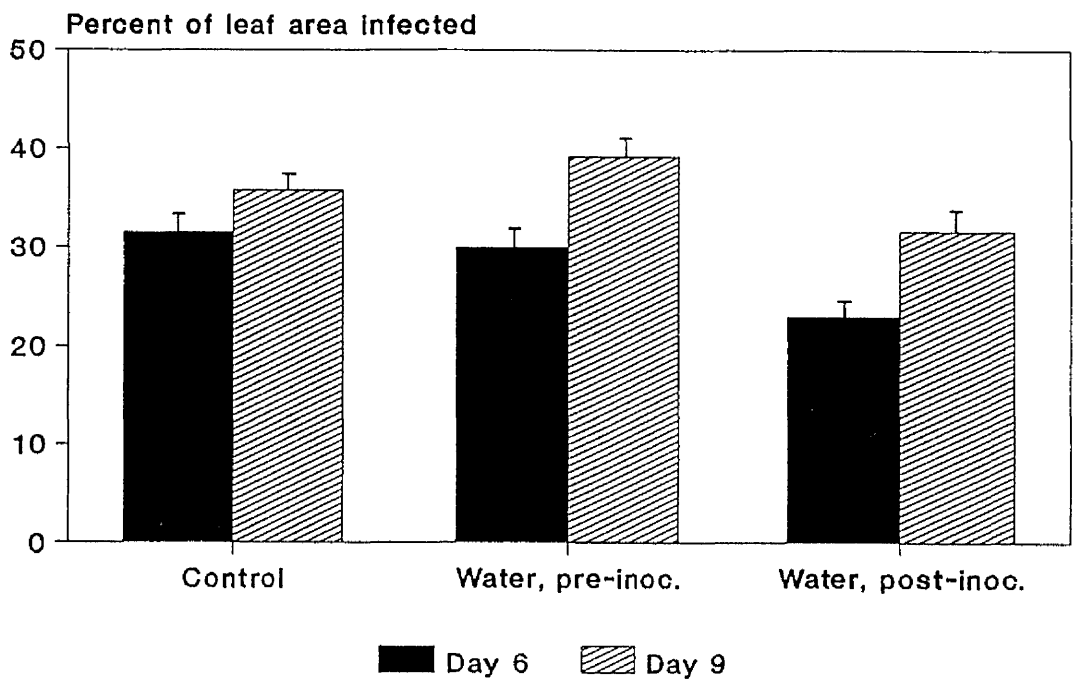
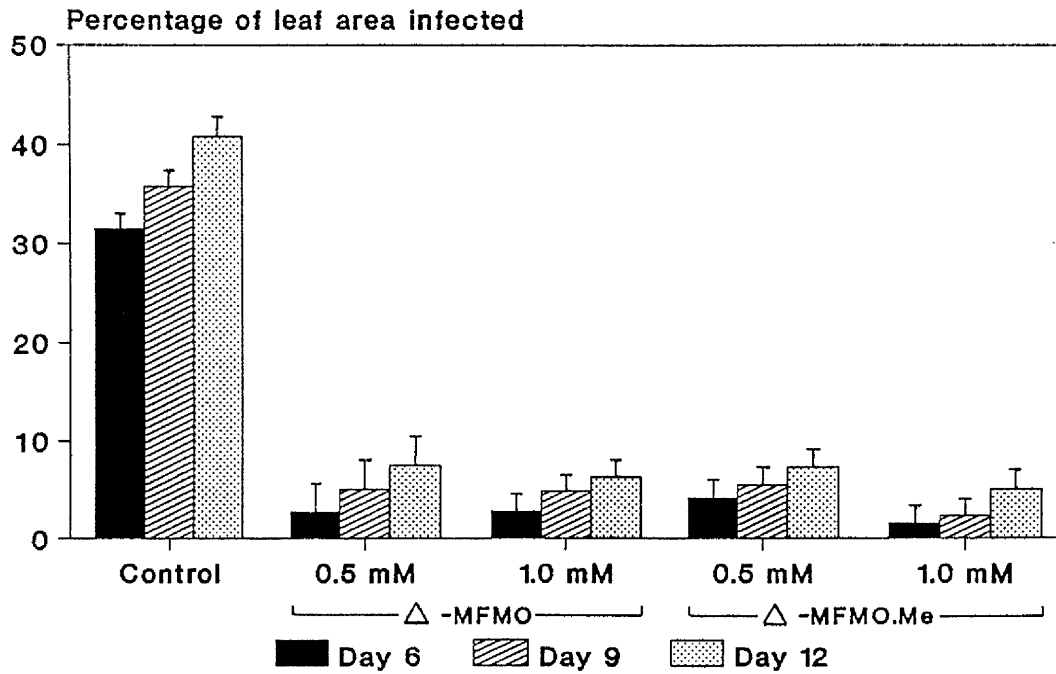
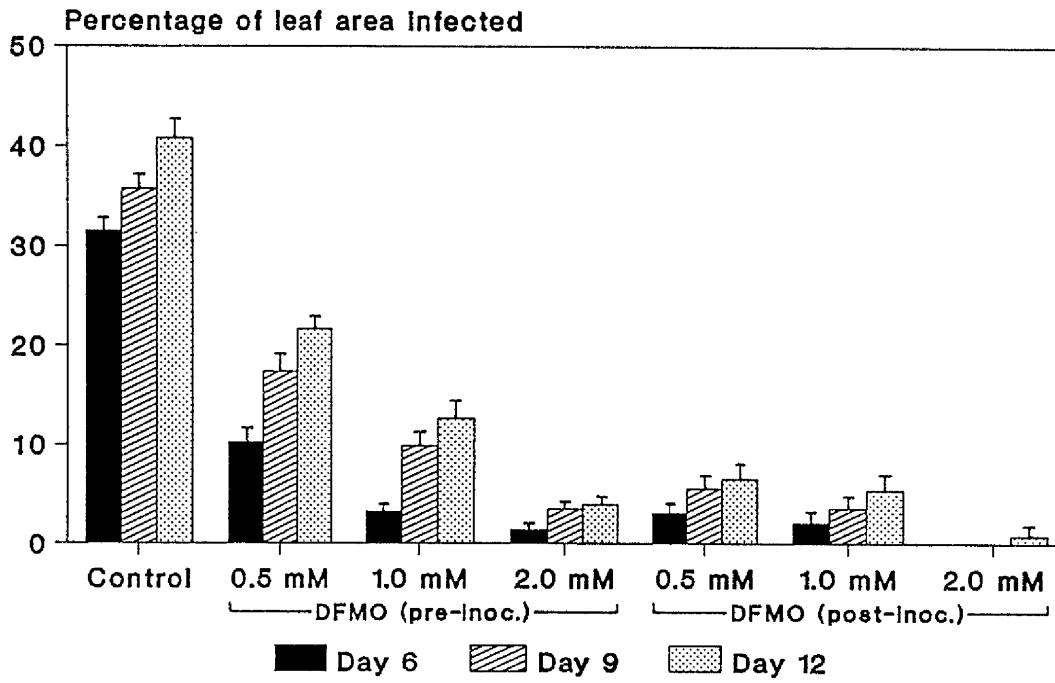


FIGURE 5. Effect of pre- or post-inoculation treatment with DFMO on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

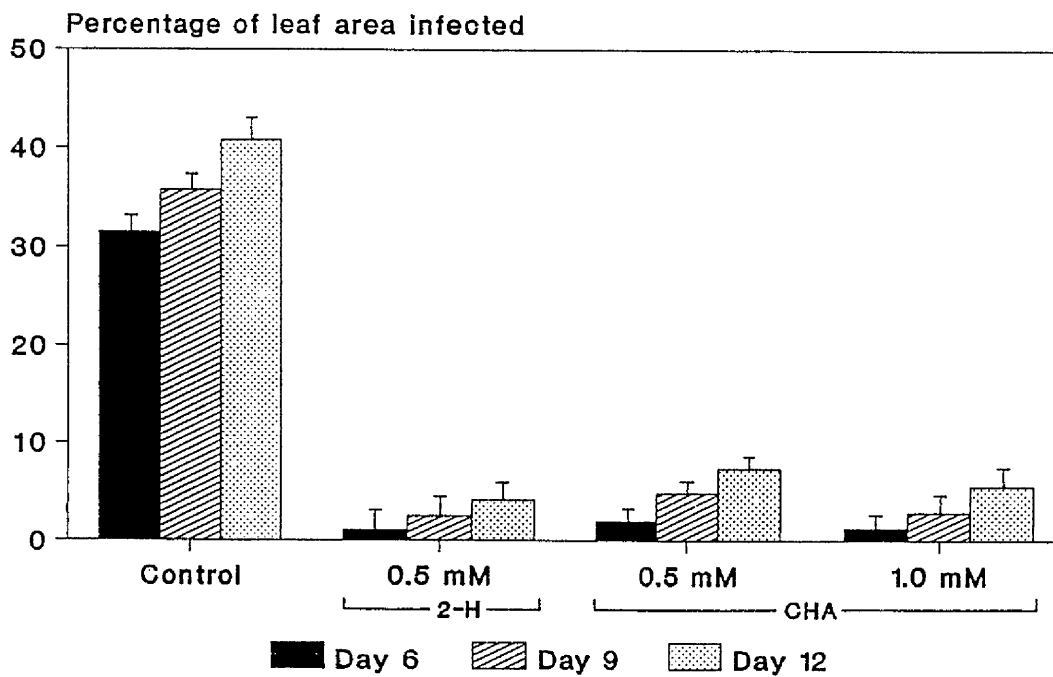
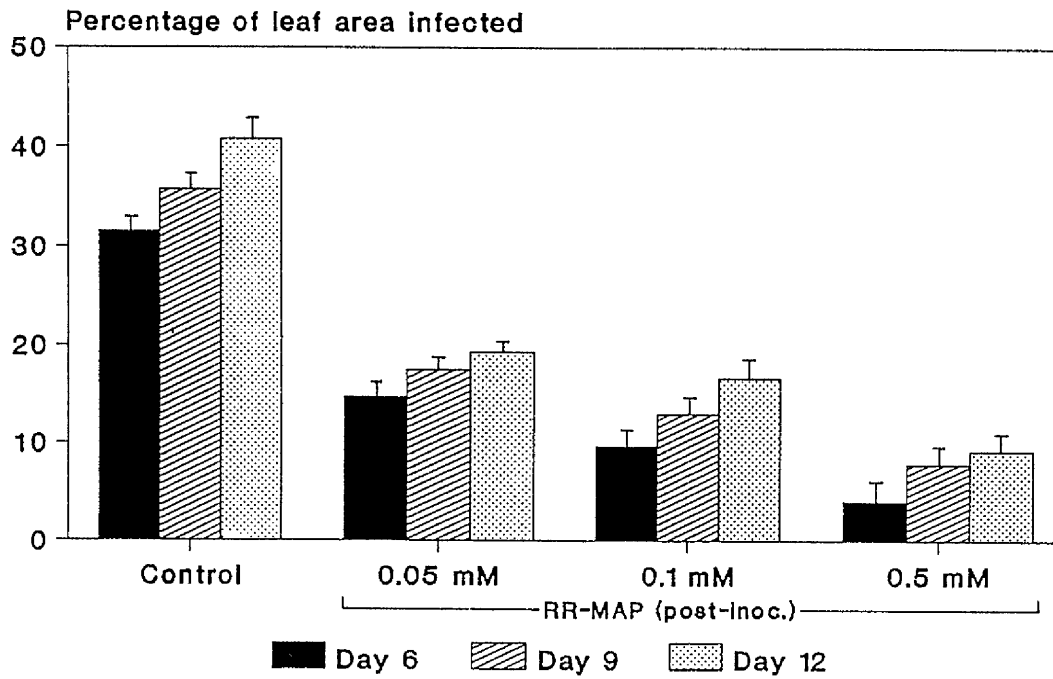
FIGURE 6. Effect of post-inoculation treatment with  $\Delta$ -MFMO and  $\Delta$ -MFMO.Me on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.



Post-Inoc.

FIGURE 7. Effect of post-inoculation treatments with RR-MAP on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

FIGURE 8. Effect of post-inoculation treatments with 2-hydrazinoornithine (2-H) and CHA on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.



Post-Inoc.



#### 3.1.4 Effect of MGBG and mixtures of DFMO + MGBG on Mildew Growth

Both pre- and post-inoculation applications of MGBG (Figures 9 and 10, Text) significantly reduced mildew infection, with the post-inoculation sprays being more efficient. Percentage of leaf area infected after treatment with a post-inoculation spray of 1.0 mM MGBG was minimal and infection was very slight after treatment with 2.0 mM of this inhibitor.

A post-inoculation application of a mixture of DFMO plus MGBG also significantly reduced the level of mildew infection (Figure 11, Text). The mixture enabled control to be attained at lower concentrations of the individual inhibitors. For example, the mixture of MGBG, 0.5 mM and DFMO, 1.0 mM reduced mildew growth to a level of 1.7% on day 9 after inoculation. MGBG, 0.5 mM limited growth to 2.7% and DFMO, 1.0 mM to 3.6% on the same day. Therefore, concentrations greater than 1.0 mM DFMO or 0.5 mM MGBG would be needed if each inhibitor were applied individually.

FIGURE 9. Effect of pre-inoculation treatments with MGBG on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

FIGURE 10. Effect of post-inoculation treatments with MGBG on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

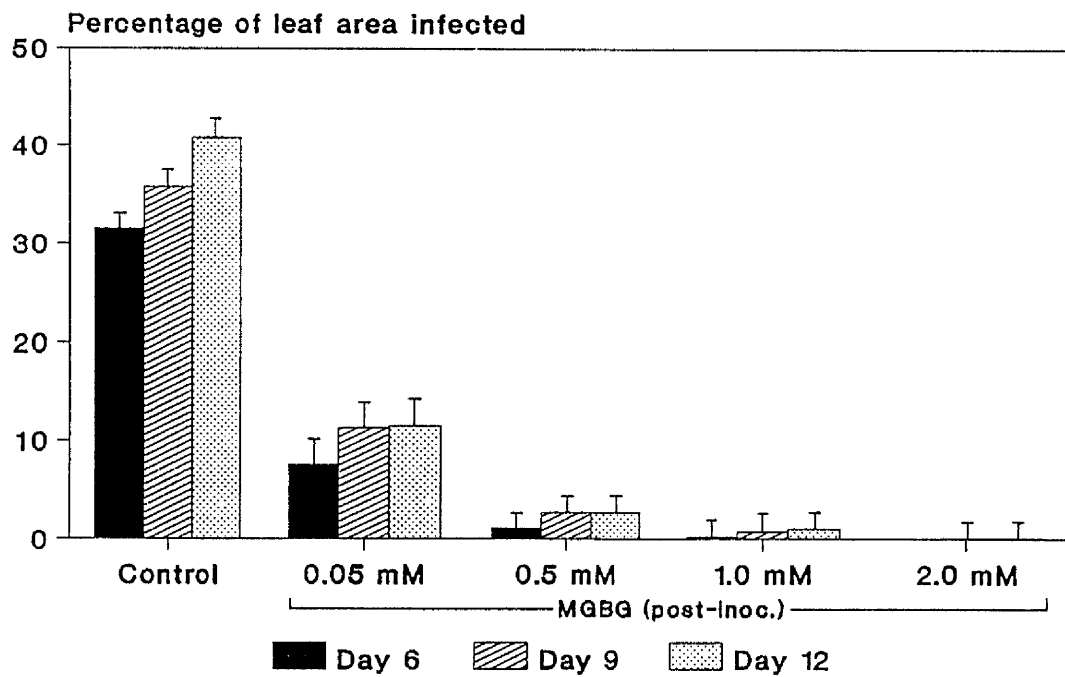
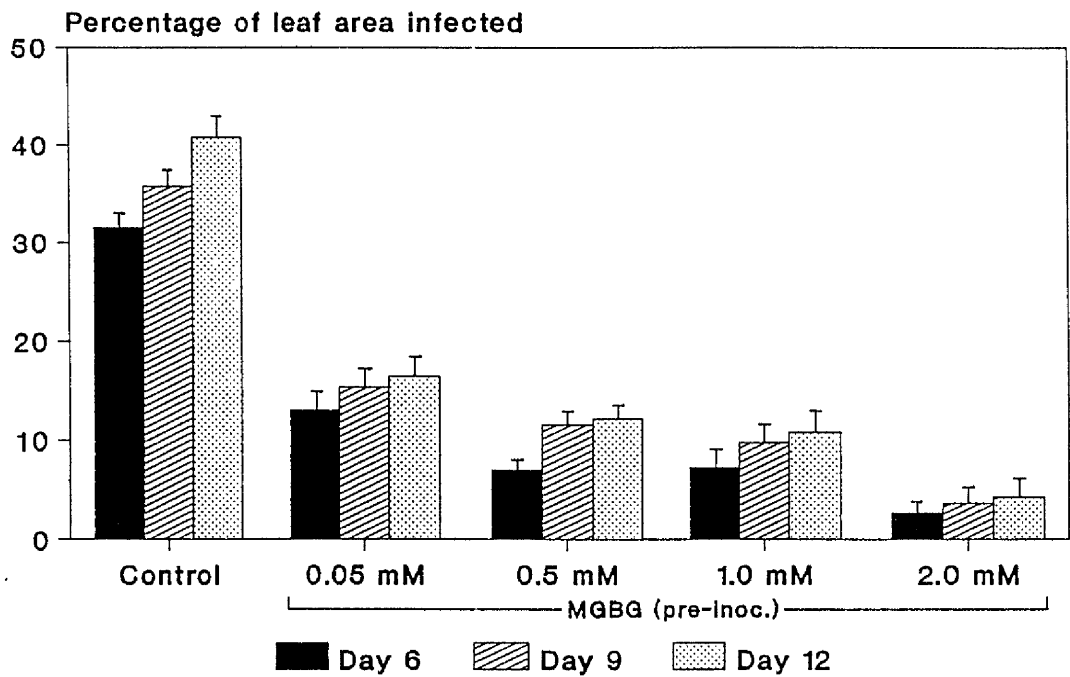
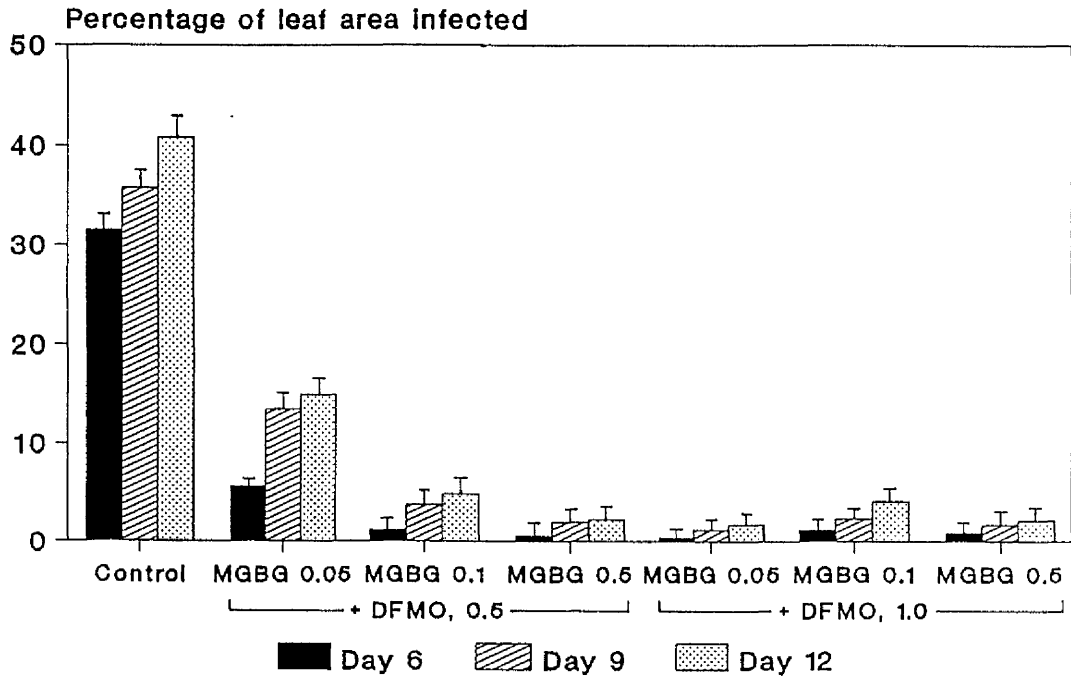


FIGURE 11. Effect of post-inoculation treatments with MGBG + DFMO on per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.



(mM) Post-inoc.

### 3.1.5 Effect of Polyamines + DFMO and Polyamines on Mildew Growth

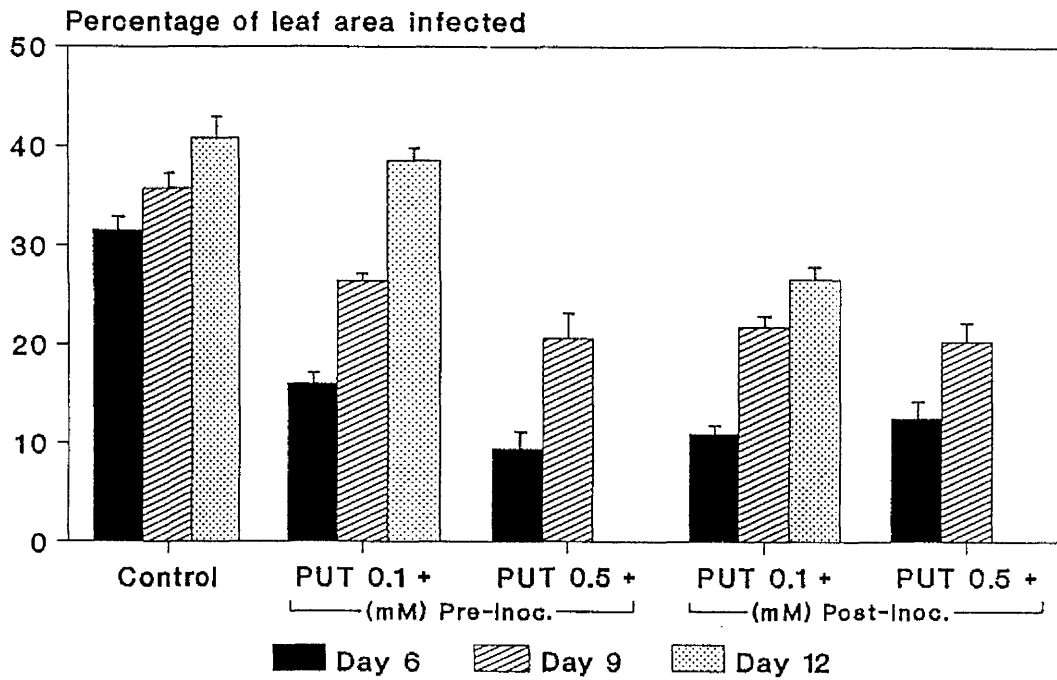
If inhibition of fungal polyamine biosynthesis is responsible for reducing fungal growth, then the effects of the inhibitors should, theoretically, be reversed by the subsequent addition of polyamines. This hypothesis was tested by giving barley leaves a pre- or post-inoculation spray of DFMO supplemented with either putrescine or spermidine. Although such treatment never completely eliminated the inhibitory effects of DFMO, both putrescine and spermidine substantially reversed the effect of DFMO.

The pre-inoculation treatment incorporating DFMO and 0.1 mM putrescine (Figure 12, Text) or spermidine (Figure 13, Text) increased mildew infection to a greater level than the 0.5 mM putrescine + DFMO or spermidine + DFMO pre-inoculation treatments, or the post-inoculation sprays of either 0.1 mM or 0.5 mM.

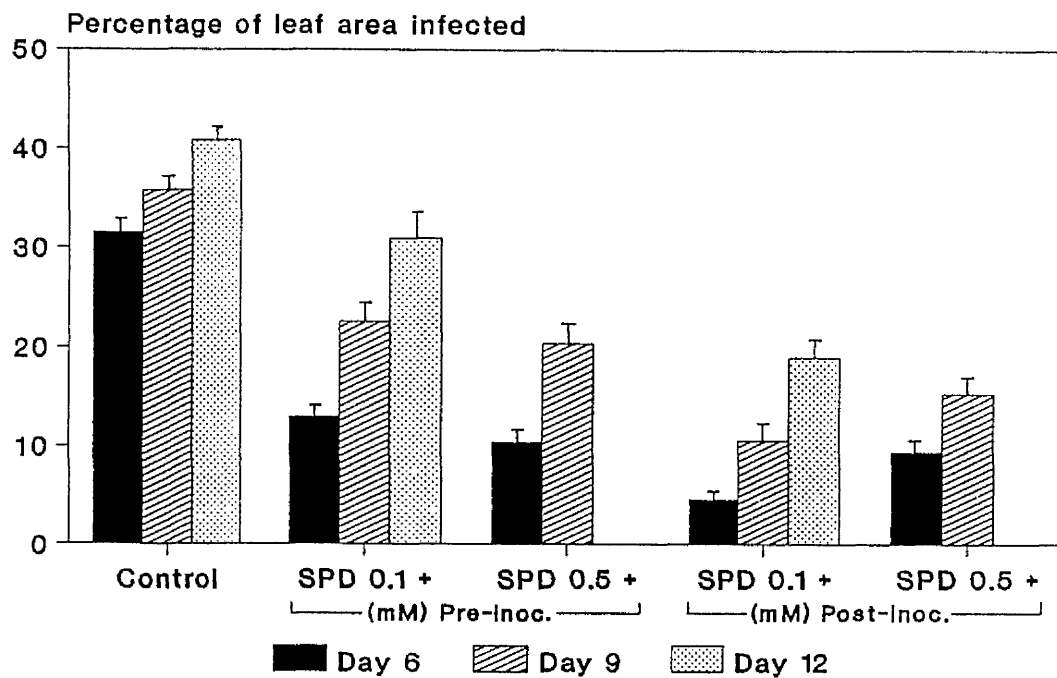
Table 2 (Text) shows the effects of putrescine and spermidine on mildew infection. The pre-inoculation treatment with putrescine increased mildew growth on days 6 and 9, but spermidine did not enhance fungal infection until day 9 after inoculation. However, these apparent increases resulting from putrescine treatment were not statistically significant. The post-inoculation treatments with putrescine and spermidine actually reduced infection.

FIGURE 12. Effect of pre- or post-inoculation treatment with DFMO + putrescine on per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

FIGURE 13. Effect of pre- or post-inoculation treatment with DFMO + spermidine on per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.



+ DFMO, 1.0 mM



+ DFMO, 1.0 mM



TABLE 2. Effect of putrescine and spermidine (0.5 mM) on infection of barley leaves by *E. graminis*. Results are expressed as percentage of leaf area infected  $\pm$  the standard error of the mean. Significant differences are shown at P = 0.02 a; P = 0.001 b.

DAYS AFTER INOCULATION	CONTROL (Inoc. only)	PUT (Pre-inoc.)	PUT (Post-inoc.)	SPD (Pre-inoc.)	SPD (Post-inoc.)
6	31.5 $\pm$ 1.51	33.2 $\pm$ 2.60	19.0 $\pm$ 1.95 <sup>b</sup>	27.0 $\pm$ 2.42 <sup>b</sup>	12.2 $\pm$ 1.61 <sup>b</sup>
9	35.7 $\pm$ 1.45	41.5 $\pm$ 2.57	29.7 $\pm$ 2.02 <sup>b</sup>	37.5 $\pm$ 2.41 <sup>a</sup>	19.7 $\pm$ 2.34 <sup>b</sup>

### 3.1.6 Timing of Inhibitor Applications

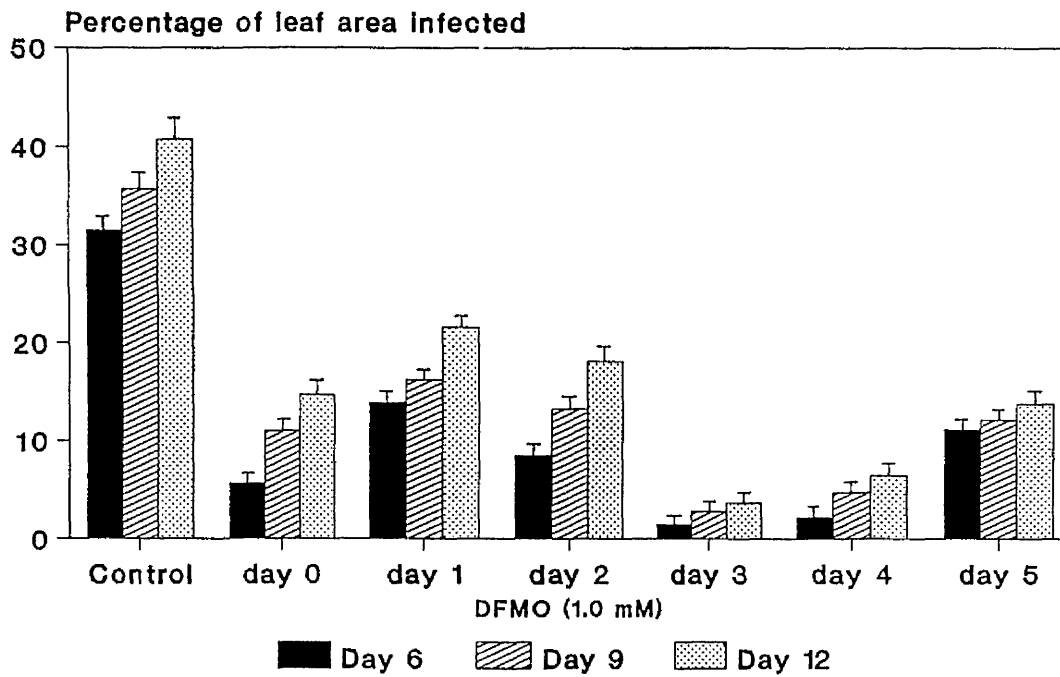
The timing of inhibitor applications was examined further by spraying with DFMO, MGBG or DFMO + MGBG just prior to inoculation (day 0, pre-inoculation spray) or 1 to 5 days after inoculation with mildew spores (Figures 14, 15 and 16, Text). The results for the different inhibitors show similar trends. The percentage infection was greatest when treatment occurred on the first day after inoculation (day 1). Mildew growth was further reduced when the inhibitors were applied on days 2 and 3, with infection increasing on leaves which were treated on days 4 and 5. The greatest reduction was obtained when leaves were sprayed 3 days after inoculation.

A similar experiment was carried out with DFMA and, although all treatments significantly reduced the per cent of leaf area infected, DFMA was most effective as a pre-inoculation treatment. The per cent of leaf area infected with mildew increased with any delay in inhibitor application (Figure 17, Text).

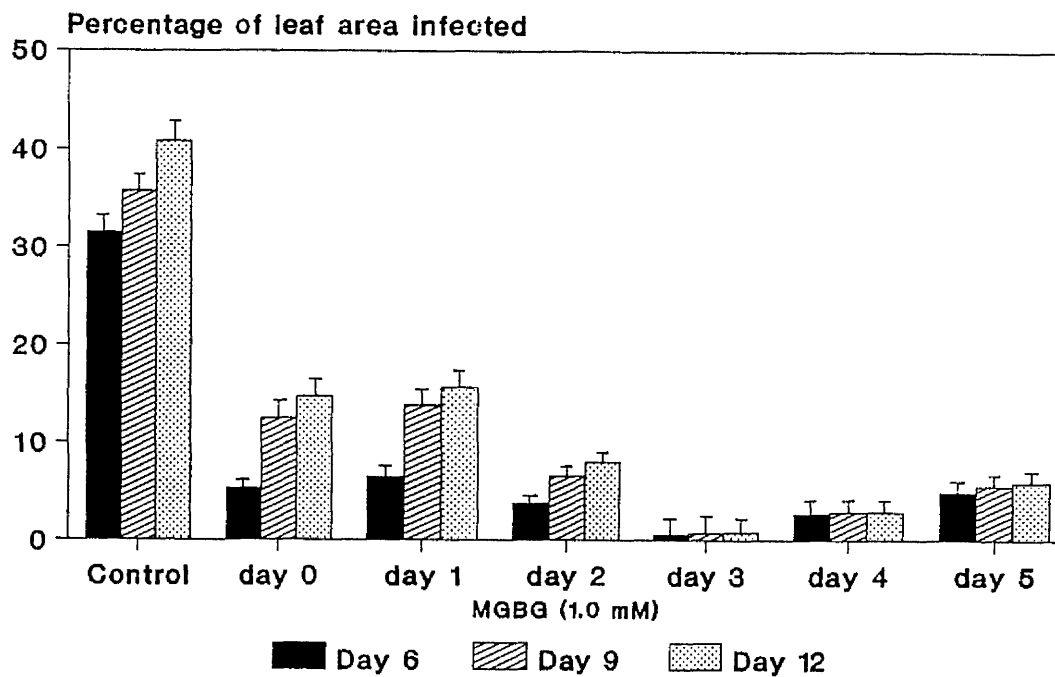
No apparent phytotoxic effects were observed on leaves which had been sprayed with inhibitor solutions in the absence of inoculum.

FIGURE 14. Effect of application of DFMO at different times on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

FIGURE 15. Effect of application of MGBG at different times on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.



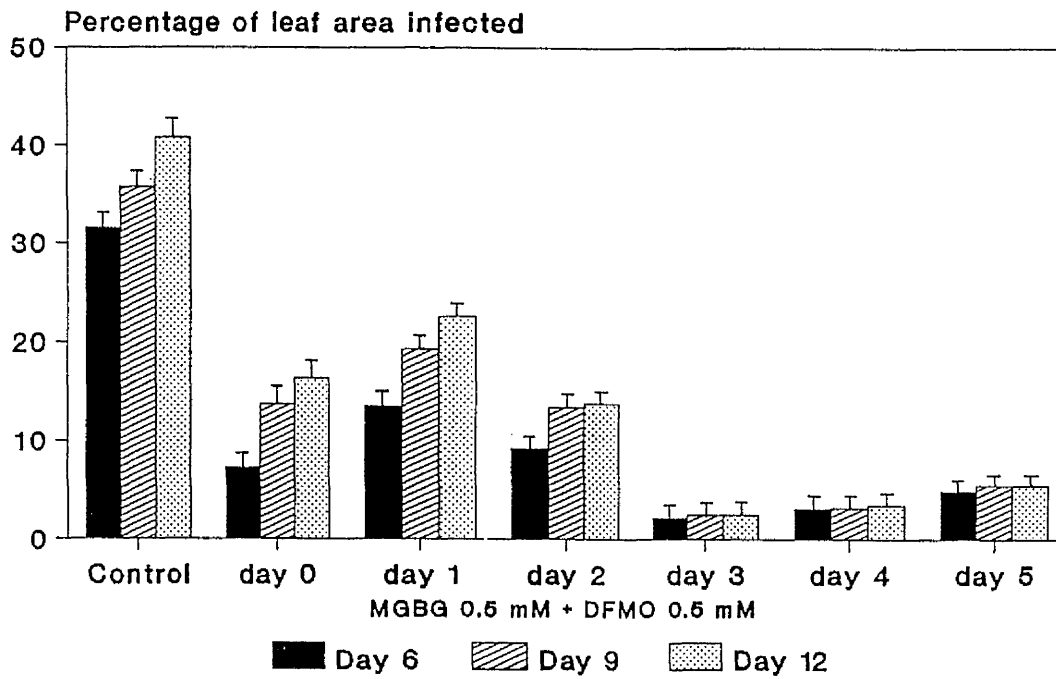
Day 0 = pre-inoc. treatment



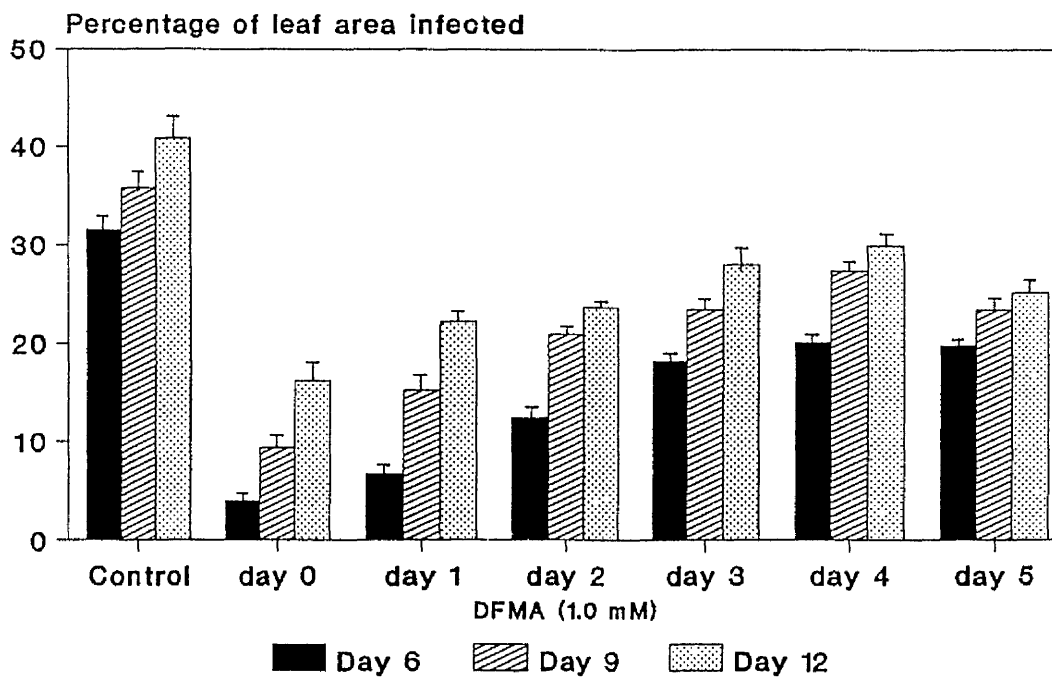
Day 0 = pre-inoc. treatment

FIGURE 16. Effect of application of MGBG + DFMO at different times on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.

FIGURE 17. Effect of application of DFMA at different times on the per cent of leaf area infected with mildew. Vertical bars represent the standard error of the mean and significant differences are  $P = 0.001$  for each treatment.



Day 0 = pre-inoc. treatment



Day 0 = pre-inoc. treatment

### 3.2 EFFECT OF THE INHIBITORS ON GROWTH OF FUNGI GROWN IN VITRO

The effects of inhibitors of polyamine biosynthesis on the mycelial growth and cell length and diameter of several species were studied.

#### 3.2.1 Statistical Analysis

The standard error of the mean was calculated and significances were determined using a t-test. Population variances were assumed to be equal and thus the following formula was used to calculate differences (taken from Parker, 1983):

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S_1^2/N_1 + S_2^2/N_2}}$$

where t has  $(N_1 + N_2) - 2$  degrees of freedom.

$\bar{X}_1$  = mean of sample 1 (control)

$\bar{X}_2$  = mean of sample 2

$N_1$  = number of replicates in sample 1

$N_2$  = number of replicates in sample 2

$S_1^2$  = variance of sample 1

$S_2^2$  = variance of sample 2.

#### 3.2.2 Effect of ODC Inhibitors on Fungal Growth

Table 3 (Text) shows the effects of ODC inhibitors on growth of P. teres, G. graminis, S. nodorum, F. culmorum and U. maydis. The inhibitory effects vary both between species and between particular inhibitors.

DFMO, at a concentration of 4.0 mM reduced growth of the grey form of P. teres by 80% and the growth of G. graminis by 30%. Although significant decreases in the growth of the orange form of P. teres and of F. culmorum were obtained with 4.0 mM DFMO, the reductions were small. DFMO had no inhibitory effect on growth of S. nodorum and 2.0 mM DFMO actually stimulated growth. Growth of the biotroph, U. maydis, was reduced by 20% at the 4.0 mM concentration of DFMO.

$\Delta$ -MFMO and  $\Delta$ -MFMO.Me reduced growth of P. teres (both forms) and F. culmorum more effectively than DFMO at the 1.0 mM concentration.

RR-MAP appeared to effect a greater reduction in growth of the orange form of P. teres and F. culmorum than DFMO. Lower concentrations of DFMO,  $\Delta$ -MFMO and  $\Delta$ -MFMO.Me caused a stimulation of growth of S. nodorum, and RR-MAP had a similar effect on the grey form of P. teres.

Mycelial growth of P. infestans was effectively controlled by 1.0 mM DFMO and particularly by a mixture of DFMO + DFMA (1.0 + 1.0 mM). DFMA administered individually, significantly increased growth of this fungus.

P. ultimum remained unaffected by DFMO and DFMA when added individually but when media was supplemented with a 1.0 + 1.0 mM mix of the two inhibitors, growth was stimulated (Table 4, Text).



Tables I to VIII (Appendix) show the effects of the ODC inhibitors on the fungi over a period of time, the length of which depended on the speed of mycelial growth. The results shown in Table V (Appendix) indicate that growth of F. culmorum was reduced on day 3 compared to that on day 2 and Table IV (Appendix) demonstrates that although growth of S. nodorum was not controlled by the inhibitors, stimulation of growth by the compounds was less on day 6 than on day 2. However, DFMO 1.0 and 2.0 mM caused an enhanced stimulation of growth on day 6.

The inhibitory effects of the compounds on the orange form of P. teres (Table II, Appendix) did not alter very much with time, although the 4.0 mM DFMO treatment did reduce growth on day 6 compared to days 2 and 4. For the grey form of this species (Table I, Appendix), a decrease in the potency of the inhibitors with time was observed, except for DFMO 2.0 and 4.0 mM treatments which gave similar levels of control with time.

Table III (Appendix) shows that the ODC inhibitors controlled growth of G. graminis more effectively on day 2 than on day 6, and U. maydis also increased growth relative to the control, on the final day of measuring (Table VIII, Appendix).

Growth of P. infestans was limited to a greater extent on day 15 than on day 9 and stimulation of growth by DFMA was less pronounced on the final day of measuring although the enhancement effect was still significant (Table VI, Appendix). In contrast, the stimulation of growth of P. ultimum (Table VII, Appendix) by DFMA and DFMO + DFMA was greater on day 2 than on day 1.

TABLE 3. Effect of ODC inhibitors on growth of P. teres, G. graminis and S. nodorum on the sixth day after inoculation, F. culmorum on the third day after inoculation and U. maydis on the twenty-first day after inoculation. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c. \*indicates that mycelium had reached the edge of the Petri dishes.

TREATMENT (nM)	<u>P. TERES</u> (grey)	<u>P. TERES</u> (orange)	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>	<u>U. MAYDIS</u>
<b>DFMO:</b>						
1.0	96.8 $\pm$ 4.8	*	86.0 $\pm$ 1.3 <sup>c</sup>	100.6 $\pm$ 0.5	98.7 $\pm$ 1.6	84.7 $\pm$ 1.6 <sup>b</sup>
2.0	61.8 $\pm$ 9.8 <sup>c</sup>	*	72.4 $\pm$ 2.4 <sup>c</sup>	105.9 $\pm$ 0.3 <sup>b</sup>	96.4 $\pm$ 1.7	-
4.0	20.4 $\pm$ 5.3 <sup>c</sup>	85.6 $\pm$ 0.7 <sup>c</sup>	69.6 $\pm$ 0.6 <sup>c</sup>	98.2 $\pm$ 1.1	78.9 $\pm$ 1.7 <sup>c</sup>	79.2 $\pm$ 1.8 <sup>b</sup>
<b><math>\Delta</math>-MFMO:</b>						
0.5	94.3 $\pm$ 3.0	87.0 $\pm$ 1.5 <sup>c</sup>	79.2 $\pm$ 2.8 <sup>c</sup>	103.5 $\pm$ 0.7 <sup>a</sup>	76.0 $\pm$ 2.9 <sup>c</sup>	-
1.0	79.6 $\pm$ 5.4 <sup>b</sup>	92.1 $\pm$ 0.8 <sup>c</sup>	92.5 $\pm$ 1.5 <sup>b</sup>	97.9 $\pm$ 0.5	84.7 $\pm$ 3.1 <sup>c</sup>	-
<b><math>\Delta</math>-MFMO.Me:</b>						
0.5	103.6 $\pm$ 2.4	93.0 $\pm$ 1.3 <sup>b</sup>	94.7 $\pm$ 1.6 <sup>a</sup>	105.9 $\pm$ 1.3 <sup>b</sup>	89.3 $\pm$ 2.9 <sup>b</sup>	-
1.0	92.1 $\pm$ 1.2 <sup>a</sup>	94.9 $\pm$ 0.6 <sup>b</sup>	96.0 $\pm$ 2.9	99.1 $\pm$ 0.6	76.6 $\pm$ 0.9 <sup>c</sup>	-
<b>RR-MAP:</b>						
0.5	110.4 $\pm$ 1.3 <sup>b</sup>	98.6 $\pm$ 1.1	95.3 $\pm$ 2.2 <sup>a</sup>	101.8 $\pm$ 1.1	90.6 $\pm$ 2.4 <sup>b</sup>	-
1.0	101.8 $\pm$ 3.2	94.9 $\pm$ 0.7 <sup>b</sup>	93.5 $\pm$ 1.2 <sup>b</sup>	98.2 $\pm$ 0.6	89.9 $\pm$ 5.2 <sup>a</sup>	-

TABLE 4. Effect of inhibitors of polyamine biosynthesis on growth of *P. ultimum* on the first day after inoculation and *P. infestans* on the fifteenth day after inoculation. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	<i>P. ULTIMUM</i>	<i>P. INFESTANS</i>
<b>DFMO:</b>		
1.0	99.3 $\pm$ 1.5	65.2 $\pm$ 3.1 <sup>a</sup>
<b>DFMA:</b>		
1.0	102.9 $\pm$ 2.7	119.1 $\pm$ 3.7 <sup>b</sup>
<b>DFMO + DFMA:</b>		
1.0 + 1.0	105.8 $\pm$ 1.2 <sup>a</sup>	56.7 $\pm$ 2.7 <sup>c</sup>

### 3.2.3 Effect of MGBG and Mixtures of DFMO + MGBG on Fungal Growth

MGBG was found to be the most powerful inhibitor of fungal growth examined. Thus, 2.0 mM MGBG caused substantial reductions in the growth of all fungi examined (Table 5, Text). G. graminis appeared to be the most susceptible to the effects of this compound: 0.5 mM MGBG reduced growth by almost 50% and no growth was observed at 2.0 mM MGBG. Growth of the grey form of P. teres was reduced by 80% compared to almost a 25% reduction in growth of the orange form. The growth of both S. nodorum and F. culmorum was significantly reduced by this inhibitor.

Mixtures of DFMO + MGBG were no more effective than MGBG on its own (Table 5, Text), although P. teres (grey form) appeared to be relatively more susceptible to the mixture at low concentrations than to either inhibitor individually at equivalent molarities.

Tables IX to XIII (Appendix) show the effects of MGBG and a mixture of DFMO + MGBG on the fungi over a period of time. The effect of MGBG on the growth of the orange form of P. teres (Table X, Appendix) was less pronounced with time and, therefore, growth was relatively less inhibited on day 6 than day 2. A similar effect on the grey form of P. teres was observed, although the 2.0 mM concentration led to a reduction in growth by day 6. G. graminis (Table XI, Appendix) was slightly less affected by MGBG with time. However, growth of both S. nodorum (Table XII, Appendix) and F. culmorum (Table XIII, Appendix) was

reduced more with time. The effects of the mixture of DFMO + MGBG on the fungi were similar to those observed for MGBG on its own.

#### 3.2.4 Effect of CHA on Fungal Growth

Growth of G. graminis was significantly reduced by CHA, especially at 2.0 and 4.0 mM, which reduced growth by 50% and 80% respectively. Although CHA caused a small reduction in growth of the orange form of P. teres, growth of the other fungi examined was not significantly affected (Table 5, Text).

Tables IX to XIII (Appendix) show that there was some variation in the effects of CHA with time, but with the exception of F. culmorum, these effects were relatively slight. Growth of F. culmorum on day 2 (Table XIII, Appendix) was greatly enhanced by the addition of CHA to the medium, but this effect was not observed on day 3.

TABLE 5. Effect of inhibitors of polyamine biosynthesis on growth of P. teres, G. graminis and S. nodorum on the sixth day after inoculation, F. culmorum on the third day after inoculation and U. maydis on the twenty-first day after inoculation. Results are expressed as percentage of control value (= 100%) ± the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT ( $\mu$ M)	<u>P. TERES</u> (grey)	<u>P. TERES</u> (orange)	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>	<u>U. MAYDIS</u>
<b>MGBG:</b>						
0.5	98.9 ± 1.2	87.6 ± 0.5 <sup>C</sup>	53.4 ± 2.2 <sup>C</sup>	80.3 ± 1.1 <sup>C</sup>	90.9 ± 1.0 <sup>b</sup>	-
1.0	95.0 ± 1.4	83.7 ± 0.5 <sup>C</sup>	11.5 ± 5.7 <sup>C</sup>	65.9 ± 1.3 <sup>C</sup>	84.4 ± 3.6 <sup>b</sup>	82.6 ± 3.4 <sup>b</sup>
2.0	20.7 ± 3.1 <sup>C</sup>	74.4 ± 0.4 <sup>C</sup>	0.0 <sup>C</sup>	50.0 ± 2.4 <sup>C</sup>	58.8 ± 5.5 <sup>C</sup>	74.3 ± 2.2 <sup>C</sup>
<b>DFMO + MGBG:</b>						
0.5+0.5	25.4 ± 2.1 <sup>C</sup>	84.8 ± 0.7 <sup>C</sup>	53.1 ± 1.0 <sup>C</sup>	81.8 ± 1.1 <sup>C</sup>	81.5 ± 2.0 <sup>C</sup>	80.6 ± 2.7 <sup>b</sup>
1.0+1.0	21.1 ± 2.9 <sup>C</sup>	80.0 ± 0.3 <sup>C</sup>	23.0 ± 4.9 <sup>C</sup>	71.8 ± 1.6 <sup>C</sup>	79.5 ± 2.0 <sup>C</sup>	72.9 ± 2.4 <sup>C</sup>
<b>CHA:</b>						
0.5	105.4 ± 1.9	92.7 ± 0.8 <sup>C</sup>	88.5 ± 2.0 <sup>C</sup>	100.3 ± 0.9	104.2 ± 0.6	-
1.0	102.1 ± 1.0	91.5 ± 0.7 <sup>C</sup>	88.2 ± 2.0 <sup>C</sup>	101.5 ± 1.9	103.2 ± 1.4	-
2.0	103.6 ± 1.4	89.3 ± 1.2 <sup>C</sup>	49.7 ± 6.9 <sup>C</sup>	102.1 ± 1.1	97.4 ± 1.4	-
4.0	83.6 ± 7.9 <sup>a</sup>	83.4 ± 2.3 <sup>C</sup>	20.8 ± 5.5 <sup>C</sup>	98.8 ± 1.1	94.2 ± 3.1	-

### 3.2.5 Effect of Polyamines and DFMO + Polyamines on Fungal Growth

Some variability was observed in the response of the various fungi to putrescine and spermidine. Thus, the addition of putrescine or spermidine to the medium caused small, but significant increases in growth of the grey form of P. teres and of F. culmorum, and growth of G. graminis was also slightly increased by exogenous spermidine. However, 1.0 mM spermidine reduced growth of the orange form of P. teres and of S. nodorum (Table 6, Text).

The addition of 1.0 mM putrescine or spermidine to 4.0 mM DFMO increased growth of the grey form of P. teres and of F. culmorum, but slightly reduced growth of the other fungi (Table 6, Text).

Tables XIV to XVIII (Appendix) show the effects of polyamines and polyamines + DFMO on growth of the fungi over a period of time. Growth of the orange form of P. teres was enhanced by the addition of putrescine on day 2, but this effect was not observed on day 6. The effects of the polyamines on G. graminis did not appreciably alter with time (Table XVI, Appendix) and although there were observable variations in effects upon the other fungi, these effects were reduced with time (for example, Table XVII, Appendix, for S. nodorum and Table XVIII, Appendix, for F. culmorum).

TABLE 6. Effect of polyamines and polyamines plus DFMO on growth of *P. teres*, *G. graminis* and *S. nodorum* on the sixth day after inoculation and *F. culmorum* on the third day after inoculation. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	<u>P. TERES</u> (grey)	<u>P. TERES</u> (orange)	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
<b>PUT:</b>					
0.5	105.4 $\pm$ 2.1	99.2 $\pm$ 0.6	99.4 $\pm$ 2.1	98.5 $\pm$ 0.5	111.7 $\pm$ 1.5 <sup>b</sup>
1.0	115.7 $\pm$ 3.2 <sup>b</sup>	102.0 $\pm$ 0.2	102.2 $\pm$ 1.2	97.1 $\pm$ 0.8	114.6 $\pm$ 1.3 <sup>c</sup>
<b>SPD:</b>					
0.5	103.9 $\pm$ 5.9	98.9 $\pm$ 0.7	105.3 $\pm$ 1.2 <sup>b</sup>	104.7 $\pm$ 0.6 <sup>b</sup>	109.7 $\pm$ 1.8 <sup>b</sup>
1.0	113.9 $\pm$ 2.7 <sup>b</sup>	94.1 $\pm$ 1.2 <sup>b</sup>	107.8 $\pm$ 2.5 <sup>a</sup>	93.2 $\pm$ 0.7 <sup>b</sup>	111.4 $\pm$ 0.9 <sup>c</sup>
<b>DFMO + PUT:</b>					
4.0+1.0	110.4 $\pm$ 6.9	95.2 $\pm$ 0.9 <sup>b</sup>	89.4 $\pm$ 2.4 <sup>b</sup>	97.1 $\pm$ 0.5	106.8 $\pm$ 2.8
<b>DFMO + SPD:</b>					
4.0+1.0	116.1 $\pm$ 5.2 <sup>a</sup>	95.8 $\pm$ 0.5 <sup>b</sup>	96.3 $\pm$ 2.2	99.1 $\pm$ 1.3	110.4 $\pm$ 1.5 <sup>b</sup>



### 3.2.6 Effect of Inhibitors and Polyamines on Cell Length and Diameter

Cell lengths of P. teres were significantly reduced by exposure to all of the inhibitors examined, as well as by putrescine (Tables 7, 8 and 9, Text). None of these treatments produced much effect on cell diameter (Tables XIX, XX and XXI, Appendix). DFMO, MGBG and a mixture of DFMO + MGBG reduced cell length in G. graminis and the ODC inhibitor  $\Delta$ -MFMO increased cell length (Tables 7 and 8, Text). Other treatments produced little effect on cell length or cell diameter in this fungus. Most of the inhibitors used increased cell lengths of S. nodorum, although CHA did not. Apart from spermidine, which also increased cell length, none of the other treatments affected cell length in this fungus. Cell diameters were not altered by exposure to inhibitors or polyamines in this fungus. In a similar vein, all of the inhibitors increased cell length of F. culmorum, although here, increases in cell diameter were also observed. Treatment with polyamines or inhibitors plus polyamines produced little effect on either length or diameter of fungal cells (Tables 7, 8 and 9, Text; XIX, XX and XXI, Appendix).

TABLE 7. Effect of ODC inhibitors on cell length of P. teres, G. graminis, S. nodorum and F. culmorum on the sixth day after inoculation  $\pm$  the standard error of the mean. Results for grey and orange forms of P. teres are pooled. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	CELL LENGTH ( $\mu$ m)			
	<u>P. TERES</u>	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
Control	41.3 $\pm$ 3.3	13.9 $\pm$ 1.3	10.6 $\pm$ 1.0	19.4 $\pm$ 1.7
<b>DFMO:</b>				
1.0	28.4 $\pm$ 2.4 <sup>b</sup>	11.4 $\pm$ 0.8	17.1 $\pm$ 1.0 <sup>c</sup>	26.6 $\pm$ 2.6 <sup>a</sup>
4.0	22.3 $\pm$ 1.7 <sup>c</sup>	10.8 $\pm$ 0.9 <sup>a</sup>	19.2 $\pm$ 1.1 <sup>c</sup>	24.9 $\pm$ 4.8 <sup>a</sup>
<b><math>\Delta</math>-MFMO:</b>				
0.5	24.3 $\pm$ 3.0 <sup>c</sup>	17.7 $\pm$ 2.2	13.5 $\pm$ 0.8 <sup>a</sup>	-
1.0	25.3 $\pm$ 1.4 <sup>c</sup>	17.1 $\pm$ 1.4	13.9 $\pm$ 0.9 <sup>a</sup>	26.6 $\pm$ 2.3 <sup>a</sup>
<b><math>\Delta</math>-MFMO.Me:</b>				
0.5	21.9 $\pm$ 3.3 <sup>c</sup>	18.4 $\pm$ 1.7 <sup>a</sup>	13.5 $\pm$ 2.2	-
1.0	24.7 $\pm$ 1.7 <sup>c</sup>	14.4 $\pm$ 1.2	14.1 $\pm$ 1.0 <sup>a</sup>	29.6 $\pm$ 2.7 <sup>c</sup>
<b>RR-MAP:</b>				
0.5	-	15.2 $\pm$ 1.3	15.4 $\pm$ 1.3 <sup>b</sup>	-
1.0	33.5 $\pm$ 1.5 <sup>a</sup>	16.0 $\pm$ 1.1	15.6 $\pm$ 1.7 <sup>b</sup>	26.6 $\pm$ 3.2 <sup>a</sup>

TABLE 8. Effect of inhibitors of polyamine biosynthesis on cell length of P. teres, G. graminis, S. nodorum and F. culmorum on the sixth day after inoculation  $\pm$  the standard error of the mean. Results for grey and orange forms of P. teres are pooled. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	CELL LENGTH ( $\mu$ m)			
	<u>P. TERES</u>	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
Control	41.3 $\pm$ 3.3	13.9 $\pm$ 1.3	10.6 $\pm$ 1.0	19.4 $\pm$ 1.7
<b>MGBG:</b>				
0.5	-	11.2 $\pm$ 1.0	-	-
1.0	28.4 $\pm$ 1.5 <sup>c</sup>	8.9 $\pm$ 0.5 <sup>b</sup>	18.2 $\pm$ 1.6 <sup>c</sup>	26.2 $\pm$ 2.8 <sup>a</sup>
2.0	29.4 $\pm$ 2.6 <sup>b</sup>	-	17.8 $\pm$ 0.9 <sup>c</sup>	22.6 $\pm$ 2.6
<b>CHA:</b>				
1.0	-	-	-	28.7 $\pm$ 4.1 <sup>a</sup>
2.0	32.0 $\pm$ 2.6 <sup>a</sup>	-	10.5 $\pm$ 0.9	24.1 $\pm$ 2.7
4.0	33.0 $\pm$ 2.0 <sup>a</sup>	-	11.0 $\pm$ 0.6	24.3 $\pm$ 2.5
<b>DFMO + MGBG:</b>				
0.5 + 0.5	31.8 $\pm$ 3.7 <sup>a</sup>	12.9 $\pm$ 1.0	17.5 $\pm$ 1.2 <sup>c</sup>	26.4 $\pm$ 2.5 <sup>a</sup>
1.0 + 1.0	30.0 $\pm$ 2.5 <sup>b</sup>	10.6 $\pm$ 0.9 <sup>a</sup>	13.1 $\pm$ 0.7 <sup>a</sup>	25.5 $\pm$ 5.8

TABLE 9. Effect of polyamines and polyamines plus DFMO on cell length of P. teres, G. graminis, S. nodorum and F. culmorum on the sixth day after inoculation  $\pm$  the standard error of the mean. Results for grey and orange forms of P. teres are pooled. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	CELL LENGTH ( $\mu$ m)			
	<u>P. TERES</u>	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
Control	41.3 $\pm$ 3.3	13.9 $\pm$ 1.3	10.6 $\pm$ 1.0	19.4 $\pm$ 1.7
<b>PUT:</b>				
0.5	24.0 $\pm$ 2.0 <sup>c</sup>	14.3 $\pm$ 1.4	11.8 $\pm$ 0.9	20.7 $\pm$ 1.9
1.0	32.4 $\pm$ 2.4 <sup>a</sup>	15.8 $\pm$ 1.3	14.1 $\pm$ 1.0	17.7 $\pm$ 1.4
<b>SPD:</b>				
0.5	36.0 $\pm$ 2.7	17.1 $\pm$ 1.8	12.4 $\pm$ 0.8	19.0 $\pm$ 1.5
1.0	43.1 $\pm$ 2.8	17.1 $\pm$ 1.5	14.4 $\pm$ 1.7 <sup>a</sup>	18.6 $\pm$ 1.2
<b>DFMO + PUT:</b>				
4.0 + 1.0	32.3 $\pm$ 3.5 <sup>a</sup>	14.6 $\pm$ 1.1	10.3 $\pm$ 0.8	17.1 $\pm$ 1.6
<b>DFMO + SPD:</b>				
4.0 + 1.0	26.8 $\pm$ 1.8 <sup>c</sup>	12.7 $\pm$ 1.2	9.7 $\pm$ 0.9	21.7 $\pm$ 2.3

### 3.3 UPTAKE OF POLYAMINES, PRECURSORS AND DFMO BY F. CULMORUM AND G. GRAMINIS

Uptake of the polyamines, arginine, ornithine and DFMO was studied in detail in F. culmorum and to some extent in G. graminis. Where possible, standard errors of the means have been shown on graphs. However, where they were unable to be graphically depicted, the standard errors have been tabulated, see Appendix, Tables XXII to XXIX.

#### 3.3.1 Statistical Analysis

The standard error of the mean was calculated and significances were determined using a t-test. As original results were obtained as counts, it was assumed that population variances were not equal. Therefore, the formulae described in section 3.1.1 were used to calculate differences.

Linear regressions were performed on data relating to the kinetics of uptake, section 3.3.4, using a Minitab program.

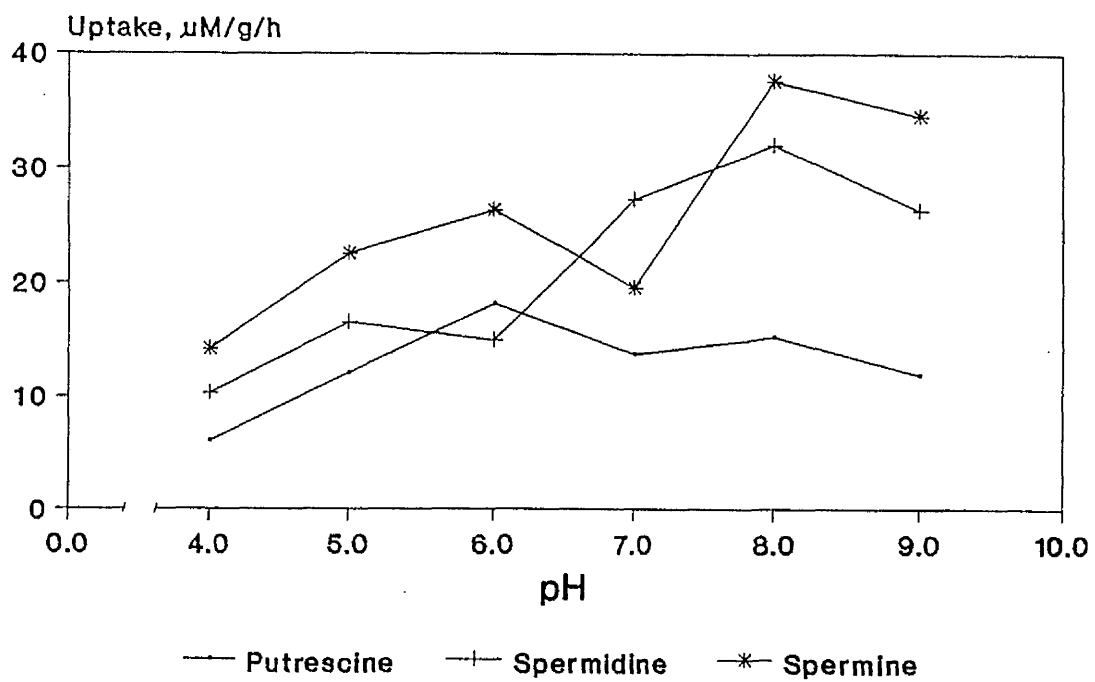
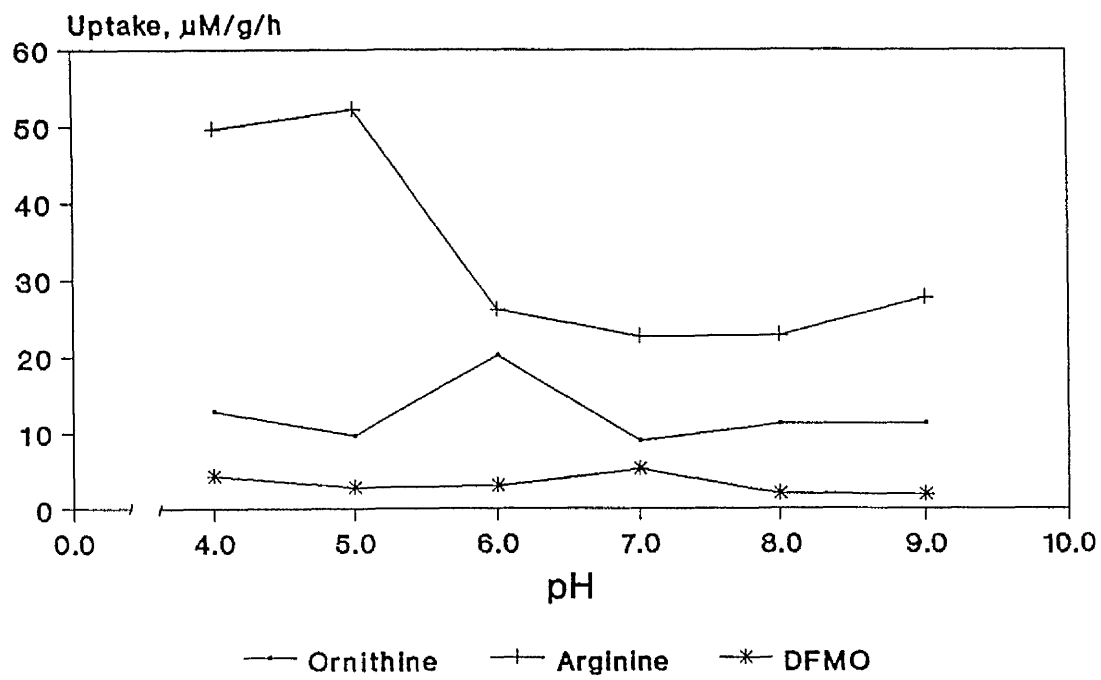
#### 3.3.2 Effect of pH on Uptake of Polyamines, Precursors and DFMO by F. culmorum

In order to determine the optimum pH at which to carry out the uptake experiments, a study of uptake over a range of pH values was performed. Uptake of the precursors and polyamines by F. culmorum appeared to be dependent upon the pH of the medium. Arginine, ornithine and DFMO (Figure 18, Text) exhibited a single peak at pH 5, 6 and 7 respectively. Putrescine, spermi-

dine and spermine each showed two maxima, the greatest of which occurred at pH 6 for putrescine and pH 8 for both spermidine and spermine (Figure 19, Text). These pH optima were used in the following uptake experiments for both F. culmorum and G. graminis. The standard errors of the means are tabulated in Table XXII, Appendix.

FIGURE 18. Effect of pH on uptake of ornithine, arginine and DFMO by F. culmorum. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXII (Appendix).

FIGURE 19. Effect of pH on uptake of polyamines by F. culmorum. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXII (Appendix).





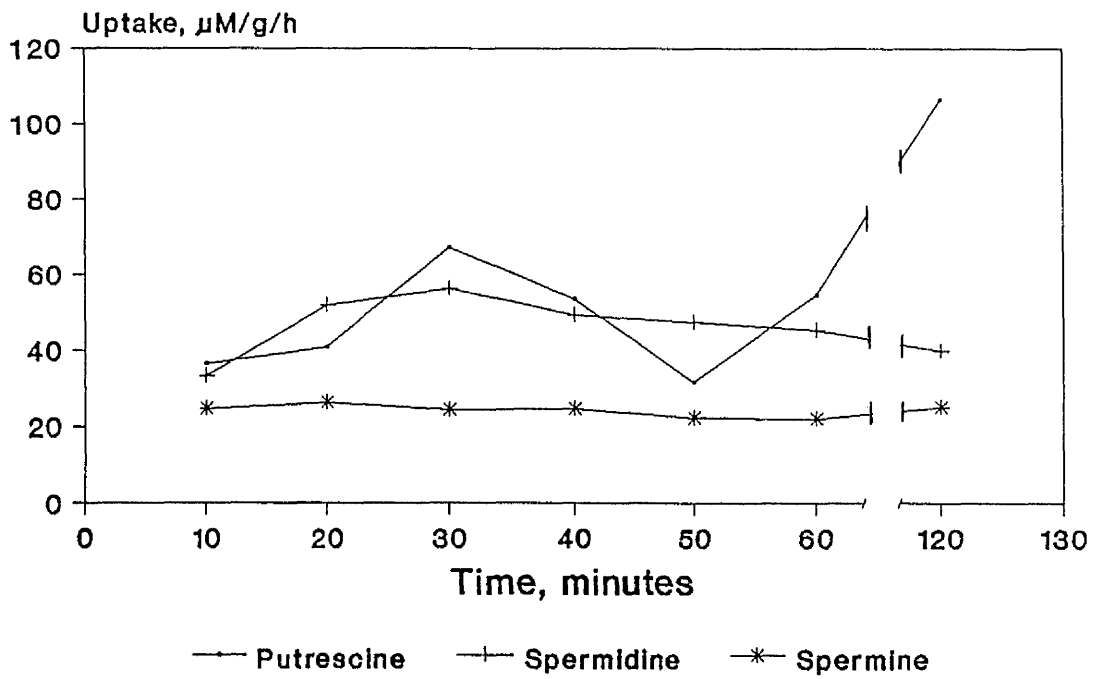
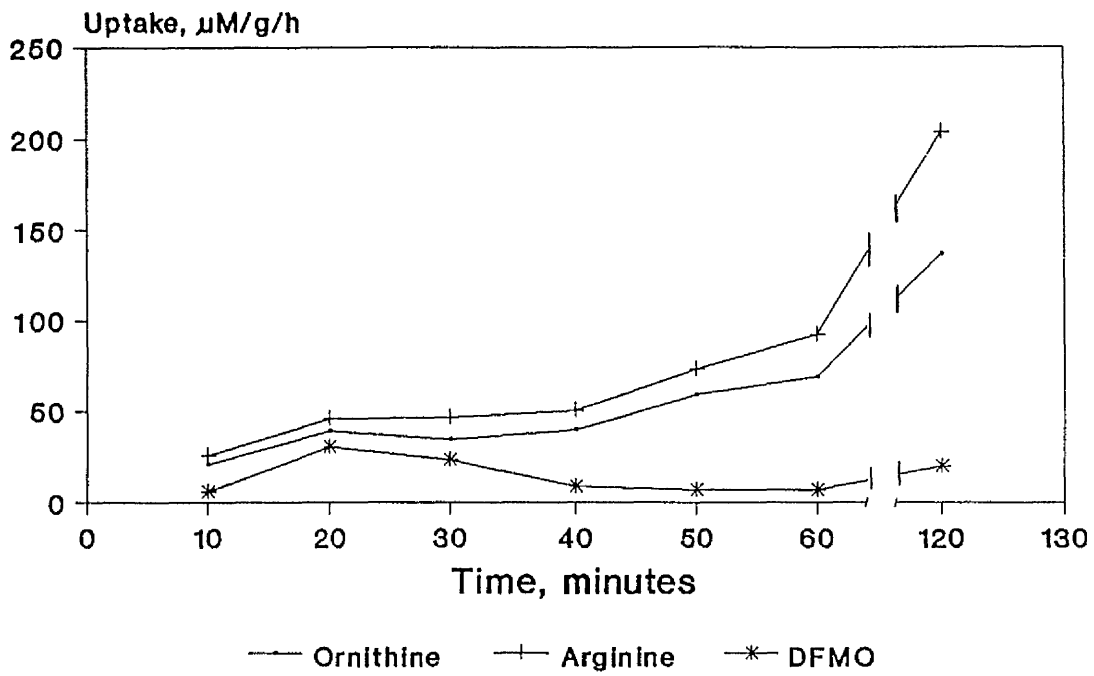
### 3.3.3 Effect of Time on Uptake of Polyamines, Precursors and DFMO by *F. culmorum*

Figures 20 and 21 (Text) depict uptake of the precursors, DFMO and polyamines by *F. culmorum* over a period of up to 120 minutes.

Uptake of ornithine and arginine followed a similar pattern with a gradual increase up to 60 minutes followed by a more marked rise to 120 minutes. DFMO uptake reached a maximum at 20 minutes and increased again at 120 minutes. Spermidine and spermine exhibited a relatively steady rate of uptake over the two hour period, although spermidine uptake did peak at 30 minutes. Putrescine, on the other hand, appeared to be taken up erratically over the time scale, reaching a maximum at 30 minutes and then rising steeply after one hour.

FIGURE 20. Time course of ornithine, arginine and DFMO uptake by F. culmorum. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXIII (Appendix).

FIGURE 21. Time course of polyamine uptake by F. culmorum. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXIII (Appendix).



### 3.3.4 Kinetics of Uptake of Polyamines, Precursors and DFMO by *F. culmorum* and *G. graminis*

The kinetics of uptake of arginine, ornithine, putrescine, spermidine, spermine and DFMO by *F. culmorum* and *G. graminis* were studied. Eleven concentrations of substrate ranging from 0.006 to 50 mM were used in detailed experiments with *F. culmorum* but only 6 concentrations were used in tests involving *G. graminis* (0.1 - 25 mM). The main aim of the experiments with *G. graminis* was to determine the  $K_m$  (affinity constant) for each substrate so that optimum quantities could be used in further experiments, thus ensuring that substrate availability would not be a limiting factor. However, from the results obtained, and by comparisons with the more detailed experiments on *F. culmorum*, certain assumptions can be tentatively made regarding data generated from studies with *G. graminis*. The affinity constants ( $K_m$ ) and maximum velocity of the uptake ( $V_{max}$ ) are shown in Tables 10 and 11 (Text).

#### 3.3.4.1 Uptake of arginine:

Uptake of this amino acid by *F. culmorum* increased with increasing concentration, with some signs of saturation occurring over the 0-1 mM concentration range (Figure 22a, Text). Similarly, uptake of arginine by *G. graminis* increased with increasing concentration, with some levelling off occurring at concentrations above 1 mM (Figure 24, Text).

Figures 23 and 25 (Text) show Eadie-Hofstee plots of arginine uptake by *F. culmorum* and *G. graminis* respectively. The

linear regression carried out on the data obtained for F. culmorum indicates that two straight lines may be fitted, suggesting a biphasic uptake system. An r-squared value of 60.7% was calculated for a single line, emphasizing the likelihood of a dual system. The Eadie-Hofstee plots of the data enabled the  $K_m$  of the systems to be calculated. Table 10 (Text) shows the values of  $K_m$  for arginine for each system. System 1, which appears to operate at low substrate concentrations has a low  $K_m$  and therefore, a higher affinity for the substrate compared to system 2 which occurs at higher concentrations and possesses a much higher  $K_m$ . With respect to F. culmorum, this observation also appears to be the case for the other substrates investigated, except for DFMO.

Table 11 (Text) shows the overall  $K_m$  calculated for G. graminis. The r-squared value obtained from the linear regression carried out on data relating to this fungus was 92.5%. The Eadie-Hofstee plot (Figure 25, Text) does not contain sufficient points to enable one to say with certainty whether the system is biphasic in this fungus. The high r-squared value indicates a good-fit of a single straight line and therefore, infers the presence of a single uptake system.

TABLE 10. Values for  $K_m$  and  $V_{max}$  (mM) for uptake of the polyamines and precursors by *E. culmorum*. Values were calculated by linear regression from Eadie-Hofstee plots.

POLYAMINES/ PRECURSORS	OVERALL		SYSTEM 1		SYSTEM 2	
	$K_m$	$V_{max}$	$K_m$	$V_{max}$	$K_m$	$V_{max}$
Arginine	2.52	0.37	1.03	0.16	8.27	0.47
Ornithine	9.43	0.52	0.58	0.04	28.15	0.95
Putrescine	2.23	0.23	0.17	0.02	32.73	0.82
Spermidine	2.49	0.21	0.34	0.04	42.17	0.58
Spermine	1.15	0.17	0.16	0.03	35.67	0.56

TABLE 11. Values for overall  $K_m$  and  $V_{max}$  (mM) for uptake of the polyamines and precursors by G. graminis. Values were calculated by linear regression from Eadie-Hofstee plots.

POLYAMINES/ PRECURSORS	$K_m$	$V_{max}$
Arginine	3.95	0.45
Ornithine	46.22	0.59
Putrescine	10.95	0.46
Spermidine	2.46	0.08
Spermine	0.34	0.08

FIGURE 22. Arginine uptake by F. culmorum as a function of substrate concentration in the external medium. (a) = uptake over the concentration range 0.006 - 50 mM; (b) = uptake over the concentration range 0.006 - 1.0 mM. Each point represents the mean of three replicates. Standard errors are tabulated, Table XXIV (Appendix).

FIGURE 23. Eadie-Hofstee plot for arginine uptake by F. culmorum. Lines were fitted by linear regression.  $V$  = arginine uptake over the concentration range 0.006 - 50 mM;  $V/[S]$  = arginine uptake divided by the substrate concentration.



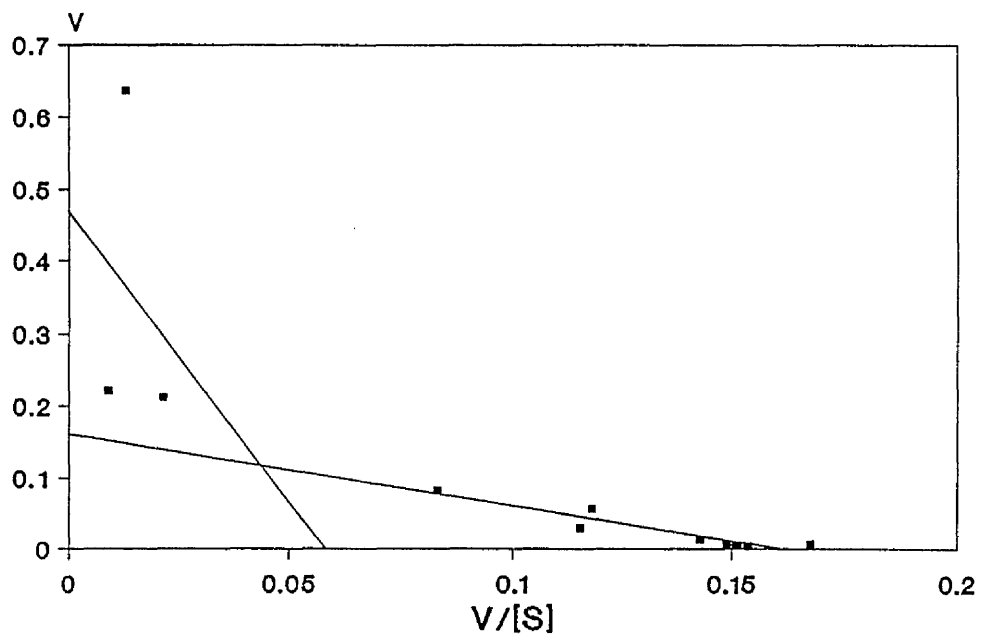
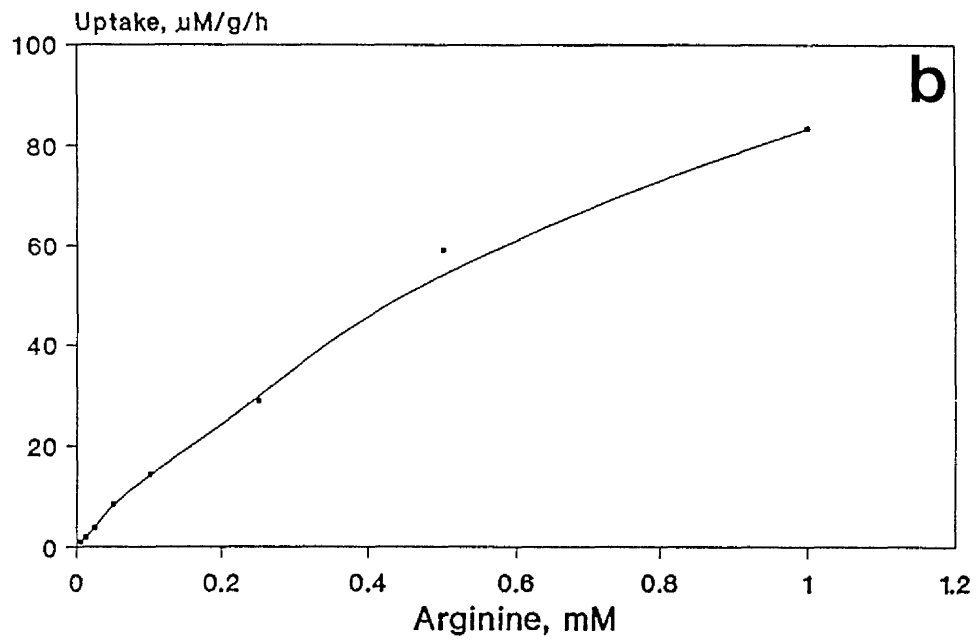
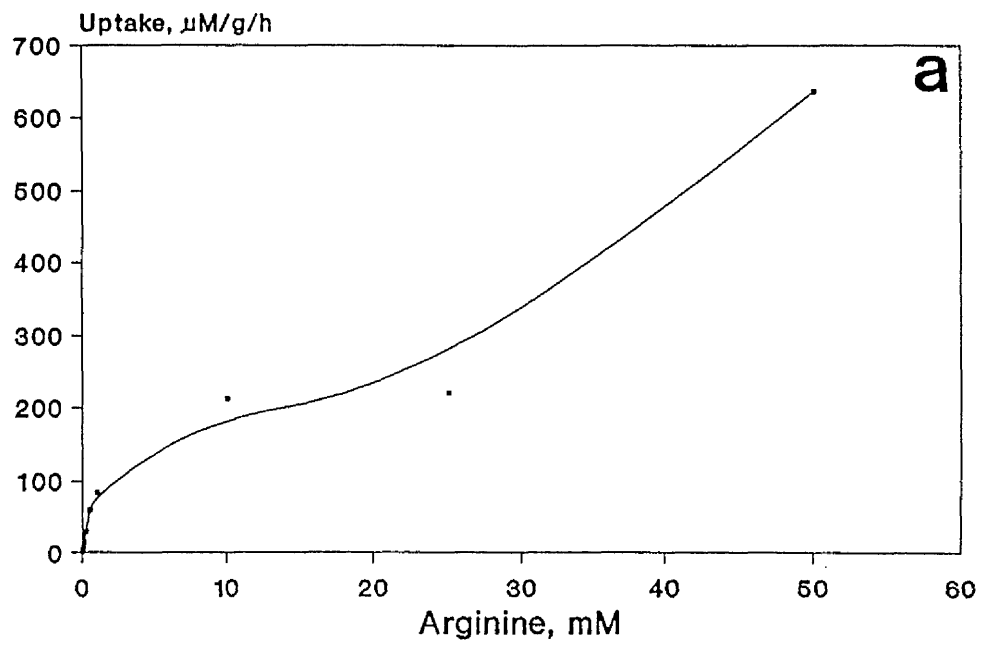
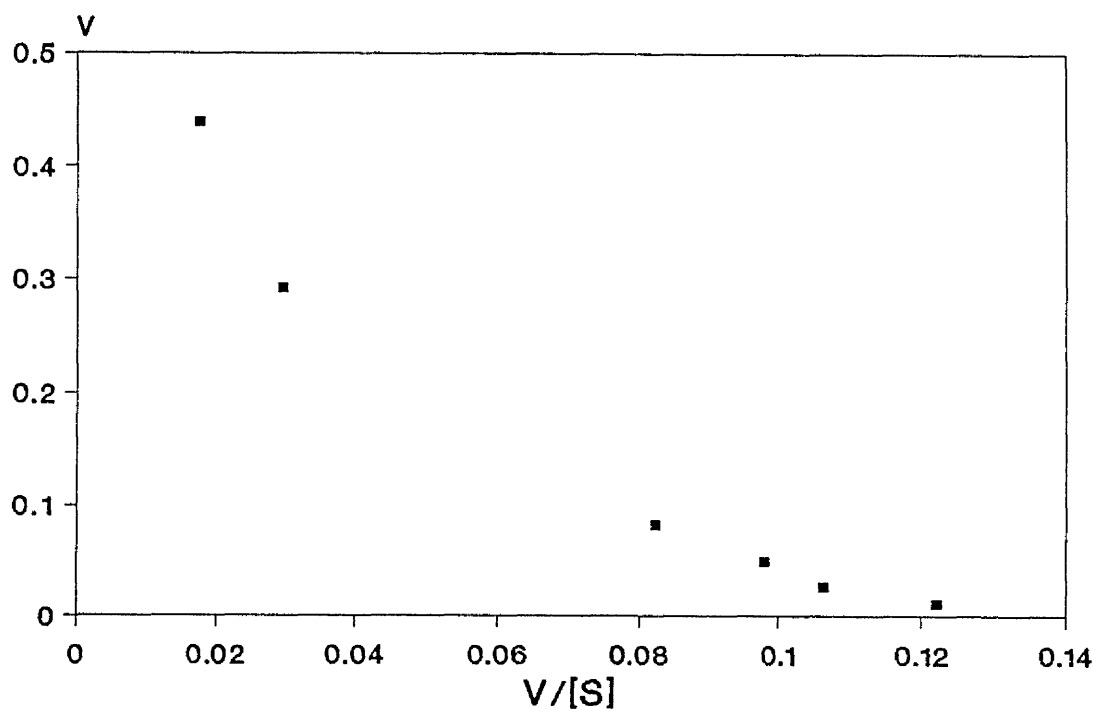
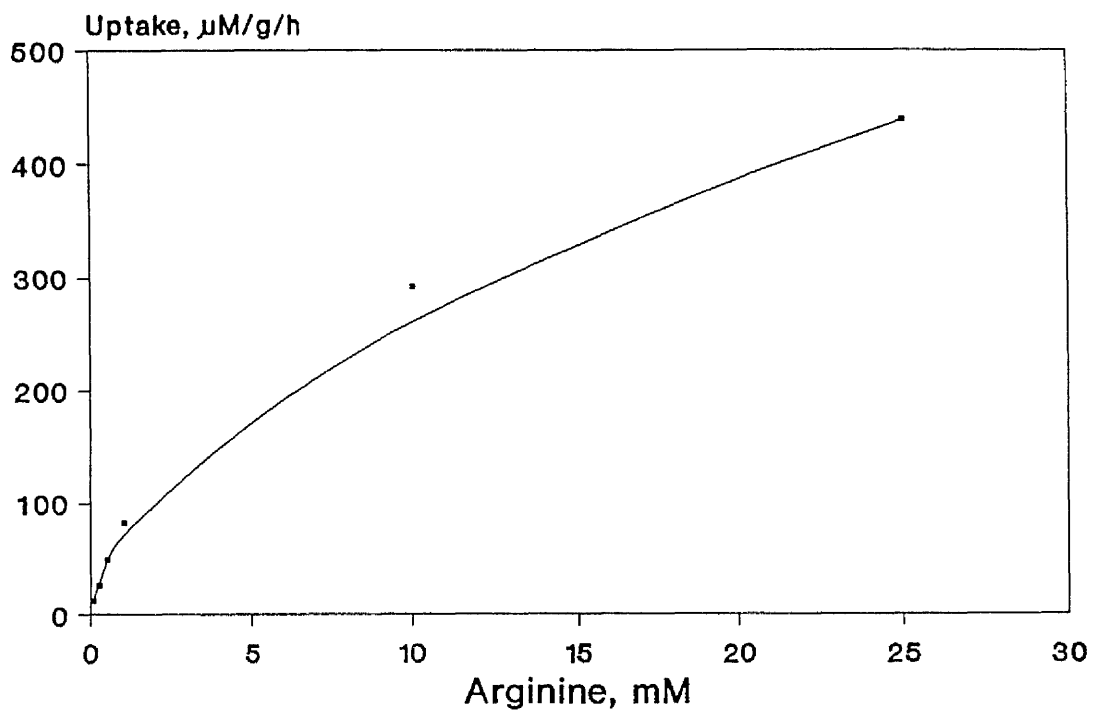


FIGURE 24. Arginine uptake by G. graminis as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.1 - 25 mM. Standard errors are tabulated, Table XXV (Appendix).

FIGURE 25. Eadie-Hofstee plot for arginine uptake by G. graminis.  $V$  = arginine uptake over the concentration range 0.1 - 25 mM;  $V/[S]$  = arginine uptake divided by the substrate concentration.



#### 3.3.4.2 Uptake of ornithine:

Uptake of ornithine by both F. culmorum (Figure 26a, Text) and G. graminis (Figure 28, Text) increased sharply with a rise in substrate concentration, showing signs of levelling off at higher concentrations. Saturation was more obvious for uptake by F. culmorum examined over the whole concentration range (0 - 50 mM; Figure 26a, Text), but was less obvious for ornithine uptake by G. graminis (Figure 28, Text). The r-squared values calculated by linear regressions were 58.5% for uptake by F. culmorum and 55.6% for uptake of G. graminis for single lines. The Eadie-Hofstee plots for each fungus (Figures 27 and 29, Text) show obvious biphasic systems. The  $K_m$  for F. culmorum is substantially smaller for the system operating at low substrate concentrations than for the second system occurring at high ornithine concentrations (Table 10, Text). Due to insufficient data, only an overall  $K_m$  could be calculated for G. graminis (Table 11, Text).

FIGURE 26. Ornithine uptake by F. culmorum as a function of substrate concentration in the external medium. (a) = uptake over the concentration range 0.006 - 50 mM; (b) = uptake over the concentration range 0.006 - 1.0 mM. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXIV (Appendix).

FIGURE 27. Eadie-Hofstee plot for ornithine uptake by F. culmorum. Lines were fitted by linear regression.  $V$  = ornithine uptake over the concentration range 0.006 - 50 mM;  $V/[S]$  = ornithine uptake divided by the substrate concentration.

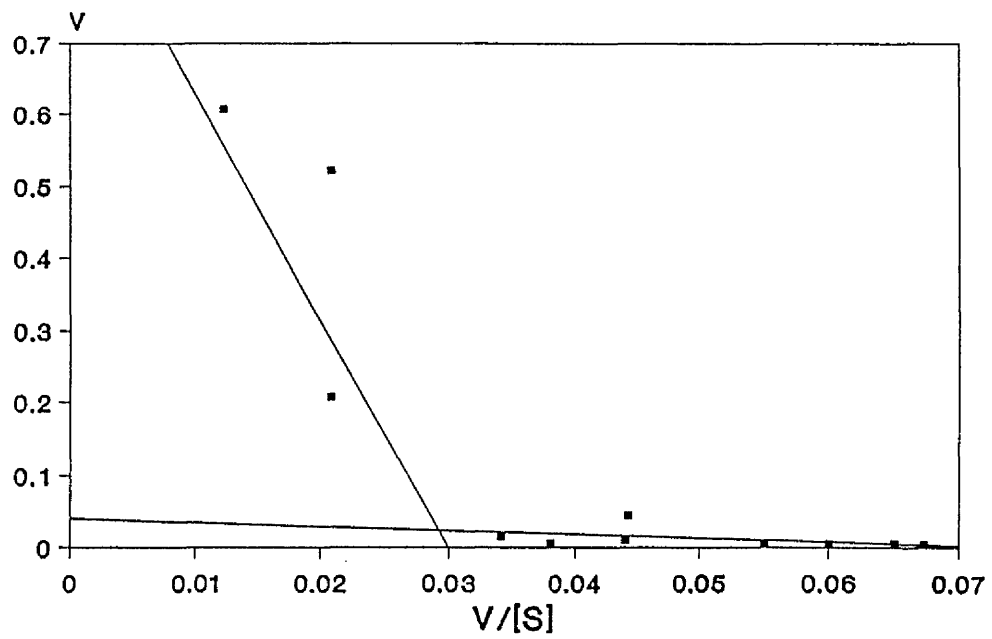
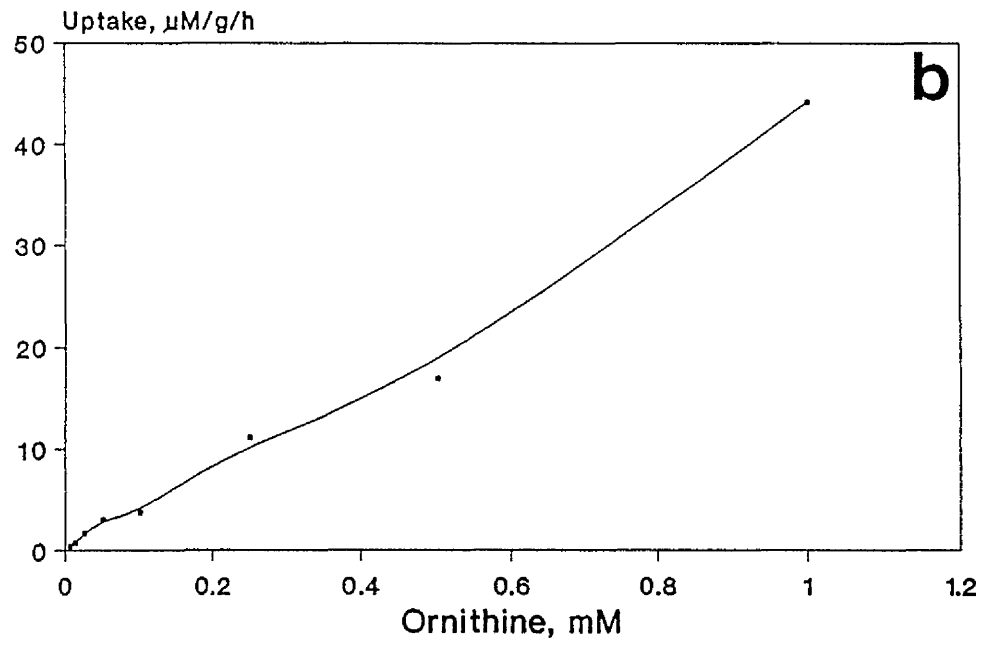
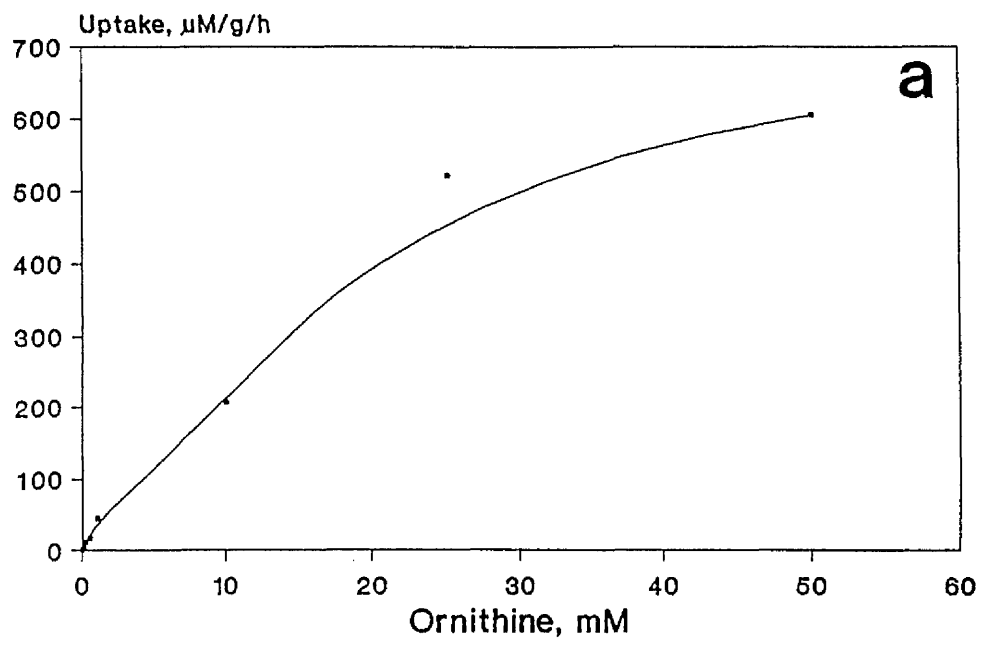
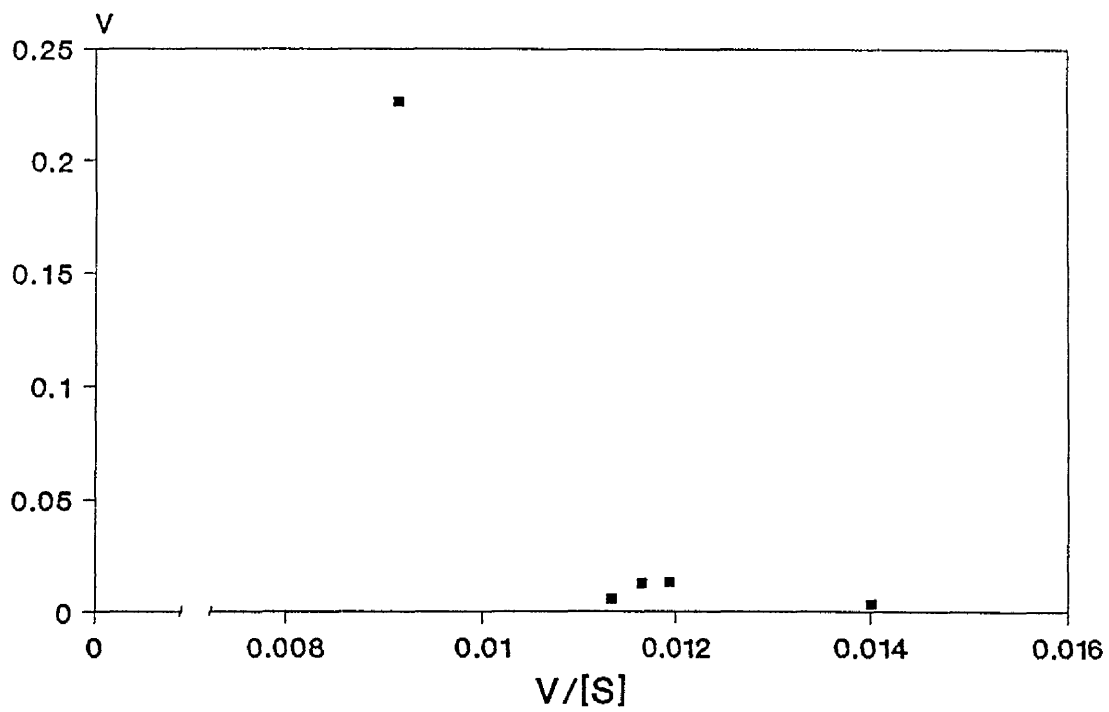
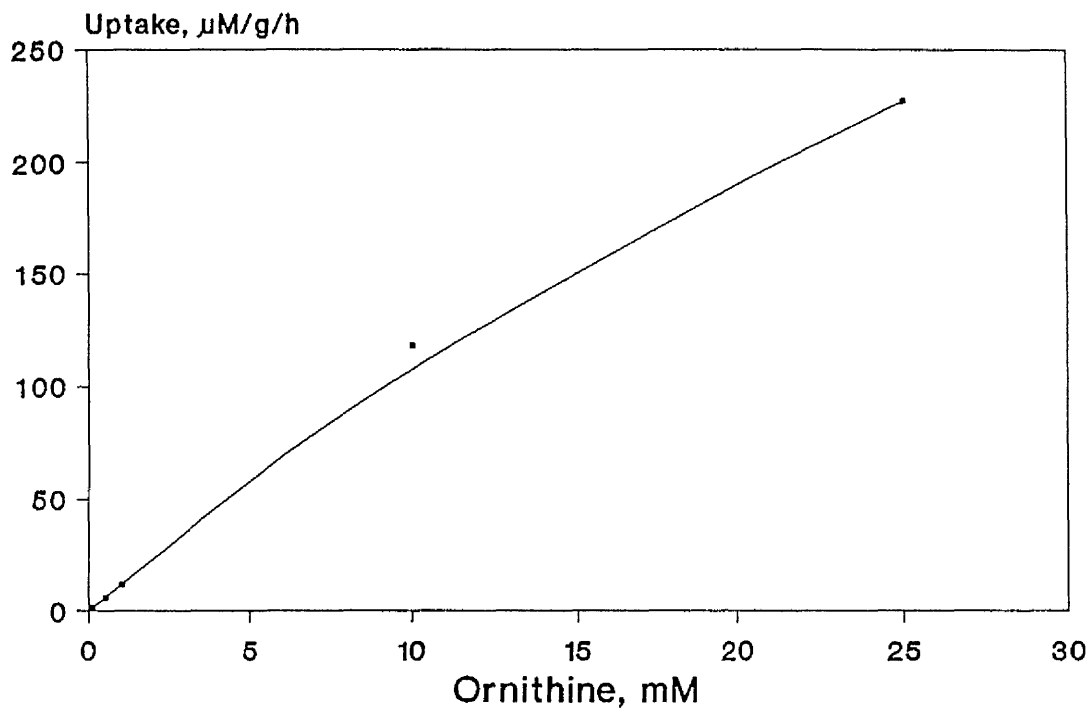




FIGURE 28. Ornithine uptake by G. graminis as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.1 - 25 mM. Standard errors are tabulated, Table XXV (Appendix).

FIGURE 29. Eadie-Hofstee plot for ornithine uptake by G. graminis.  $V$  = ornithine uptake over the concentration range 0.1 - 25 mM;  $V/[S]$  = ornithine uptake divided by the substrate concentration.





### 3.3.4.3 Uptake of putrescine:

Uptake of this substrate by both F. culmorum and G. graminis was biphasic. The linear regression allowed the calculation of an r-squared value of 29.4% and 46.4% for one line for F. culmorum and G. graminis respectively. The poor goodness-of-fit of a single line to the Eadie-Hofstee plots indicates that two systems were employed. Lines for uptake systems 1 and 2 were fitted on the Eadie-Hofstee plot for F. culmorum by linear regression (Figure 31, Text). The calculated Km value for uptake of putrescine by F. culmorum was very high for system 2 (10 - 50 mM) at 32.73 mM and low for system 1 (0.006 - 1.0 mM) at 0.17 mM (Table 10, Text).

FIGURE 30. Putrescine uptake by F. culmorum as a function of substrate concentration in the external medium. (a) = uptake over the concentration range 0.006 - 50 mM; (b) = uptake over the concentration range 0.006 - 1.0 mM. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXIV (Appendix).

FIGURE 31. Eadie-Hofstee plot for putrescine uptake by F. culmorum. Lines were fitted by linear regression.  $V$  = putrescine uptake over the concentration range 0.006 - 50 mM;  $V/[S]$  = putrescine uptake divided by the substrate concentration.

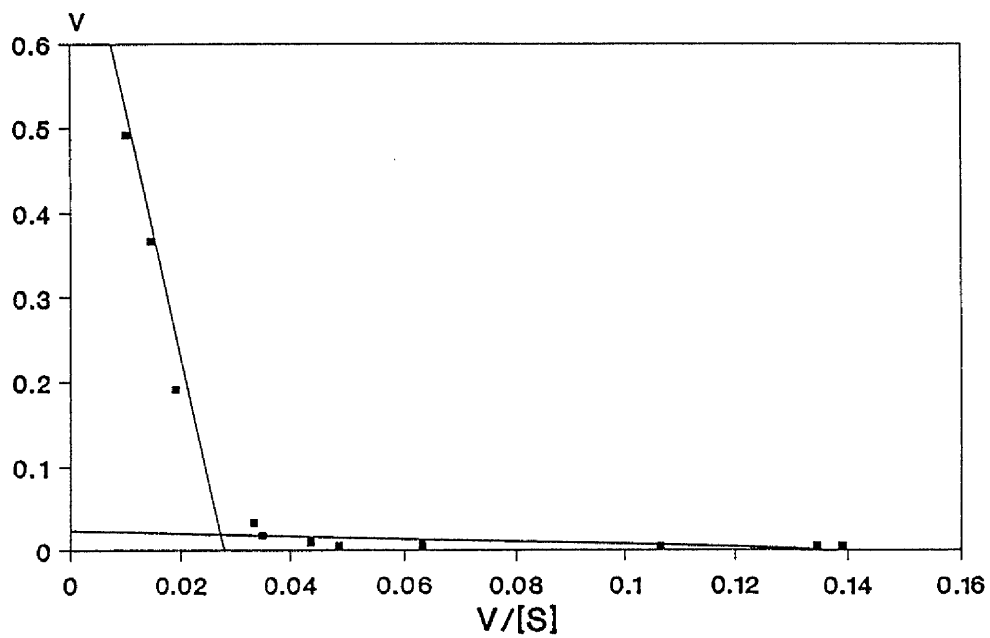
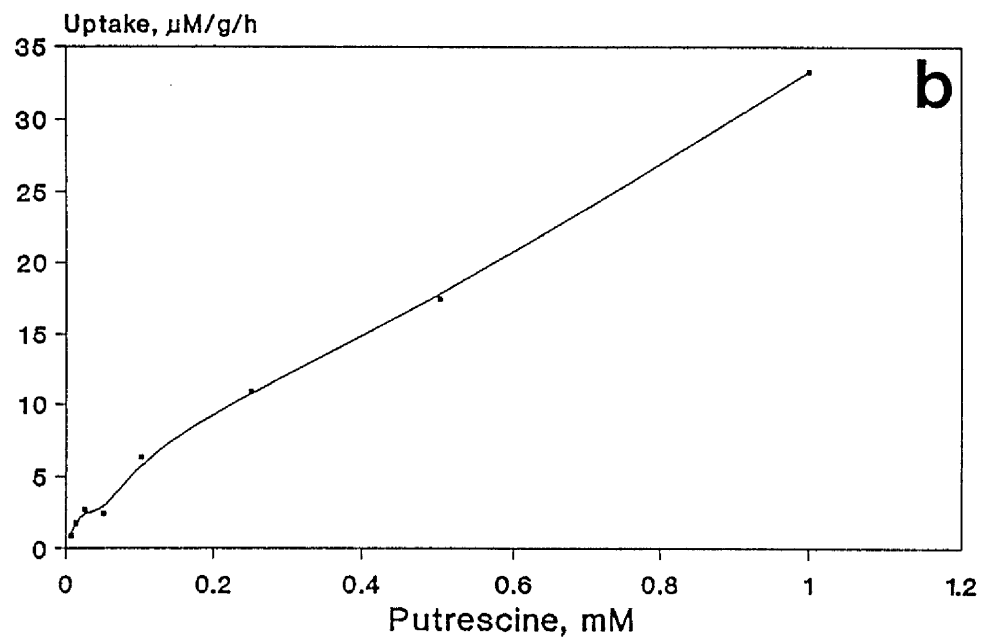
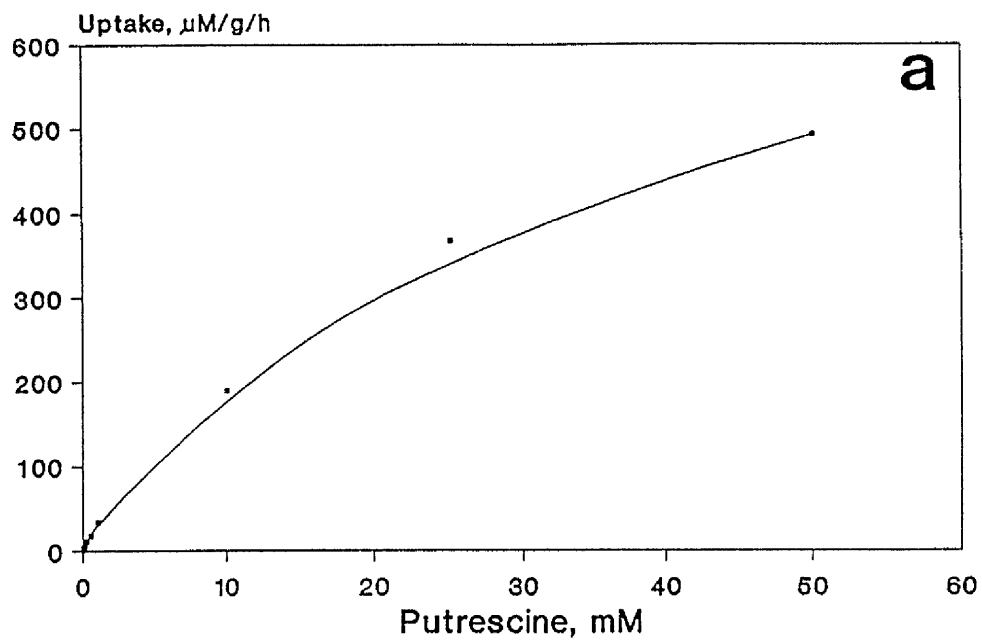
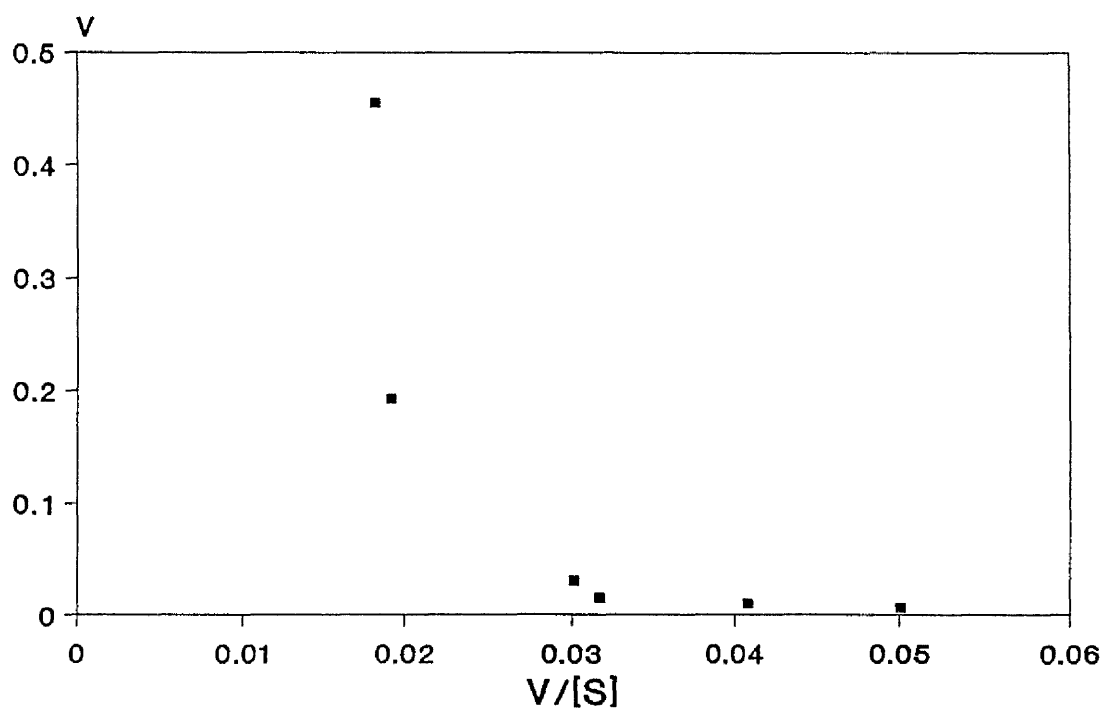
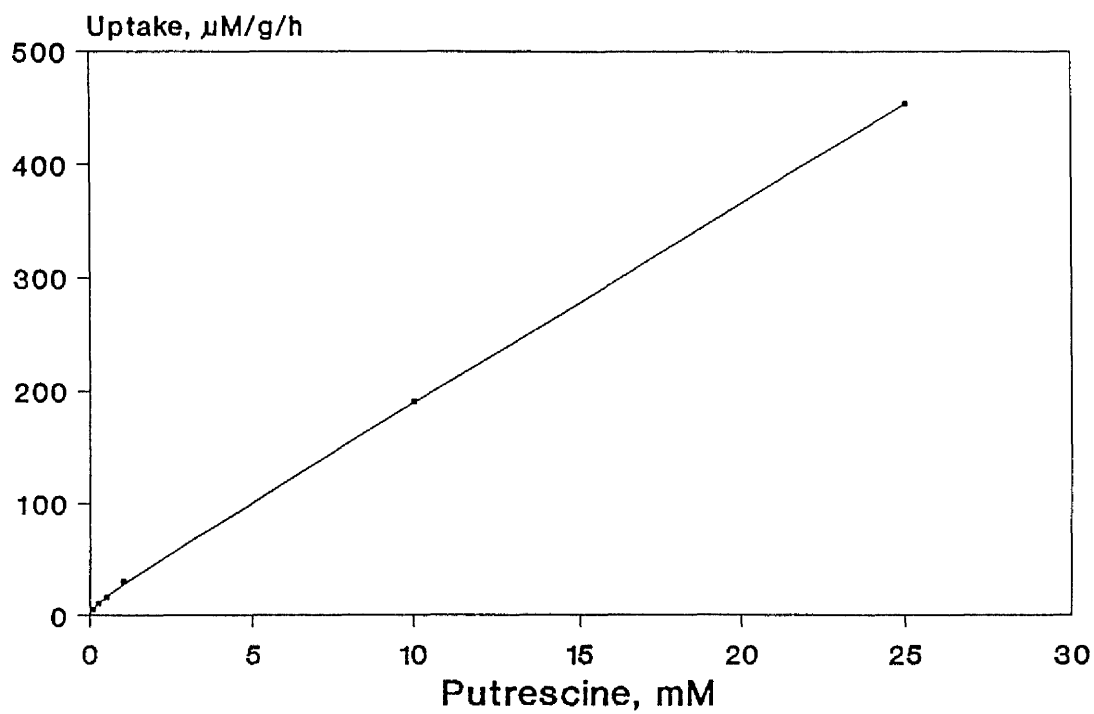


FIGURE 32. Putrescine uptake by G. graminis as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.1 - 25 mM. Standard errors are tabulated, Table XXV (Appendix).

FIGURE 33. Eadie-Hofstee plot for putrescine uptake by G. graminis.  $V$  = putrescine uptake over the concentration range 0.1 - 25 mM;  $V/[S]$  = putrescine uptake divided by the substrate concentration.



#### 3.3.4.4 Uptake of spermidine:

Uptake of spermidine by F. culmorum appeared to be biphasic. The r-squared value calculated by linear regression was 50.3% for one line, thus indicating that two systems were operating. This can be clearly seen in Figure 35 (Text) which shows an Eadie-Hofstee plot fitted with lines depicting the two systems. As has been observed for other substrates where two systems have been reported, one occurs at low substrate concentrations (system 1) and the other at high spermidine concentrations (system 2). However, from the results available for spermidine uptake by G. graminis, a single system would seem to be functioning. The r-squared value calculated by linear regression was 94.9% indicating a good-fit of a single line. However, whether this is actually the case, or whether it is a result of using insufficient substrate concentrations remains undetermined.

FIGURE 34. Spermidine uptake by F. culmorum as a function of substrate concentration in the external medium. (a) = uptake over the concentration range 0.006 - 50 mM; (b) = uptake over the concentration range 0.006 - 1.0 mM. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXIV (Appendix).

FIGURE 35. Eadie-Hofstee plot for spermidine uptake by F. culmorum. Lines were fitted by linear regression.  $V$  = spermidine uptake over the concentration range 0.006 - 50 mM;  $V/[S]$  = spermidine uptake divided by the substrate concentration.



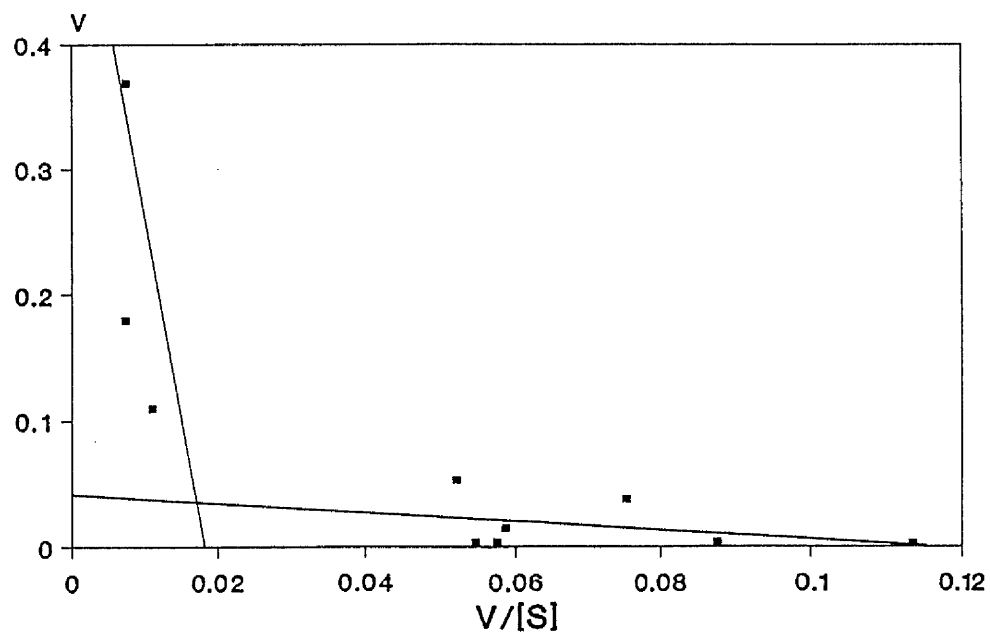
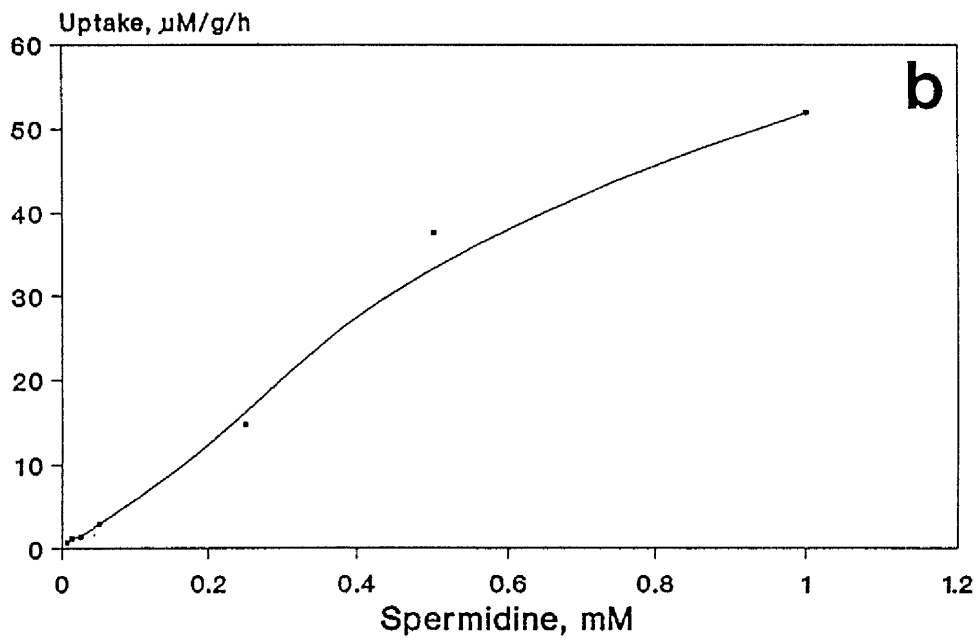
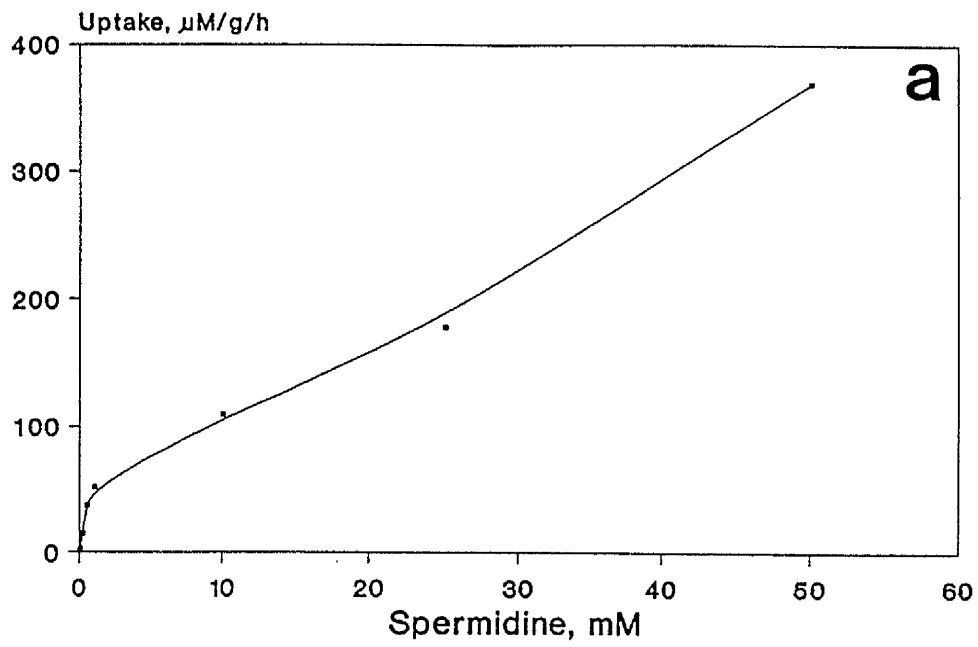
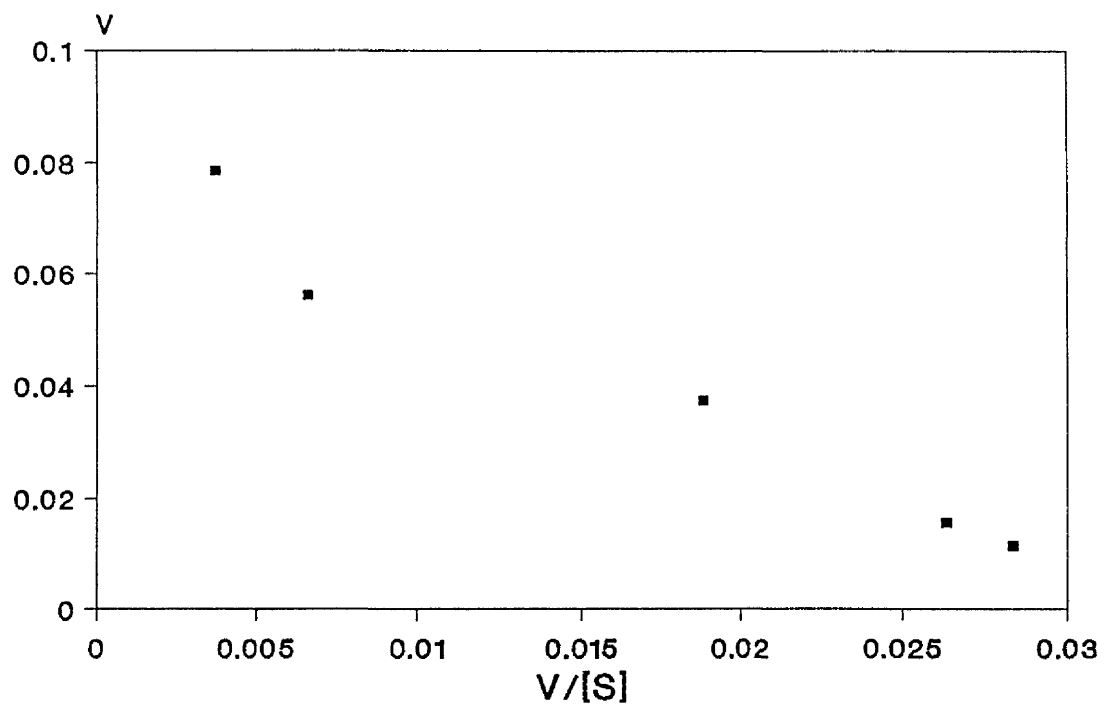
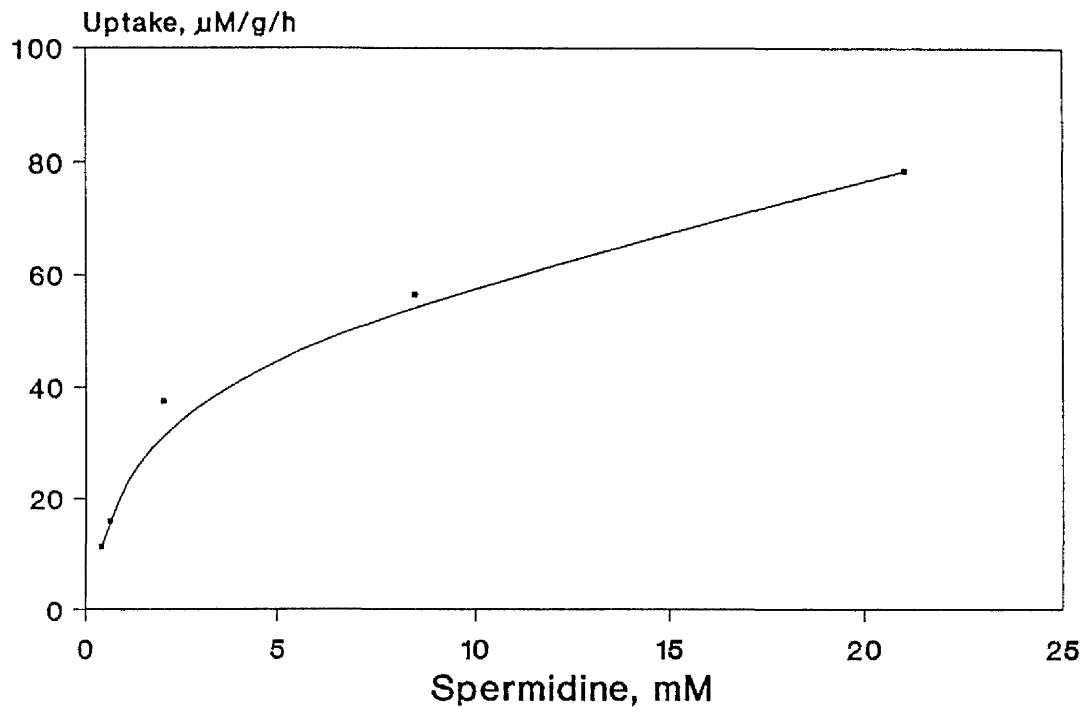


FIGURE 36. Spermidine uptake by G. graminis as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.4 - 21 mM. Standard errors are tabulated, Table XXVI (Appendix).

FIGURE 37. Eadie-Hofstee plot for spermidine uptake by G. graminis.  $V$  = spermidine uptake over the concentration range 0.4 - 21 mM;  $V/[S]$  = spermidine uptake divided by the substrate concentration.



#### 3.3.4.5 Uptake of spermine:

Spermine uptake by F. culmorum showed clear signs of saturation at low concentrations but levelling off of uptake was less obvious at higher concentrations (Figures 38a and 38b, Text). A similar response was observed for G. graminis, although here, there was more evidence of saturation at higher concentrations (Figure 40, Text). The Eadie-Hofstee plots for each fungus indicate a biphasic system dependent on the substrate concentration. Lines depicting each system were plotted for uptake by F. culmorum using a linear regression. Insufficient high substrate concentrations were used in the experiment with G. graminis to enable similar lines to be fitted, but the general trend of the plots (Figures 40 and 41, Text) suggest a biphasic system. The r-squared values for a single uptake system calculated for F. culmorum and G. graminis were 37.3% and 60.8% respectively.

FIGURE 38. Spermine uptake by F. culmorum as a function of substrate concentration in the external medium. (a) = uptake over the concentration range 0.006 - 50 mM; (b) = uptake over the concentration range 0.006 - 1.0 mM. Each point represents the mean of 3 replicates. Standard errors are tabulated, Table XXIV (Appendix).

FIGURE 39. Eadie-Hofstee plot for spermine uptake by F. culmorum. Lines were fitted by linear regression.  $V$  = spermine uptake over the concentration range 0.006 - 50 mM;  $V/[S]$  = spermine uptake divided by the substrate concentration.

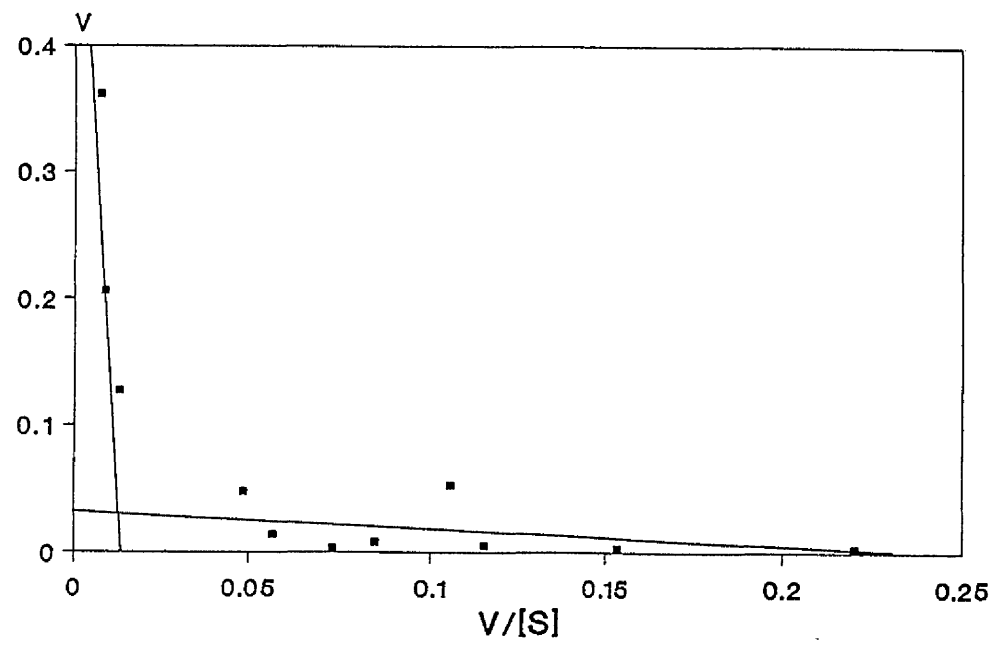
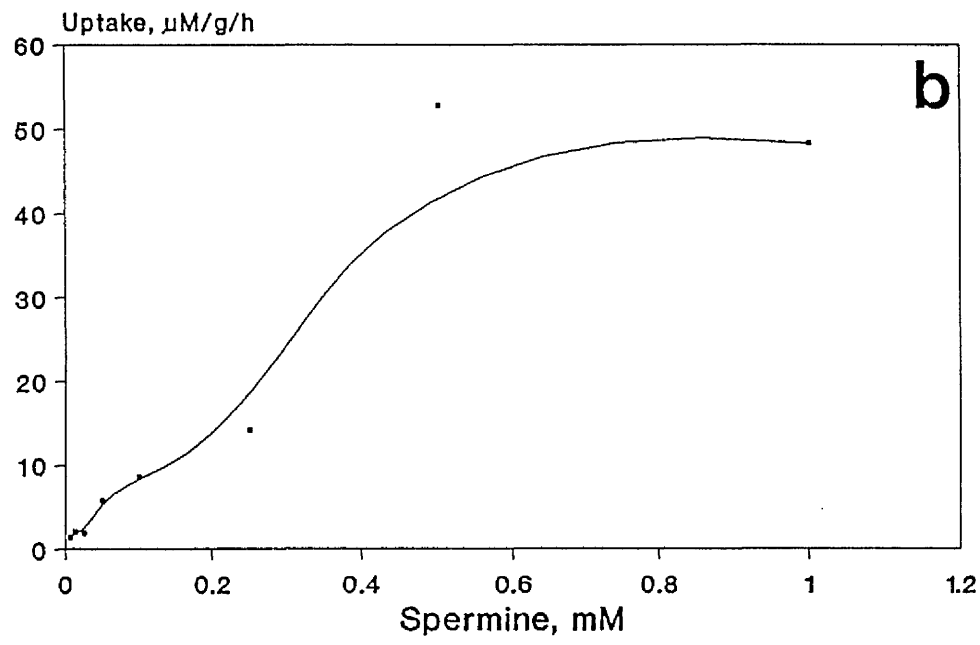
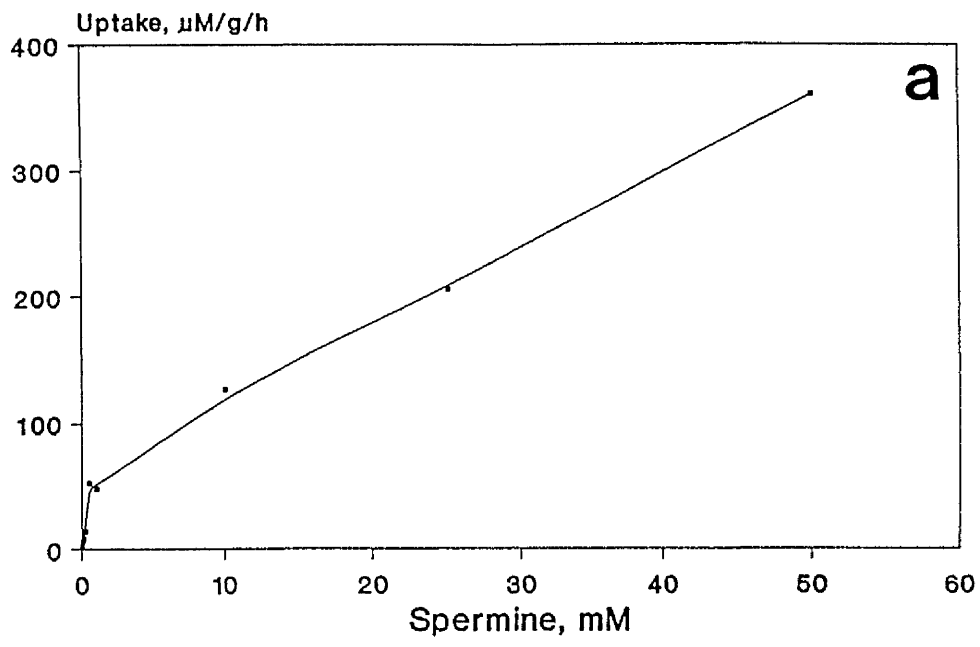
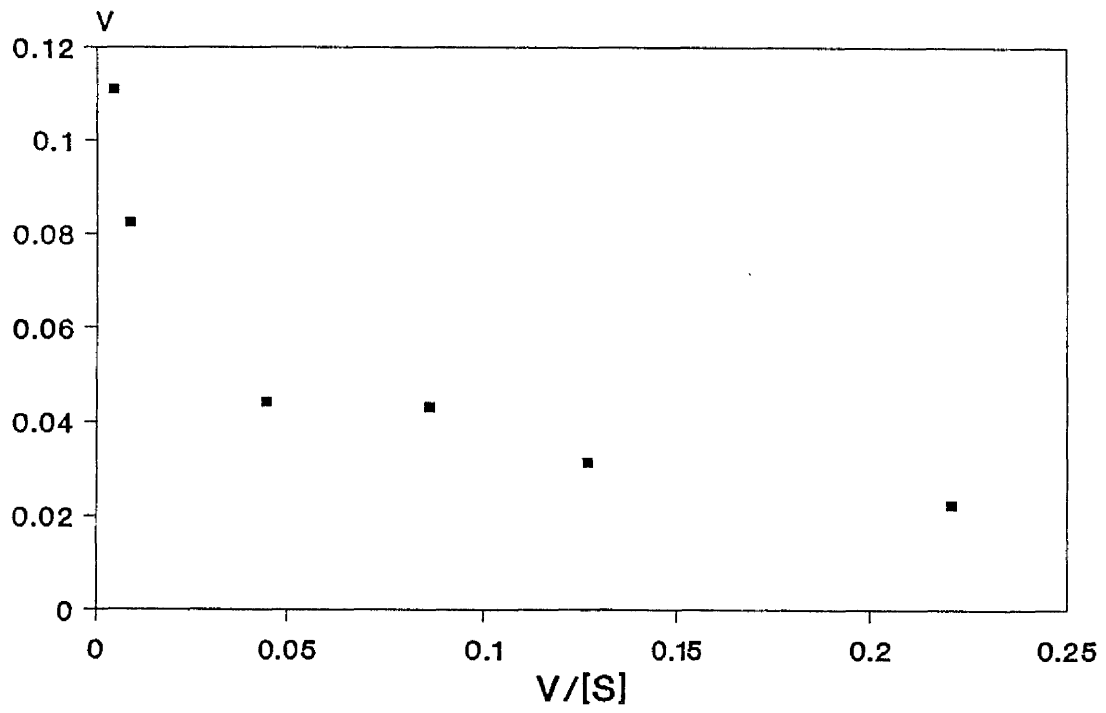
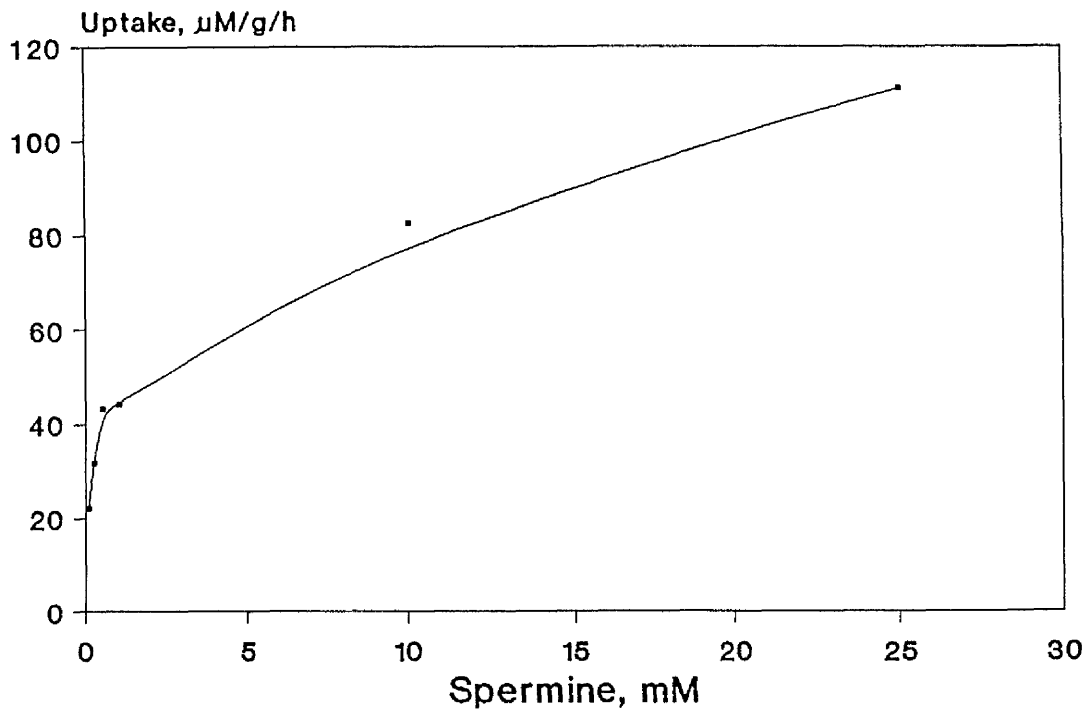


FIGURE 40. Spermine uptake by G. graminis as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.1 - 25 mM. Standard errors are tabulated, Table XXV (Appendix).

FIGURE 41. Eadie-Hofstee plot for spermine uptake by G. graminis.  $V$  = spermine uptake over the concentration range 0.1 - 25 mM;  $V/[S]$  = spermine uptake divided by the substrate concentration.



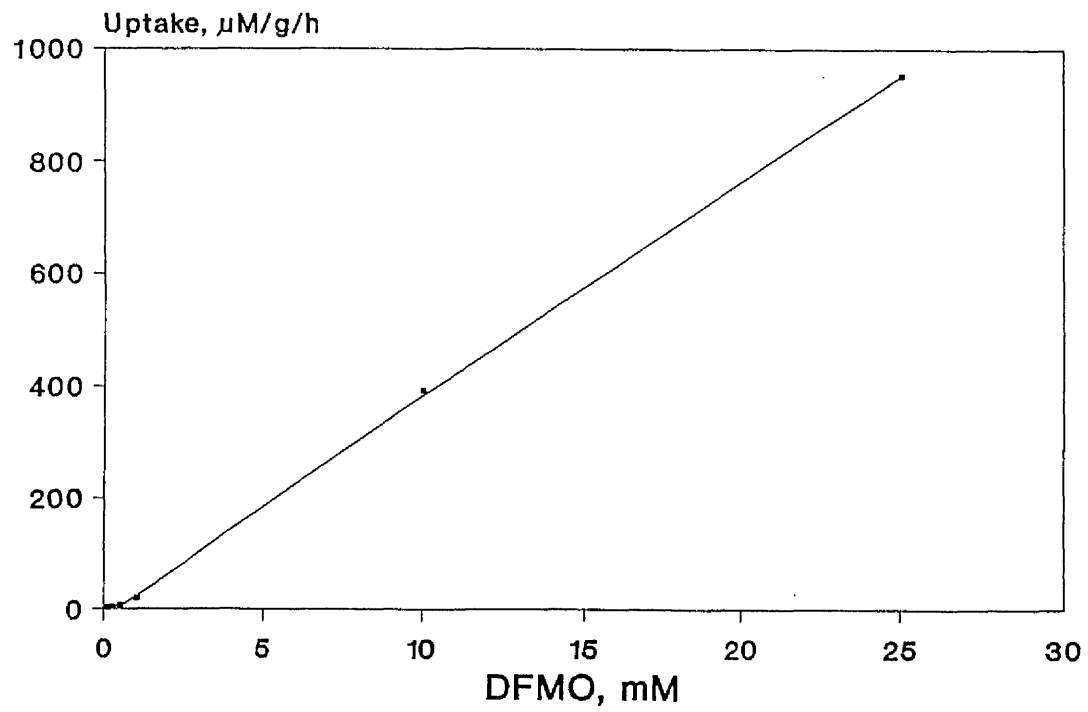
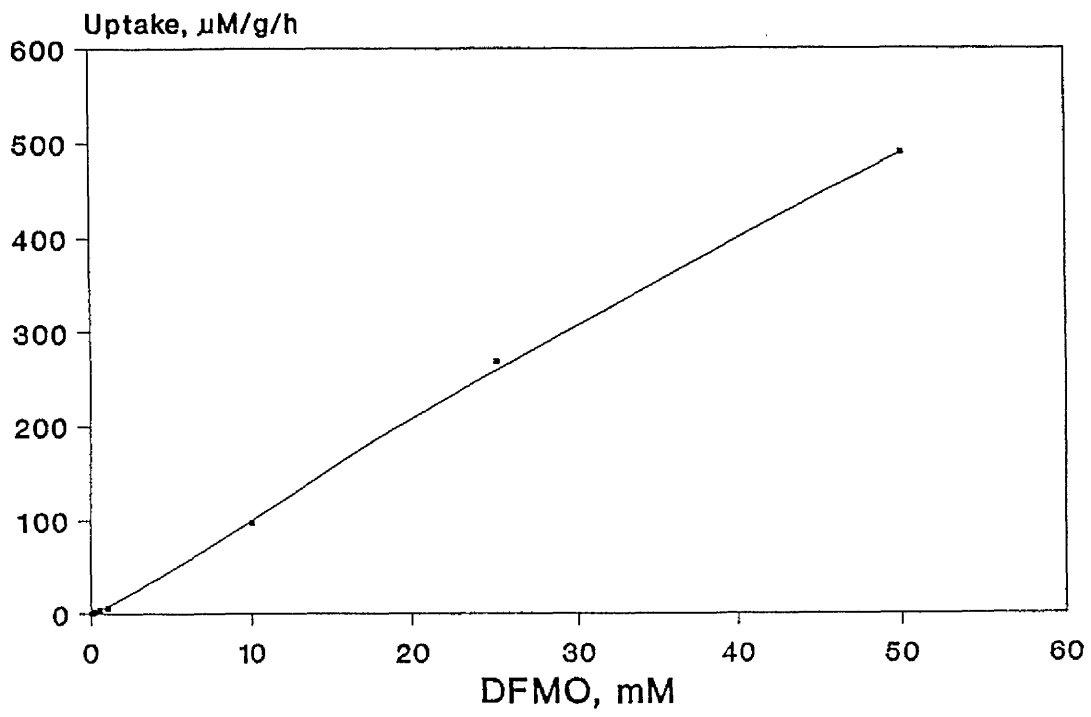


#### 3.3.4.6 Uptake of DFMO:

Uptake of this ODC inhibitor by both F. culmorum and G. graminis was linear and did not appear to be saturable (Figures 42 and 43, Text). As no  $V_{max}$  was apparently reached, it was not possible to calculate a  $K_m$  for the substrate or to graph Eadie-Hofstee plots. Uptake would appear to be non-saturable and attained perhaps via a single system.

FIGURE 42. DFMO uptake of F. culmorum as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.006 - 50 mM. Standard errors are tabulated, Table XXIV (Appendix).

FIGURE 43. DFMO uptake by G. graminis as a function of substrate concentration in the external medium. Each point represents the mean of 3 replicates over the concentration range 0.1 - 25 mM. Standard errors are tabulated, Table XXV (Appendix).



### 3.3.5 Effect of a Respiratory Inhibitor on Uptake of Polyamines and DFMO

Uptake of putrescine, spermidine and DFMO by F. culmorum was studied both in the presence and absence of the respiratory inhibitor, sodium azide (1.0 mM concentration). Uptake of putrescine and DFMO was significantly reduced when sodium azide was added to the uptake medium. Spermidine uptake appeared to be inhibited by the presence of sodium azide, but the reduction was not statistically significant (Figure 44, Text). The results indicate the existence of an energy requiring component of the uptake process for putrescine and DFMO (and perhaps spermidine), albeit small.

### 3.3.6 Antagonistic Effects of Polyamines, Ornithine and DFMO on Uptake of Putrescine, Spermidine and DFMO

Uptake of putrescine, spermidine and DFMO by F. culmorum was examined in the presence of unlabelled polyamines, ornithine and DFMO. Figure 45 (Text) shows that cold spermidine, ornithine and DFMO did not inhibit uptake of labelled putrescine by F. culmorum. Similarly, cold putrescine, ornithine and DFMO did not inhibit uptake of spermidine by the fungus. Rather, in both cases, uptake was actually increased substantially in the presence of the other polyamine, ornithine or DFMO. However, ornithine, putrescine and spermidine all appeared to inhibit uptake of DFMO, with ornithine exerting less of an effect than the two polyamines.

### 3.3.7 Effect of Growing Fungi in Inhibitor Amended Medium on Uptake of Polyamines

It would be expected that fungi grown in conditions which would lead to a depletion of intracellular polyamines would exhibit an increase in uptake of polyamines should these compounds be added to the medium.

Figures 46, 47 and 48 (Text) show uptake of putrescine, spermidine and spermine by F. culmorum after the fungus had been grown in the presence of inhibitors of polyamine biosynthesis. The only inhibitor to cause a significant change in uptake of all three polyamines was MGBG. However, this was a decrease in uptake rather than an increase. Uptake of spermidine was most affected by growth of the fungus in the presence of MGBG. DFMO (2.0 mM) caused a significant increase in spermine uptake (Figure 48, Text). DFMO also caused superficial increases in putrescine and spermidine uptake by F. culmorum but statistical analysis proved these changes not to be significant.

Enhanced uptake of putrescine by G. graminis after the fungus had been grown in 1.0 mM DFMO was observed but a 4.0 mM concentration of the inhibitor led to a significant decrease in putrescine uptake as did the MGBG treatment. Significant decreases in spermidine uptake by G. graminis were observed after growth in DFMO and MGBG. Spermine uptake by G. graminis appeared to be unchanged irrespective of the inhibitor in which the fungus was grown. More detailed experiments using higher concentrations of MGBG or DFMO + MGBG were not possible as the fungus would not grow in liquid culture under such conditions.

FIGURE 44. Effect of sodium azide on uptake of putrescine, spermidine and DFMO by F. culmorum. Vertical bars represent the standard error of the mean and significant differences are shown at  $P = 0.1 - 0.05^*$ .

FIGURE 45. Antagonistic effects of polyamines, ornithine and DFMO on uptake of putrescine, spermidine and DFMO by F. culmorum. Standard errors are tabulated, Table XXVII (Appendix). Significant differences are shown at  $P = 0.02 - 0.01^{**}$ ;  $P = 0.002 - 0.001^{***}$ .

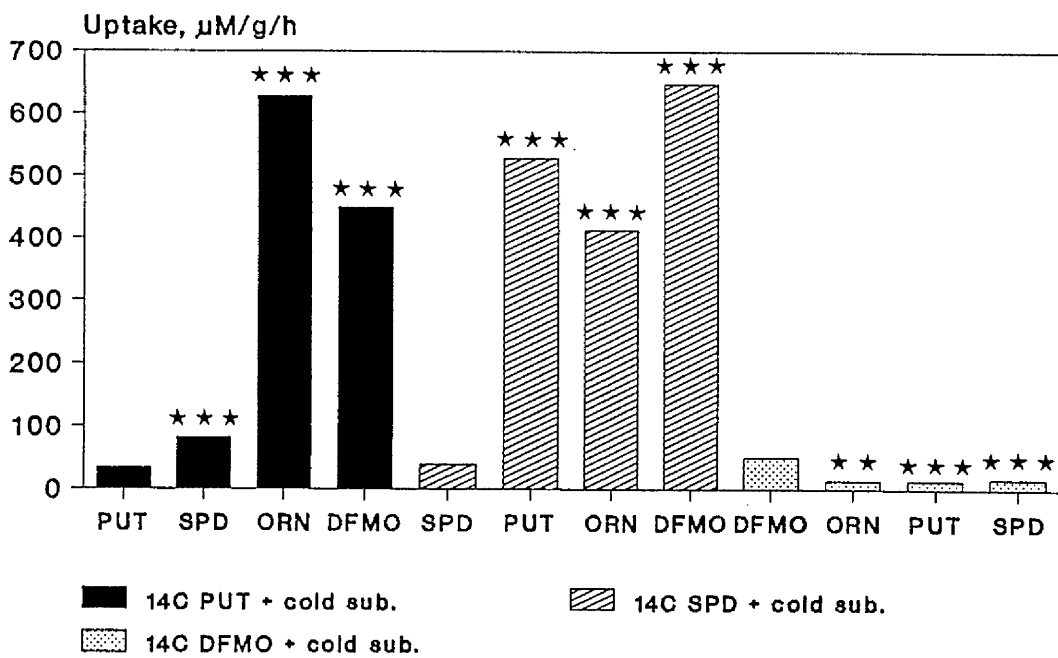
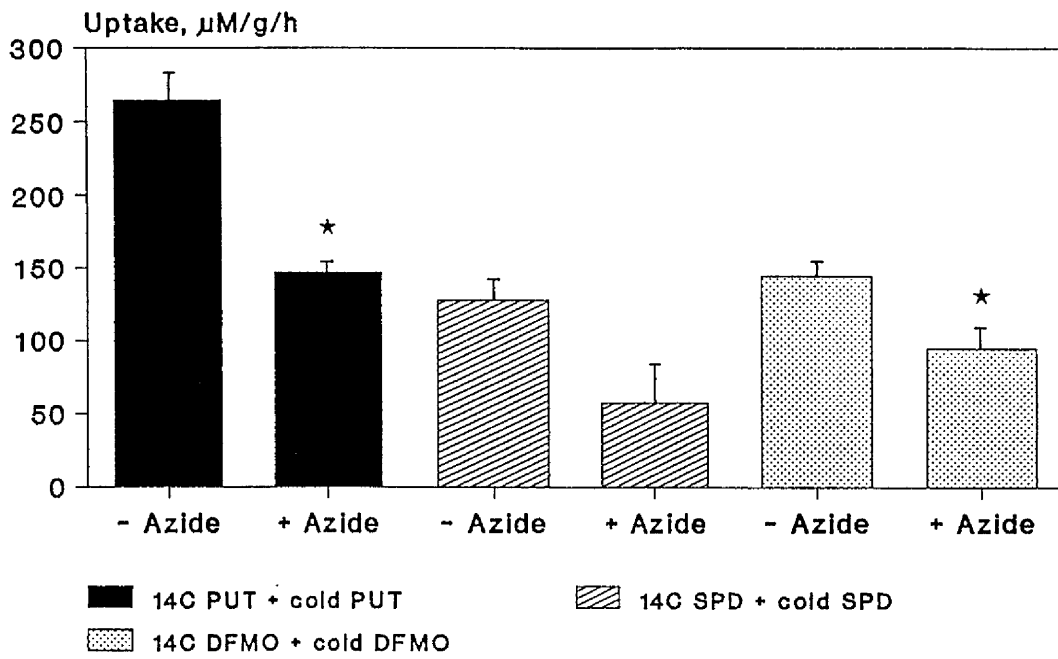


FIGURE 46. Effect of growing F. culmorum in the presence of DFMO, MGBG and DFMO + MGBG on the uptake of putrescine by the fungus. Vertical bars represent the standard error of the mean and significant differences are shown at  $P = 0.02 - 0.01^{**}$ .

FIGURE 47. Effect of growing F. culmorum in the presence of DFMO, MGBG and DFMO + MGBG on the uptake of spermidine by the fungus. Vertical bars represent the standard error of the mean and significant differences are shown at  $P = 0.02 - 0.01^{**}$ .



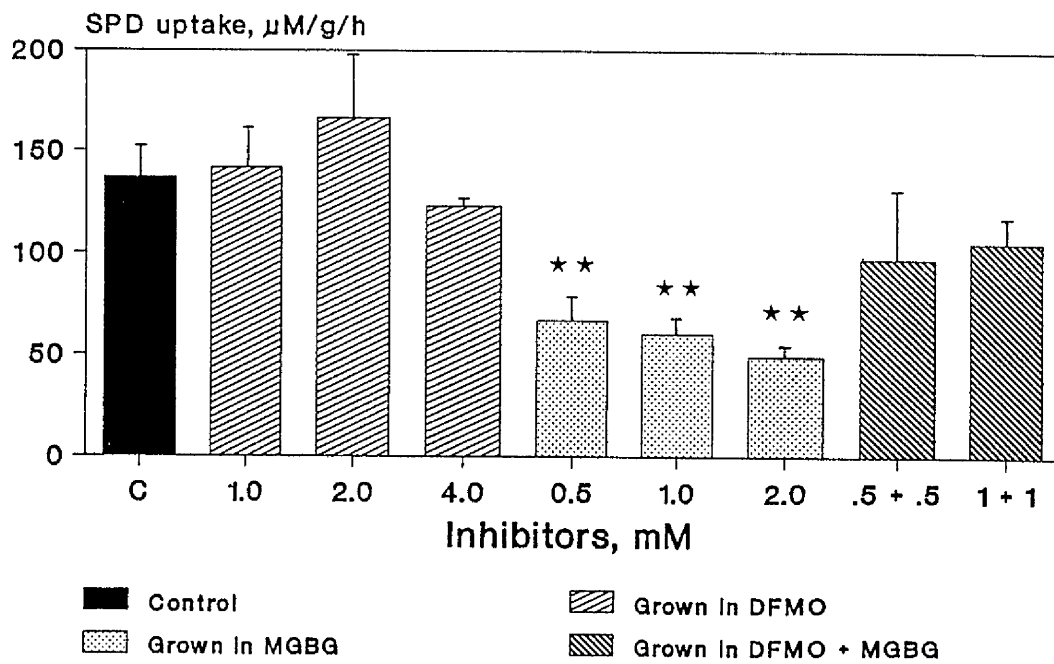
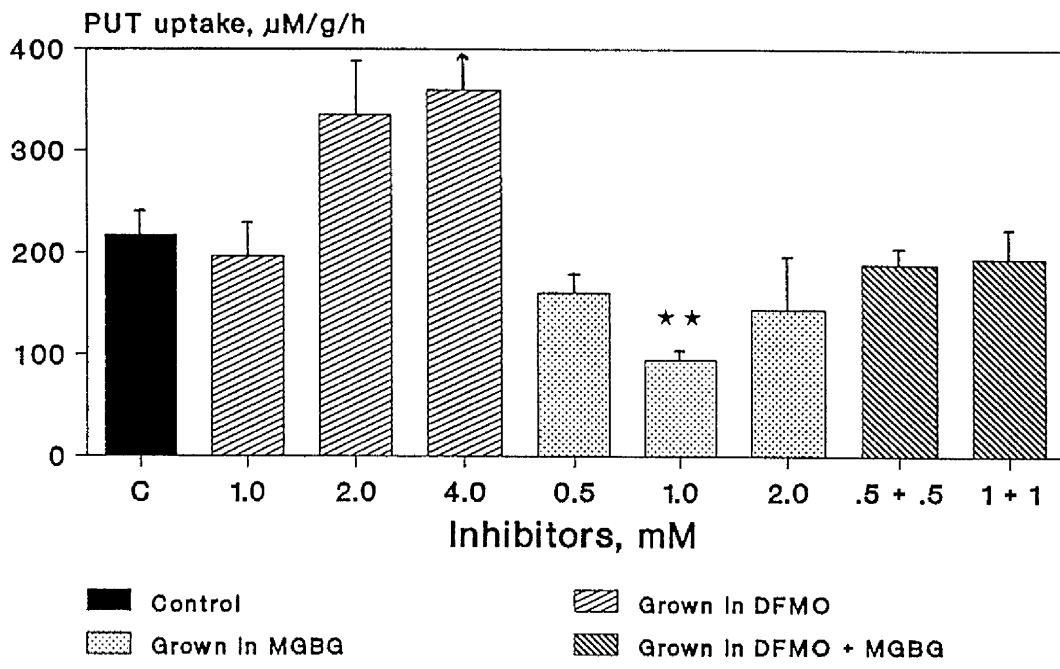
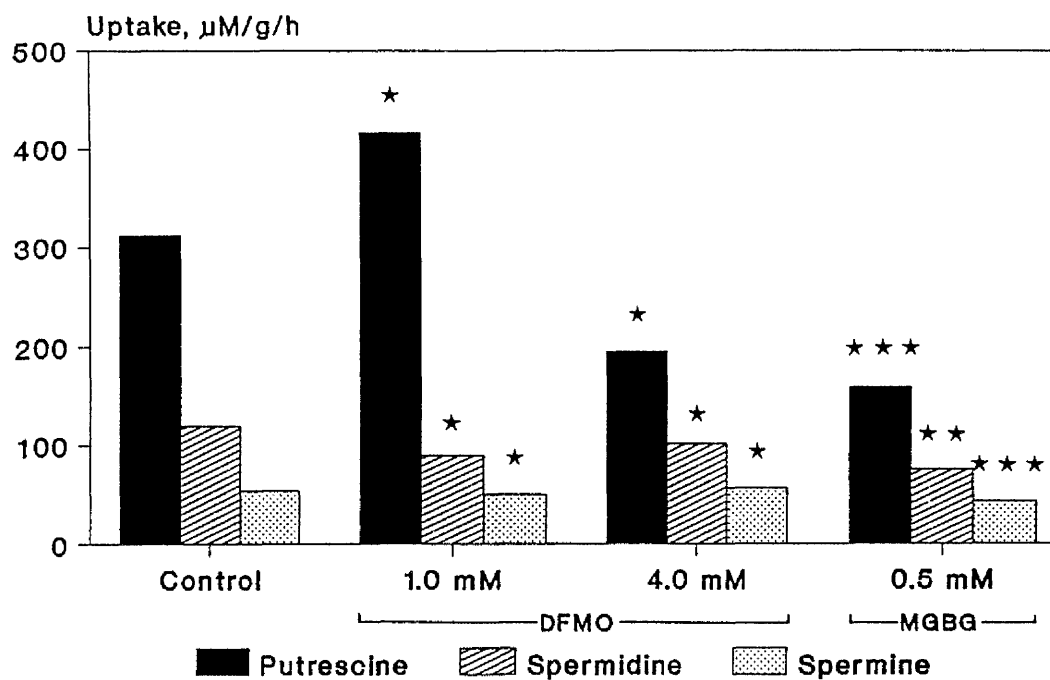
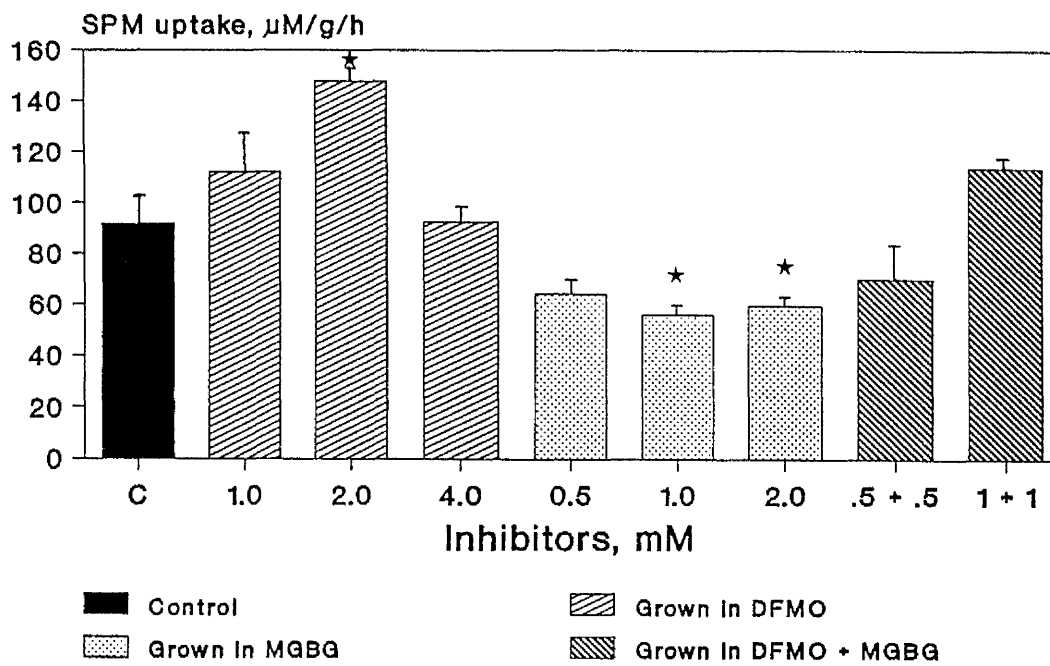


FIGURE 48. Effect of growing F. culmorum in the presence of DFMO, MGBG and DFMO + MGBG on the uptake of spermine by the fungus. Vertical bars represent the standard error of the mean and significant differences are shown at  $P = 0.1 - 0.05^*$ .

FIGURE 49. Effect of growing G. graminis in the presence of DFMO and MGBG on the uptake of putrescine, spermidine and spermine by the fungus. Standard errors are tabulated, Table XXVIII (Appendix). Significant differences are shown at  $P = 0.1 - 0.05^*$ ;  $P = 0.02 - 0.01^{**}$ ;  $P = 0.002 - 0.001^{***}$ .



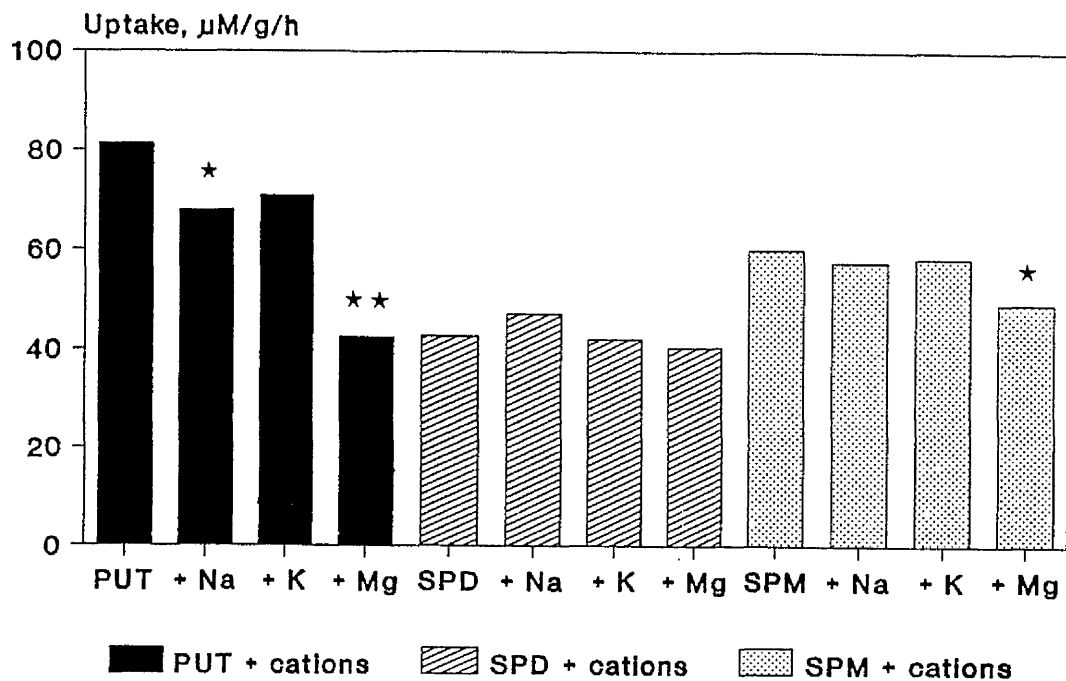
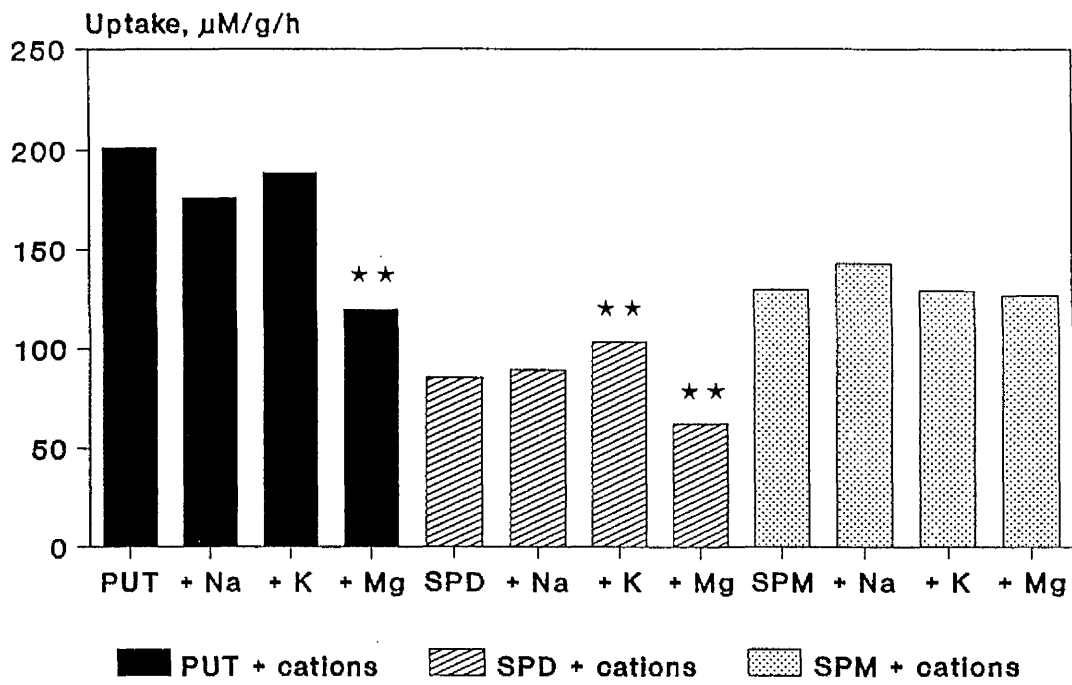
### 3.3.8 Competition by Cations Within the Growth Medium on Uptake of Polyamines

The effect of individual cations present within the Czapek Dox medium used was studied. Uptake of polyamines by F. culmorum and G. graminis from a MOPS buffer which had been individually amended with Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>2+</sup> was determined.

Figure 50 (Text) shows that uptake of putrescine and spermidine by F. culmorum was inhibited by the presence of Mg<sup>2+</sup> whilst K<sup>+</sup> actually led to enhanced uptake of spermidine. Uptake of putrescine and spermine by G. graminis was inhibited by Mg<sup>2+</sup> and putrescine uptake was also reduced in the presence of Na<sup>+</sup>.

FIGURE 50. Effect of the presence of cations within the assay medium (MOPS buffer) on uptake of polyamines by F. culmorum. Standard errors are tabulated, Table XXIX (Appendix). Significant differences are shown at  $P = 0.02 - 0.01^{**}$ .

FIGURE 51. Effect of the presence of cations within the assay medium (MOPS buffer) on uptake of polyamines by G. graminis. Standard errors are tabulated, Table XXIX (Appendix). Significant differences are shown at  $P = 0.1 - 0.05^{*}$ ;  $P = 0.02 - 0.01^{**}$ .



SECTION 4

DISCUSSION

#### 4.1 EFFECT OF THE INHIBITORS ON INFECTION OF BARLEY BY ERYSIPHE GRAMINIS

All of the inhibitors of polyamine biosynthesis used on leaves inoculated with mildew, significantly reduced the level of infection. DFMO gave good control of fungal growth, the post-inoculation sprays being particularly effective in comparison with the pre-inoculation treatments.

A similar result was reported by Walters (1986) who studied the effect of DFMO on infection of Vicia faba by the bean rust fungus Uromyces viciae-fabae and also by Weinstein et al. (1987) for infection by Puccinia recondita (leaf rust), P. graminis f.sp. tritici (stem rust) and E. graminis (powdery mildew). A possible explanation for this effect proposed by Weinstein et al. (1987) is that when a pre-inoculation spray of DFMO is applied, the drug enters the leaf and reacts with ornithine decarboxylase within the plant cell. The DFMO is thus unavailable when the leaf is inoculated with fungal spores. Post-inoculation sprays of MGBG were also more effective than pre-inoculation treatments. The hypothesis suggested by Weinstein et al. could equally apply to applications of MGBG, since this inhibitor may be rendered unavailable by binding to S-adenosylmethionine decarboxylase within the leaf. However, post-inoculation sprays of water also reduced the level of mildew infection more efficiently than pre-inoculation sprays. It is an accepted fact that mildew spores do not germinate readily if conditions are wet (Perera and Wheeler, 1975). It is, therefore, not surprising that a post-inoculation treatment of water will reduce infection. Although water appears to act as a fungi-



cide for the reason stated, the action of the polyamine biosynthetic inhibitors far outweighs the inhibitory effects of the water.

As DFMO was originally synthesized as an anti-cancer drug and has since been used in the treatment of trypanosomal infection, most of the previous work has been in connection with these. During such work, several limitations of DFMO have been discovered, namely, that the inhibitor does not deplete the cell of spermine, but only of putrescine and spermidine. This may be because of an incomplete blockage in the synthetic pathway, or it may be due to the stimulation of S-adenosylmethionine decarboxylase activity, which may result when DFMO is applied (Pera et al., 1986). Therefore, DFMO is considered to be cytostatic rather than cytotoxic (Mamont et al., 1984). The efficacy of DFMO may be reduced further by other compensatory mechanisms which it may induce. Thus, in addition to secondary enzyme production, there may also be an overproduction of ornithine decarboxylase itself (Janne et al., 1985). Pegg et al. (1982) discuss the occurrence of an increased level of decarboxylated S-adenosylmethionine resulting when transformed mouse fibroblasts were treated with DFMO. The authors indicate that this may be a general phenomenon and not restricted to mammalian cells. The consequences of this are an inhibition of methyl-transferase activity and the possibility of reactions to compensate for a reduced spermidine content. This phenomenon may also result after treatment with other ornithine decarboxylase inhibitors and not be restricted to DFMO.

The other ornithine decarboxylase inhibitors such as  $\Delta$ -MFMO,  $\Delta$ -MFMO.Me and RR-MAP are relatively recent introductions and

again, most work has been carried out on animal systems. Studies on these systems suggest that they have a greater efficacy than DFMO. Indeed, RR-MAP has the advantage of being able to reduce spermine pools and should theoretically be cytotoxic to cells. However, the results of this study show that although very low concentrations of RR-MAP controlled mildew, the inhibitor was not as effective as post-inoculation sprays of DFMO. In contrast to these findings, Mamont et al. (1984), showed RR-MAP to be more potent than DFMO when applied to rat hepatoma cells. These workers found that intracellular accumulation of RR-MAP was greater than that of DFMO.  $\Delta$ -MFMO.Me has the same properties as  $\Delta$ -MFMO, except that the additional methyl ester of the former inhibitor leads to an increased uptake into animal cells, where the compound is enzymatically de-esterified (Pera et al., 1986). The results of this study show that  $\Delta$ -MFMO.Me gave greater control than  $\Delta$ -MFMO at a concentration of 1.0 mM, but there was very little difference at the lower concentration. DFMO appeared to be slightly more potent at the lower concentration but there was little difference in effectiveness between the new ornithine analogues and DFMO. As mentioned above, Pera et al. (1986) and Bacchi et al. (1987) found the new inhibitors to be much more powerful than DFMO in animal systems. Finally, the commercially available ornithine decarboxylase inhibitor, 2-hydrazinoornithine, gave better control of mildew than the other ornithine analogues.

Depletion of cellular spermidine is an important goal in research on cancer chemotherapy, since this polyamine appears to be functionally related to cell proliferation (Porter and Sufrin, 1986). Since spermidine is the most common polyamine found in fungi and

is important for cell division (Stevens and Winther, 1979), its depletion in fungal cells should prevent growth. Thus, it was interesting to find that CHA, an inhibitor of spermidine synthase was also very efficient in reducing mildew infection. However, in discussion of possible disease control, emphasis has been put upon the inhibition of S-adenosylmethionine <sup>decarboxylase</sup> because of its importance as a rate-limiting biosynthetic enzyme in fungal polyamine biosynthesis, especially spermidine. MGBG inhibits this enzyme and the results of this study show that the post-inoculation sprays were particularly effective. The major disadvantage of using MGBG is that it has been reported to inhibit cellular respiration. Thus, the inhibitory effects may be a result of mitochondrial damage and not solely due to an inhibition of S-adenosylmethionine (Janne et al., 1985). The applications of MGBG plus DFMO mixtures were also very efficient in controlling mildew. The two compounds should, theoretically, exert a synergistic effect and be more effective than either DFMO or MGBG alone. Indeed, with the exception of MGBG at 0.05 mM plus DFMO at 0.5 mM, the combination was more effective than low concentrations of DFMO and MGBG applied separately. However, applications of high levels of MGBG were more effective than the mixture of the two inhibitors, although, using high levels of MGBG will lead to effects other than those connected with polyamine metabolism.

The results obtained when polyamines were added to DFMO show that the effect of the inhibitors can be reversed. Growth of the mildew was not restored to control levels but it was greater than that after the comparable DFMO treatment. Such partial reversal may be due to compartmentation of the exogenously applied amines

within the leaf, rendering a proportion of these compounds unavailable for fungal uptake. The higher levels of spermidine and putrescine added with DFMO did not encourage growth to the same extent as the lower polyamine concentrations. This may be due to some non-specific toxic effect of the amines, or possibly a result of feedback inhibition brought about by the availability of the exogenous polyamines and, perhaps, endogenous polyamines within the plant cells.

Post-inoculation sprays of putrescine and spermidine reduced mildew infection quite considerably. This may be partially due to the inhibitory effects of applying water as a post-inoculation spray and to feedback inhibition or toxicity of the polyamines as already discussed. The pre-inoculation treatment of putrescine increased mildew infection although the result was not statistically significant. However, the pre-inoculation treatment of spermidine produced a significant increase in mildew infection by day 9.

The results obtained from experiments carried out to determine the most effective time of inhibitor application showed similar trends for DFMO, MGBG and the combination of MGBG and DFMO, with the greatest reduction in mildew growth occurring after leaves were sprayed three days after inoculation. It is possible that much less effective inhibition occurs with pre-inoculation treatments because inhibitor is rendered unavailable by reacting with ornithine decarboxylase within the leaf, consequently reducing the inhibitor titres. Mildew did not show the same response to treatment with DFMA. Nevertheless the fungus was significantly con-

trolled by this inhibitor, with the pre-inoculation treatment being most efficient. The inhibitory effect was similar to that after treatment with DFMO on the first day after inoculation. However, whereas mildew growth was reduced even more after treatment with DFMO on day 2, mildew infection increased after DFMA application on the same day. It is possible that a limited conversion of DFMA to DFMO by arginase occurred within the plant (see Weinstein et al., 1987). Thus, DFMO formed within the plant would reduce the initial growth of the mildew. However, as the fungus developed, the DFMO produced by this conversion would not be sufficient to reduce fungal growth to the same extent as direct application of DFMO. It is unlikely that mildew possesses biosynthetic arginine decarboxylase activity since ornithine decarboxylase inhibitors are so effective in reducing fungal growth.

No visible phytotoxic effects were observed in this work but this does not necessarily mean that ornithine decarboxylase activity within leaves was not affected. However, Walters (1986) found no effect of 4.0 mM DFMO on growth and polyamine levels in Vicia faba in short term experiments. What is needed are longer term studies of the effects of these inhibitors on growth, yield and polyamine metabolism in selected crop plants.

#### 4.2 EFFECT OF THE INHIBITORS ON GROWTH OF FUNGI GROWN IN VITRO

The effect on mycelial growth of inhibitors of polyamine biosynthesis is dependent upon the particular fungus. Birecka et al. (1986) stated that genus-dependent differences in sensitivity to inhibitors may be due to differences in uptake of the inhibitor, ornithine decarboxylase sensitivity and polyamine requirements. However, work carried out in this study shows that DFMO is taken up in a linear non-saturable fashion by both F. culmorum and G. graminis. These two fungi exhibit different growth responses when exposed to DFMO, thus suggesting the importance of other factors such as enzyme sensitivity. Rajam and Galston (1985) also found that different genera had varying response patterns to these inhibitors. Of the species studied here, several treatments actually enhanced growth of some fungi whereas the same treatment inhibited others. Effects of the inhibitors also varied with time according to the particular inhibitor and fungal species.

The main trends observed were ones of increasing potency with time, decreasing effectiveness with time and no alteration in the degree of growth reduction. This study takes these genus-dependent differences a step further because variations have been observed between mutations of the same species. P. teres differentiated, possibly due to segregation of nuclei, or mutation, thus allowing observations of orange or grey forms of the fungus derived from the same stock. Growth of the orange form was inhibited by CHA, whereas growth of the grey form was increased by the same treatment. Interestingly, recent work in this laboratory has shown that the oat-infecting fungus Pyrenophora avenae was

very susceptible to inhibition of polyamine biosynthesis (Foster and Walters, unpublished results). It would be useful to understand the mechanisms underlying such differences within the same genus.

DFMO was less effective in controlling mycelial growth than  $\Delta$ -MFMO and  $\Delta$ -MFMO.Me in P. teres (both forms) and F. culmorum, and DFMO,  $\Delta$ -MFMO and  $\Delta$ -MFMO.Me actually increased growth of S. nodorum. However, high rates of DFMO (4.0 mM) gave good control of P. teres (grey form) compared to the orange forms of this species.

$\Delta$ -MFMO was more efficient than  $\Delta$ -MFMO.Me in reducing growth of P. teres (both forms) and G. graminis. Low concentrations of  $\Delta$ -MFMO were more efficient with regard to S. nodorum and F. culmorum, but the methylated form ( $\Delta$ -MFMO.Me) proved to be slightly more potent at the higher concentration used.

The greater inhibitory effect of  $\Delta$ -MFMO is interesting, as the addition of the methyl ester to the  $\Delta$ -MFMO should theoretically enhance uptake of the inhibitor into the cell. Thus,  $\Delta$ -MFMO.Me would have been expected to exhibit greater control of mycelial growth. Indeed, this was the case for F. culmorum at the highest concentration studied, but not the case for the other species. The work carried out on mildew infection of barley indicated, in contrast to the observed results with the necrotrophs, that  $\Delta$ -MFMO.Me was more efficient than  $\Delta$ -MFMO at concentrations of 1.0 mM but not lower than that. It would seem that the addition of the methyl ester to the inhibitor does not enhance uptake by the fungi grown in vitro, or perhaps the various fungi exhibit

differences in ability to de-esterify  $\Delta$ -MFMO.Me. These two inhibitors ( $\Delta$ -MFMO and  $\Delta$ -MFMO.Me) were slightly more effective than DFMO at comparable concentrations although growth of G. graminis was reduced a greater amount by DFMO.

In some cases increases in growth beyond that of the controls were observed with treatments of ornithine decarboxylase inhibitors and particularly with CHA. This phenomenon is possibly a result of incomplete blocking of the synthetic pathway, or to enhanced production of S-adenosylmethionine decarboxylase (Pera et al., 1986). As already mentioned in section 4.1, it is known that cells can compensate for the effects induced by ornithine decarboxylase inhibitors, by means of increasing the activities of other enzymes of polyamine biosynthesis and by overproducing the target enzyme.

The putrescine analogue, RR-MAP, which has proved to be a potent inhibitor when used on rat hepatoma cells (Mamont et al., 1984), was only moderately successful in inhibiting growth of F. culmorum and G. graminis.

The results obtained using DFMO and DFMA on P. ultimum and P. infestans show that for the former fungus, neither of the inhibitors used affected growth. However, a mixture of the two inhibitors resulted in a slight increase in growth. It is possible that DFMA was converted to DFMO by arginase within the fungus, thus effectively increasing the level of DFMO present. The enhanced inhibitor concentration could have caused the fungus to compensate and therefore, increase its growth response. In contrast, P.



infestans suffered a reduction in growth when grown in the presence of DFMO. On the other hand, DFMA caused a significant increase in mycelial growth. Again, this may be a result of a conversion of DFMA to DFMO within the fungus, and the smaller concentration of DFMO may have encouraged the fungus to compensate for the disturbance of polyamine metabolism. The mixture of DFMO plus DFMA led to a greater growth reduction in P. infestans compared with the DFMO, 1.0 mM treatment. It is possible that DFMA in the mixture was converted to DFMO, thus increasing the effective intracellular concentration of DFMO. It is clear that further work must be carried out to determine the responses of the enzymes responsible for the biosynthesis of polyamines when fungi are treated with inhibitors.

MGBG appeared to be the inhibitor with the greatest overall efficacy, controlling all of the fungi. The mixture of MGBG and DFMO also exhibited good control of fungal growth, although in many cases the highest concentration of MGBG produced greater growth reductions. It is a matter of debate as to whether the effects of MGBG are solely due to the inhibition of S-adenosylmethionine decarboxylase or whether they are a result of a reduction in cellular respiration due to possible mitochondrial damage caused by MGBG (Janne et al., 1985). This damage may be reduced by using mixtures of MGBG and DFMO which act synergistically, allowing lower concentrations to be used. This synergistic effect is noticeable in P. teres (grey form) but does not appear to be apparent in the other genera. Janne et al. (1985) discuss the fact that MGBG may only be moderately effective when used in combination with DFMO, because MGBG can re-establish the polyamines

that are depleted as a result of the action of DFMO. It is possible that this phenomenon occurred in the fungi other than P. teres (grey form). It is particularly interesting that the orange form of P. teres did not appear to exhibit the effects of a synergistic reaction.

The growth of the biotroph U. maydis was not controlled any more effectively by the inhibitors than growth of the necrotrophs. Considering the success achieved in reducing mildew infection of barley and a range of rust diseases (Rajam, Weinstein and Galston, 1985; Walters, 1986), it would have been expected that U. maydis should have responded in a similar manner. However, the fact that this fungus can be cultured in vitro suggests differences and reduced specificity in nutritional requirements. Perhaps, therefore, similarity to the results obtained using necrotrophs is not really surprising.

In experiments where polyamines were added to DFMO, spermidine and putrescine additions increased mycelial growth over that of the 4.0 mM DFMO treatment and, in some cases, even beyond that of the control (P. teres, grey form and F. culmorum). The reversal of inhibition indicates that polyamines are necessary for fungal growth (Tabor, 1981). The results obtained from additions of spermidine and putrescine to the media, indicate that the polyamines enhanced growth over that of the control. However, spermidine decreased growth of P. teres (orange form) and S. nodorum at the highest concentration used and this may be due to a non-specific toxic effect of the polyamine.

Rajam and Galston (1985) found that cell size of Botrytis spp. and Monilinia fructicola were altered by treatment with inhibitors. Cell lengths were reduced and diameters increased by inhibitors whereas both cell length and diameter were increased when the fungi had been exposed to polyamines. Results from work carried out in this study show that cell size of G. graminis barely changed, although some treatments (for example, MGBG) caused a decrease in cell length. P. teres also showed reductions in lengths on exposure to several inhibitors. This is in contrast to S. nodorum and F. culmorum which exhibited increased cell lengths as a result of inhibitor treatments; the diameters of F. culmorum were also increased. These two main trends do not appear to be connected with the efficacy with which the inhibitors reduced mycelial growth. Thus, G. graminis was relatively sensitive to the inhibitors and yet cell sizes were mostly unchanged.

#### 4.3 UPTAKE OF POLYAMINES, PRECURSORS AND DFMO BY F. CULMORUM AND G. GRAMINIS

In order to fully understand the mechanisms involved in inhibiting polyamine biosynthesis, the essential features of the synthesis and the transport of the amines must be elucidated. Therefore, once it has been established that the inhibitors of the biosynthetic enzymes exhibit fungicidal action, further fundamental questions must be asked. It is clear that the inhibitors do act upon the polyamine biosynthetic pathway. The fact that the action of the inhibitors can be reversed by adding polyamines to the medium is indicative of this. However, what has yet to be determined is how the fungicidal properties of the inhibitors are

effected and why some fungal species are more susceptible than others to these drugs. The answers lie in either the susceptibility of the relevant enzymes to inhibitors or in differences in uptake of the inhibitors. In an attempt to answer at least some of these questions, uptake systems of the polyamines, ornithine, arginine and DFMO were studied in some detail. It is important to appreciate that uptake and not transport of the compounds was described and will be discussed. The term uptake includes transport, distribution and metabolism. However, in this text the terms uptake and transport will be synonymous and will thus be interchangeable. Since the inhibitors are analogues of either basic amino acids or the polyamines themselves, it is important to understand the uptake mechanisms for these compounds to allow rational design of new inhibitors which would be transported efficiently to the target site.

Uptake of the amino acids and polyamines by *F. culmorum* was dependent upon the pH of the external medium. The data indicated that uptake of the higher polyamines, spermidine and spermine, increased with greater pH values, both reaching optimum uptake at pH 8.0. In contrast, transport of the diamine putrescine, gradually increased with rising pH, until the optimum value of pH 6.0 was attained. Thereafter, uptake by *F. culmorum* declined. The amino acids, arginine and ornithine, were taken up most rapidly at pH values 5.0 and 6.0 respectively. Uptake of the amino acids decreased with increasing alkalinity. Thus, the general trends were ones of more rapid transport of polyamines with increasing pH and a slowing down of uptake of amino acids with rising alkalinity. On the other hand, DFMO showed very

little dependence on the pH of the external medium. Although amino acids have been thoroughly studied with regard to fungal nutrition, little work has been carried out on uptake into fungal cells. Nor has very much work been completed on transport of polyamines by fungal cells. However, the results described in this thesis relating to optimum pH values for uptake of the amino acids, are in agreement with work carried out on Sacch. cerevisiae at the Institut Pasteur, which found that many amino acids have an optimum pH of between 5.0 and 6.5 (Burnett, 1968). Davis and Ristow (1988) studied the effect of pH on uptake rates of polyamines by N. crassa and concluded that putrescine transport depended only slightly on pH, but spermidine showed an optimum of pH 7.2. These results are not dissimilar to the findings of this study which shows a pH optimum for spermidine uptake of 8.0. In this respect, spermidine uptake in F. culmorum more closely resembles spermidine uptake in Saintpaulia petals, which also showed an optimum at pH 8.0 (Pistocchi, Bagni and Creus, 1986).

Although putrescine transport peaked at pH 6.0, the dependence on the pH of the medium, from values 5.0 to 9.0 was less than that observed for the higher polyamines. Other reports of polyamine uptake being pH dependent exist. This is the case in, for example, Saintpaulia petals (Bagni and Pistocchi, 1985), carrot protoplasts and vacuoles (Pistocchi et al., 1988), Anacystis nidulans (Guarino and Cohen, 1979), the lichen Evernia prunastri (Legaz and Escribano, 1987) and the human platelet (Nadler and Takahashi, 1985). Bagni and Pistocchi (1985) observed two uptake maxima for putrescine, one at pH 5.0-5.5 and another at pH 8.0. Each maximum was concentration dependent, the former being noted

for uptake at low substrate concentrations whereas the latter was connected with uptake at higher putrescine concentrations. These workers explain this effect in terms of the presence of uncharged putrescine at pH 8.0 and positively charged diamine at acidic pH values. In addition, they state that at acidic pH values, putrescine may be acetylated before passing across the cell membrane and this could change the cationic nature of the molecules. Pistocchi et al. (1988) discuss pH dependent uptake in terms of basic substances crossing membranes at pH values where they are less protonated, and Legaz and Escribano (1987) found that lichen cells take up putrescine as a mono-protonated form which is not degraded and as a di-protonated form (at pH 5.0) which is broken down. It is thus tempting to suggest that in this study, spermidine and spermine were present within the medium in a less protonated form at higher pH values and therefore, were taken up more readily by the fungal cells at those values.

However, even at pH 8.0, most of the polyamines would remain highly protonated (Pistocchi, Bagni and Creus, 1986). These authors suggest that peak in polyamine uptake observed at basic pH values could be correlated to the pH optimum of tonoplast ATPase. In the absence of the appropriate experimental data, this remains as mere speculation.

The results obtained from timing experiments indicated that uptake of both spermidine and spermine was relatively constant over time. The rate of transport by F. culmorum was constant over a 2 hour period. This infers a controlled uptake mechanism. Putrescine, on the other hand was taken up rather erratically, showing a

maximum uptake at 30 minutes and then falling after that. However, transport into the fungal cells rose dramatically after 50 minutes and continued to increase linearly up to 2 hours, when the experiment was stopped. It is possible that when the initial surge of uptake occurred, putrescine was adhered to cell walls and nucleic acids within the cell itself. When these sites were filled, putrescine was transported less rapidly into the fungal cells at a rate controlled by the metabolic needs of the cell for the diamine.

The rapid linear increase observed after 50 minutes could have been due to enhanced synthesis of higher polyamines, in addition to catabolism of putrescine by diamine oxidases and/or excretion of excess substrate back into the external medium. The linear increase in uptake of putrescine coincided with a very small decrease in transport of spermidine, indicating the possibility of enhanced synthesis of spermidine by the cell. However, the increase in putrescine uptake was considerably greater than the corresponding decrease in spermidine transport. Interestingly, work carried out in this laboratory has shown that uptake of spermidine by isolated cell walls of *F. culmorum* does not increase with time, over a span of 15 seconds to 1 hour (D.R. Walters, unpublished results). In other words, maximum attachment of polyamines to the cell wall occurred within 15 seconds and no further increases in polyamine binding associated with walls occurred after this time.

Therefore, the remaining spermidine transported into the cell will be bound to nucleic acids and used in metabolic processes. The

non-dependence on time infers that uptake of the higher polyamines is highly regulated. There are many reports of fungal cell walls retaining cations which are later transported into the cell.

These stored compounds apparently do not affect the permeability of the cell wall. However, according to Marchant (1966), cited by Burnett (1968), F. culmorum possesses an outer gel-like layer which acts as an enlarged absorbing area and may inhibit uptake for a short while. Thus, the reduced uptake of putrescine following the initial surge, may have been due to a prevention of the substrate from being transported into the cell from the gel layer. As this inhibition is a delaying response rather than a preventative reaction, the linear uptake occurring after 50 minutes could have been the result of transport of the diamine into the fungal cells thereby freeing sites within the layer of gel. As putrescine is the precursor for the higher polyamines, it is not surprising that uptake of this diamine was greater than that of spermidine and spermine.

Uptake of the amino acids increased up to 20 minutes and then levelled off, but increased again after 40 minutes. Both ornithine and arginine followed the same pattern of uptake over the time course. According to Pall (1970), four uptake systems exist in N. crassa for the main amino acids.

L-arginine may be transported by two systems, whilst ornithine is taken up by one. However, these systems have not as yet been described and it is simply accepted from competition studies that they exist. The fact that arginine can be taken up by two systems



may be indicative of the greater degree of transport observed for this amino acid than for ornithine. That is of course, assuming that both systems can operate simultaneously. In the present work in contrast to the pattern of ornithine uptake, transport of its analogue, DFMO, did not increase with time. It would be expected that because of its structural similarity to ornithine, DFMO would utilize the ornithine transport system.

However, according to work carried out on Sacch. cerevisiae, competitive inhibition between amino acids is not a function of their structure (Burnett, 1968), and hence, similarity between DFMO and ornithine may not necessarily mean that similar uptake systems are utilized.

Uptake as a function of substrate concentration was studied for the amino acids, polyamines and DFMO and the  $K_m$  values (or affinity constants) for the compounds were calculated. However, the  $K_m$  of DFMO could not be derived, as uptake of this compound was linear, with the consequence that no  $V_{max}$  was reached over the concentrations studied. Uptake by both F. culmorum and G. graminis was examined, although a greater substrate concentration range was studied for F. culmorum. The data obtained was graphed, to produce in most cases typical curves indicative of saturation kinetics. The data was also manipulated to produce Eadie-Hofstee plots which emphasized the presence of biphasic systems for uptake of the compounds, and enabled the affinity constants ( $K_m$ ) to be directly calculated. As already indicated, the substrate concentration range for uptake by F. culmorum was relatively wide. Thus, a  $K_m$  value for each uptake system could be calculated for

the compounds studied. A high and low affinity system was found to be present, the former operating at low substrate concentrations, whilst the latter was observed at high substrate concentrations. The  $K_m$  values calculated for each system were very different. For example, for spermidine uptake by F. culmorum, the  $K_m$  for the high affinity system (system 1) was 0.34 mM whilst that for the low affinity system (system 2) was 42.17 mM.

This trend was the same for each compound.  $K_m$  values for each system for G. graminis could not be calculated due to the narrow range of the substrate concentrations used. It is interesting to note that the affinity for ornithine and putrescine by G. graminis appeared to be less than that of F. culmorum. Eadie-Hofstee plots were graphed in order that the overall  $K_m$  could be calculated. However, these plots clearly show the biphasic properties of uptake of most of the compounds except for arginine and spermidine. The data for these substrates is indicative of a single system, but in light of the results obtained for other compounds, and for the same compounds relating to F. culmorum, it would be fair to speculate that two systems do actually exist. The plots of the unmanipulated data for these substrates show curves, the appearance of which suggest biphasic systems.

The existence of biphasic uptake systems for polyamines has also been shown by Pistocchi, Bagni and Creus (1987) who found that external polyamine concentrations up to 100 mM yielded two systems with different affinities at high and low substrate concentrations in carrot cell cultures. In addition, Munro, Bell and Lederman (1974) reported the existence of multiple transport components for

putrescine in E. coli and Davis and Ristow (1988) discussed two uptake systems for polyamines in N. crassa in terms of saturable and non-saturable components. The former term refers to uptake over a low concentration range and the latter at higher concentrations. These workers suggest that part of the concentrative nature of uptake may be due to binding of the polyamines to cell constituents. Work carried out in this laboratory to determine the level of binding to fungal cell walls, showed that almost two-thirds of accumulated spermidine could be removed from the cells by washing them in 0.25 M sodium chloride (D.R. Walters, unpublished results). However, it is important to study total uptake rather than immediate transport into the cells, as this is more indicative of what actually occurs in a dynamic situation in vivo. Indeed, the substrate adhering to the cell walls will probably be utilized by the fungus at a later date.

DFMO did not exhibit similar kinetic properties to ornithine, as would have been expected by the very nature of its structure. Instead, the inhibitor was taken up in a linear fashion by both F. culmorum and G. graminis. These findings agree with work carried out by Erwin and Pegg (1982) when they looked at uptake of DFMO by mouse fibroblasts. They found transport of the inhibitor to be slow and non-saturable and suggested passive diffusion to be the mechanism by which the ornithine analogue enters the cells. The workers also concluded that the reversal effects observed when exogenous polyamines were added to DFMO treated cells, were not a result of interference with drug uptake. These results were corroborated by Bitonti et al. (1986) when they found uptake of DFMO by T. brucei brucei to be non-saturable and not antagonized by

additions of amino acids. However, Phillips and Wang (1987) report the existence of a mutant strain of T. brucei brucei which is resistant to DFMO. This is unusual in light of the extreme susceptibility of trypanosomes to the inhibitor. The resistance was inferred on the mutant due to its inability to transport DFMO. The workers found the same level of ornithine decarboxylase activity, the same rate of ornithine decarboxylation, the same membrane potential and the same degree of ornithine decarboxylase susceptibility to DFMO in both the mutant and wild-type. The fact that this organism is able to reduce DFMO uptake would infer that the transport mechanism is not wholly one of passive diffusion as proposed by previous workers.

Phillips and Wang (1987) suggest the possibility that DFMO may be transported by a specific protein which is non-functional in the mutant strain, or, that the presence of DFMO leads to an increase in protein density within the membrane, which could block non-saturable diffusion. This latter phenomenon has already been reported in Chinese hamster ovary cells, where mutant lines fail to take up colchicine, a drug transported by passive diffusion in wild-type cells (Carlsen, Till and Ling, 1976).

The only other work carried out to date on the uptake of DFMO was by Walters and Kingham (1989) who studied transport in barley seedlings and its effects on mildew infection. These workers found that DFMO uptake by roots was biphasic and saturable. They also found that uptake was reduced in the dark and they speculated on the possibility of an energy requirement for the transport.

In addition, they found that ornithine antagonized uptake of DFMO, an interesting finding which leads to speculation that DFMO uptake may actually be via an amino acid transport mechanism in this system. The antagonistic effect on DFMO uptake of other compounds has not previously been found. Nevertheless in the present study, it was observed that DFMO uptake by F. culmorum was inhibited by the presence of ornithine (similar to that observed by Walters and Kingham (1989) for barley), putrescine and spermidine within the medium.

However, the reverse was not true and DFMO did not antagonize putrescine or spermidine uptake. This observation does not agree with the work carried out on trypanosomes or mouse fibroblasts, although the non-saturable aspect of the uptake is similar to that found in those systems. It is possible that DFMO enters a cell via passive diffusion and/or, transport may be facilitated. Speculating about the uptake mechanisms of DFMO is complicated further by the fact that the addition of sodium azide (a respiratory inhibitor) caused a reduction, albeit small, in uptake by F. culmorum. There appeared to be, therefore, an energy requirement for transport of the inhibitor.

The mechanisms involved are apparently more complex and specific than were previously thought. Transport of putrescine also appears to possess an active component, as addition of sodium azide to the medium reduced uptake by F. culmorum. Spermidine uptake by this fungus did not exhibit a significant decrease when the respiratory inhibitor was added to the medium. This is interesting as Pistocchi, Bagni and Creus (1987) found that

spermidine uptake by carrot culture cells was affected more by metabolic inhibitors than was putrescine. Uptake of polyamines by E. coli is energy dependent (Tabor and Tabor, 1966), but to date, the energy donor has not been described. However, Kashiwagi, Kobayashi and Igarashi (1986) propose that polyamines are transported into E. coli by proton motive force and the process is unidirectional because of the formation of strong binding complexes with nucleic acids.

This work on F. culmorum also showed that putrescine uptake was not antagonized by spermidine, ornithine or DFMO, nor was spermidine uptake antagonized by putrescine, ornithine or DFMO. It would thus appear that putrescine, spermidine and ornithine have different uptake systems as they do not inhibit transport of each other. Spathas, Pateman and Clutterbuck (1981) found that neither spermidine or spermine inhibited putrescine uptake, but spermidine transport was inhibited by putrescine and spermine in Aspergillus nidulans. Therefore, in this fungus spermidine or spermine are not taken up by the putrescine system, although putrescine and spermine can be transported via the spermidine system. In contrast, uptake of each polyamine by F. culmorum appears to be via its own specific system.

Theoretically, if a fungus were to be exposed to inhibitors of polyamine biosynthesis for a prolonged period of time, some effect on uptake of polyamines from the medium should be observed. Thus, F. culmorum and G. graminis were cultured in the presence of inhibitors. The range of inhibitors used for G. graminis was limited, as the fungus did not grow well in liquid culture when

the inhibitors were present. In contrast, F. culmorum grew readily.

From the results obtained, it can be seen that the only inhibitor to cause any significant effect on uptake of the polyamines by F. culmorum was MGBG. This, in fact, led to a decrease in uptake when the polyamines were added to the medium. It is possible that long term exposure to MGBG caused functional damage to the fungal mitochondria and uptake was reduced for reasons other than those related to polyamine biosynthesis.

Thus, it is also possible that damage to the fungal mitochondria caused by MGBG might suppress any active component of the transport system. However, this does not seem to be a plausible explanation for these results, as it was suggested earlier that spermidine probably did not possess an active uptake system in F. culmorum. Yet this polyamine was the most affected by the exposure to MGBG.

It is interesting to note that uptake of spermidine was most significantly affected, with spermine and then putrescine uptake being reduced. It is possible that the transport systems were affected by the MGBG and the aliphatic chain length of the polyamine may have some bearing on the uptake of the compounds. Thus, the shorter the chain length, the greater the likelihood of being transported into the cells.

Exposure to DFMO had little overall effect on uptake of polyamines by F. culmorum. One or more concentrations of the three polyamines superficially appear on the graphs as having a greater

uptake than the control treatment. Statistically though, the increases are not significant. However, according to Erwin and Pegg (1982) who studied uptake of DFMO by mouse fibroblasts, exposure of cells to DFMO under normal growth conditions can lead to inactivation of the inhibitor. DFMO can bind covalently to ornithine decarboxylase, thus rendering the inhibitor inactive. However, with respect to DFMO activity, this would probably have a negligible effect because of the relatively small quantities of ornithine decarboxylase protein within cells. DFMO could also attach itself to other protein molecules within the cell. However, it is most likely that the inhibitor undergoes a non-enzymatic decarboxylation by pyridoxal phosphate, thus creating a product which reacts with nucleophiles within the cell, or within water. Indeed, Erwin and Pegg (1982) state that after 12 hours of exposure to radioactive DFMO, only 50 per cent of the total label was recoverable again. This phenomenon could help to explain the ineffectiveness of DFMO against certain fungi in this study.

It is perhaps not surprising that little difference in uptake of polyamines was observed in F. culmorum after exposure to inhibitors, when one considers the effects of DFMO on growth of the fungus. Likewise, since DFMO is unlikely to have depleted intracellular polyamine concentrations in F. culmorum, it should not be surprising that uptake of polyamines was not increased. On the other hand, G. graminis proved to be more susceptible to the inhibitors than did F. culmorum. Indeed, exposure of G. graminis to 1.0 mM DFMO actually led to an enhanced uptake of putrescine, and growth in 4.0 mM DFMO caused an enhanced uptake of spermine.



However, all other treatments resulted in significant reductions in polyamine transport.

F. culmorum and G. graminis showed differing susceptibilities to the inhibitors used throughout this research. It seems clear from the experiments carried out here that both fungal species react differently to the inhibitors and yet transport of the drug into the cells was similar.

It is important when attempting to define and understand uptake mechanisms, that conditions remain constant throughout the experiments. Differences in medium, pH or even fungal line may alter the kinetics of the uptake. Davis and Ristow (1988) whilst working on polyamine transport in N. crassa, found that uptake from the growth medium was very slow and was in fact being inhibited by cations within the medium. To determine whether or not cations within the liquid Czapek Dox + mycological peptone medium used in this study were having an effect on polyamine transport, an uptake experiment was carried out using MOPS free acid buffer as the assay medium. It was found that magnesium ions affected uptake of putrescine by F. culmorum and G. graminis and sodium ions affected uptake of this diamine by G. graminis. Magnesium and potassium ions competed with spermidine uptake by F. culmorum and magnesium ions inhibited spermine uptake by G. graminis.

Magnesium ions appeared to have the greatest inhibitory effect on uptake of polyamines with putrescine being more effected in both F. culmorum and G. graminis. This is possibly a function of similarity of charge of the magnesium ions and the diamine. It is

interesting to note that magnesium ions also inhibit uptake of spermidine by F. culmorum and spermine by G. graminis although to a lesser extent than putrescine. The enhancement of spermidine uptake by F. culmorum when subjected to potassium ions could be related to possible specific cation-activated ATPase activity leading to increased transport (see Lindberg, 1980).

It was decided to carry out the uptake studies in Czapek Dox medium + peptone in spite of competitive effects of cations present. This was partly to reduce fungal shock, by keeping the growth and uptake medium the same, and in order that meaningful comparisons could be made with the growth studies previously performed. Moreover, any competitive effects would be relative to the control treatments throughout.

#### 4.4 FUTURE CONSIDERATIONS

The work carried out in this thesis indicates that the inhibitors used have potential as fungicides. It was also established that species-dependent growth responses to these inhibitors were not a result of variations in uptake of the compound. Nevertheless it is clear that there is much work yet to be carried out with respect to uptake processes. For example, a study of membrane potential would be useful, as would an attempt to describe the carrier mechanisms involved. Uptake could be looked at in the presence of compounds such as chloramphenicol in an attempt to block carrier mechanisms, which may yield more specific results than those obtained by using a respiratory inhibitor. Uptake of DFMO must also be characterized further and in addition, it is

necessary to study transport of other, more recent inhibitors. Once the carrier mechanisms of polyamines and ornithine are characterized, inhibitors can be modelled which can utilize these components more efficiently. It is clear that uptake of DFMO and ornithine differ, in spite of their close structural relationship.

In addition to considering uptake further, it is perhaps more important to become familiar with alterations in enzyme content and susceptibility of the enzymes to the inhibitors. If variations in growth responses are not due to differences in uptake by the fungal species, then the key must lie in the susceptibility of the enzymes to the inhibitor, rates of decarboxylation of ornithine, efficiency in compensating for polyamine deficits, polyamine requirements of the fungi, or ability to metabolize the inhibitors into ineffective forms.

Indeed, work recently carried out in this laboratory has shown that Pyrenophora avenae, a necrotroph which is susceptible to ornithine decarboxylase inhibitors, shows reduced intracellular ornithine decarboxylase levels and lower concentrations of polyamines when subjected to these inhibitors (Foster and Walters, unpublished results).

Another vital future consideration is the fungal/plant relationship and effects of the inhibitors on this interaction. It is necessary to know whether fungi will be able to utilize a supply of polyamines from the plant itself, and also, the effects of the inhibitors on polyamine and enzyme content of the plant must be considered.

To determine the potential of the inhibitors of polyamine biosynthesis, these questions must be answered and a true understanding of the mechanisms involved gained. This will enable resistance responses to be predicted and inhibitors rationally designed to achieve maximum effect.

BIBLIOGRAPHY

ADAS (1982). Cereal mildew. Leaflet number 579. Ministry of Agriculture, Fisheries and Food.

ADAS (1983). Fusarium diseases of cereals. Leaflet number 854. Ministry of Agriculture, Fisheries and Food.

ADAS (1984). Use of fungicides and insecticides on cereals. Booklet number 2257 (84). Ministry of Agriculture, Fisheries and Food.

ALHONEN-HONGISTO, L. (1980). Regulation of S-adenosylmethionine decarboxylase by polyamines in Ehrlich ascites-carcinoma cells grown in culture. Biochemical Journal 190, 747-754.

ALHONEN-HONGISTO, L. and JANNE, J. (1980). Polyamine depletion induces enhanced synthesis and accumulation of cadaverine in cultured Ehrlich ascites-carcinoma cells. Biochemical and Biophysical Research Communications 93, 1005-1013.

ALHONEN-HONGISTO, L., KALLIO, A., SINERVIRTA, R., JANNE, O.A., GAHMBERG, C.G. and JANNE, J. (1985). Tumorigenicity, cell-surface glycoprotein changes and ornithine decarboxylase gene pattern in Ehrlich ascites-carcinoma cells. Biochemical Journal 229, 711-715.

ALHONEN-HONGISTO, L., POSO, H. and JANNE, J. (1980). Inhibition of derivatives of diguanidines of cell-proliferation in Ehrlich ascites-cells grown in culture. Biochemical Journal 188, 491-501.

- ALHONEN-HONGISTO, L., SEPPANEN, P., HOLTTA, E. and JANNE, J. (1982). Replacement of natural polyamines by cadaverine and its aminopropyl derivatives in Ehrlich ascites carcinoma cells. Biochemical and Biophysical Research Communications 106, 291-297.
- ALTMAN, A. (1982). Retardation of radish leaf senescence by polyamines. Physiologia Plantarum 54, 189-193.
- ALTMAN, A., KAUR-SAWHNEY, R. and GALSTON, A.W. (1977). Stabilization of oat leaf protoplasts through polyamine-mediated inhibition of senescence. Plant Physiology 60, 570-574.
- ATKINS, J.F., LEWIS, J.B., ANDERSON, C.W. and GESTLELAND, R.F. (1975). Enhanced differential synthesis of proteins in a mammalian cell-free system by addition of polyamines. Journal of Biological Chemistry 250, 5688-5695.
- ATMAR, V.J., DANIELS, G.R. and KUEHN, G.D. (1978). Polyamine stimulation of phosphorylation of nonhistone acid protein in nuclei and nucleoli from Physarum polycephalum. European Journal of Biochemistry 90, 29-37.
- BACCHI, C.J., GARAFALO, J., MOCHENHAUPT, D., McCANN, P.P., DIEKEMA, K. A., PEGG, A.E., NATHAN, H.C., MULLANEY, E.A., CHUNOSOFF, L., SJOERDSMA, A. and HUNTER, S.J. (1983). In vivo effects of DL- $\alpha$ -difluoromethylornithine on the metabolism and morphology of Trypanosoma brucei brucei. Molecular and Biochemical Parasitology 7, 209-225.

BACCHI, C.J., NATHAN, H.C., CLARKSON, A.B., BIENEN, E.J., BITONTI, A. J., McCANN, P.P. and SJOERDSMA, A. (1987). Effects of the ornithine decarboxylase inhibitors DL- $\alpha$ -difluoromethylornithine and  $\alpha$ -monofluoromethyldehydroornithine methyl ester alone and in combination with suramin against Trypanosoma brucei brucei central nervous system. American Journal of Tropical Medicine and Hygiene 36, 46-52.

BACCHI, C.J., NATHAN, H.C., HUNTER, S.H., McCANN, P.P. and SJOERDSMA, A. (1980). Polyamine metabolism: a potential therapeutic target in trypanosomes. Science 210, 332-334.

BAGNI, N. (1970). Metabolic changes of polyamines during the germination of Phaseolus vulgaris. New Phytologist 69, 159-164.

BAGNI, N., MALUCELLI, B. and TORRIGIANI, P. (1980). Polyamines, storage substances and abscisic acid-like inhibitors during dormancy and very early activation of Helianthus tuberosus tuber tissues. Physiologia Plantarum 49, 341-345.

BAGNI, N. and PISTOCCHI, R. (1985). Putrescine uptake in Saintpaulia petals. Plant Physiology 77, 398-402.

BAGNI, N. and PISTOCCHI, R. (1988). Polyamines as growth substances in higher plants. In: Progress in Polyamine Research; Novel Biochemical, Pharmacological and Clinical Aspects, Eds V. Zappia and A.E. Pegg, pp 547-558. Plenum Press, London.



- BAGNI, N., SERAFINI-FRACASSINI, D. and TORRIGIANI, P. (1981). Polyamines and growth in higher plants. In: Advances in Polyamine Research. Vol 3. Ed. C.M. Caldarera, pp 377-388. Raven Press, New York.
- BAGNI, N., TORRIGIANI, P. and BARBIERI, P. (1981). Effect of various inhibitors of polyamine synthesis on the growth of Helianthus tuberosus. Medical Biology 59, 403-409.
- BAKANASHVILI, M., BARKAI-GOLAN, R., KOPELIOVITCH, E. and APELBAUM, A. (1987). Polyamine biosynthesis in Rhizopus-infected tomato fruits: possible interaction with ethylene. Physiological and Molecular Plant Pathology 31, 41-50.
- BALLAS, S.K., MOHANDAS, N., MARTON, L.J. and SHOHET, S.B. (1983). Stabilization of erythrocyte membranes by polyamines. Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences 80, 1942-1946.
- BALLINGER, D.J. and KOLLMORGEN, J.F. (1986). Control of take-all of wheat in the field with benzimidazole and triazole fungicides applied at seeding. Plant Pathology 35, 67-73.
- BATCHELOR, K.W., SMITH, R.A. and WATSON, N.S. (1985). Dicyclohexylamine is not an inhibitor of spermidine synthase. Biochemical Journal Letters 233, 307-308.

- BEHE, M. and FELSENFELD, G. (1981). Effects of methylation on a synthetic polynucleotide - the B-Z transition in poly (DG-M5DC). Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences 78, 1619-1623.
- BEN-ARIE, R., LURIE, S. and MATTOO, A.K. (1982). Temperature dependent inhibitory effects of calcium and spermine on ethylene biosynthesis in apple discs correlate with changes in microsomal membrane micro-viscosity. Plant Science Letters 24, 239-247.
- BEY, P., DANZIN, C. and JUNG, M. (1987). Inhibition of basic amino acid decarboxylases involved in polyamine biosynthesis. In: Inhibition of Polyamine Metabolism. Eds P.P. McCann, A.E. Pegg and A. Sjoerdsma, pp 1-31. Academic Press.
- BIASI, R., BAGNI, N. and COSTA, G. (1988). Endogenous polyamines in apple and their relationship to fruit set and fruit growth. Physiologia Plantarum 73, 201-205.
- BIRECKA, H., GARRAWAY, M.O., BAUMANN, R.J. and McCANN, P.P. (1986). Inhibition of ornithine decarboxylase and growth of the fungus Helminthosporium maydis. Plant Physiology 80, 798-800.
- BITONTI, A.J., BACCHI, C.J., McCANN, P.P. and SJOERDSMA, A. (1986). Uptake of  $\alpha$ -difluoromethylornithine by Trypanosoma brucei brucei. Biochemical Pharmacology 35, 351-354.

- BITONTI, A.J., BUSH, T.L. and McCANN, P.P. (1989). Regulation of polyamine biosynthesis in rat hepatoma (HTC) cells by a bisbenzyl (polyamine) analogue. Biochemical Journal 257, 769-774.
- BITONTI, A.J., DUMONT, J.A., BUSH, T.L., EDWARDS, M.L., STEMERICK, D. M., McCANN, P.P. and SJOERDSMA, A. (1989). Bis (benzyl) polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (Plasmodium falciparum) in vitro and in combination with  $\alpha$ -difluoromethylornithine cure murine malaria. Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences 86, 651-655.
- BLOOMFIELD, V.A. and WILSON, R.W. (1981). In: Polyamines in Biology and Medicine. Eds D.R. Morris and L.J. Marton, pp 183-206. Dekker, New York.
- BOUHOT, D. and SMITH, I.M. (1988). Pythium spp. In. European Handbook of Plant Diseases. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 200-202. Blackwell Scientific Publications.
- BOWMAN, W.H., TABOR, C.W. and TABOR, H. (1973). Spermidine biosynthesis, purification and properties of propylamine transferase from Escherichia coli. Journal of Biological Chemistry 248, 2480-2486.
- BURNETT, J.H. (1968). Fundamentals of Mycology. Edward Arnold, London.

- BUSHNELL, M.E. and BULL, A.T. (1974). Polyamine, magnesium and ribonucleic acid levels in steady-state cultures of the mould Aspergillus nidulans. Journal of General Microbiology 81, 271-273.
- CANELLAKIS, E.S., VICEPS-MADONE, D., KYRIAKIDIS, D.A. and HELLER, J.S. (1979). Regulation and function of ornithine decarboxylase and of the polyamines. Current Topics in Cell Regulation 15, 155-202.
- CARLSEN, S.A., TILL, J.E. and LING, V. (1976). Modulation of membrane drug permeability in Chinese hamster ovary cells. Biochimica et Biophysica Acta 455, 900-912.
- CASARA, P., DANZIN, C., METCALF, B. and JUNG, M. (1985). Stereospecific synthesis of (2R, 5R) - hept-6-yne-2, 5-diamine - a potent and selective enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC). Journal of the Chemical Society - Perkin Transactions 1, 2201-2207.
- CASSINI, F. and SMITH, I.M. (1988). Ustilago maydis (DC.) Corda. In: European Handbook of Plant Disease. Eds I.M. Smith, J. Dunez, R.A. Lelliot, D.H. Phillips and S.A. Archer, pp 469-470. Blackwell Scientific Publications.
- CHAFFEE, R.R.J., ARINE, R.M. and ROCHELLE, R.H. (1979). Possible role of intracellular polyamines in mitochondrial metabolic regulation. Biochemical and Biophysical Research Communications 86, 293-299.

- CHUN, P.W., RENNERT, O.M., SAFFERN, E.E. and TAYLOR, W.J. (1976). Effect of polyamines on electrokinetic properties of red blood cells. Biochemical and Biophysical Research Communications 69, 1095-1101.
- C.M.I. (1964). Fusarium culmorum. Description number 26. Commonwealth Mycological Institute.
- C.M.I. (1965). Ustilago maydis. Description number 79. Commonwealth Mycological Institute.
- C.M.I. (1966). Leptosphaeria nodorum. Description number 86. Commonwealth Mycological Institute.
- C.M.I. (1967). Erysiphe graminis. Description number 153. Commonwealth Mycological Institute.
- C.M.I. (1973). Gaeumannomyces graminis var. tritici. Description number 383. Commonwealth Mycological Institute.
- C.M.I. (1973). Pyrenophora teres. Description number 390. Commonwealth Mycological Institute.
- C.M.I. (1985). Phytophthora infestans. Description number 838. Commonwealth Mycological Institute.
- COHEN, E., ARAD, S.M., HEIMER, Y.M. and MIZRAHI, Y. (1982). Participation of ornithine decarboxylase in early stages of tomato fruit development. Plant Physiology 70, 540-543.

- COHEN, E., ARAD, S.M., HEIMER, Y.M. and MIZRAHI, Y. (1984). Polyamine biosynthetic enzymes in the cell cycle of Chlorella. Correlation between ornithine decarboxylase and DNA synthesis at different light intensities. Plant Physiology 74, 385-388.
- COHEN, S.S. and GREENBERG, M.L. (1981). Spermidine, an intrinsic component of turnip yellow mosaic-virus. Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences 78, 5470-5474.
- COHEN, A.S., POPOVIC, R.B. and ZALIK, S. (1979). Effects of polyamines on chlorophyll and protein content, photochemical activity, and chloroplast ultrastructure of barley leaf discs during senescence. Plant Physiology 64, 717-720.
- COHN, M.S., TABOR, C.W. and TABOR, H. (1978). Isolation and characterization of Saccharomyces cerevisiae mutants deficient in S-adenosylmethionine decarboxylase, spermidine and spermine. Journal of Bacteriology 134, 208-213.
- CORTI, A., DAVE, C., WILLIAMS-ASHMAN, H.G., MIHICH, E. and SCHENONE, A. (1974). Specific inhibition of enzymic decarboxylation of S-adenosylmethionine by methylglyoxal bis (guanylhydrazone) and related substances. Biochemical Journal 139, 351-357.
- COSTA, G. and BAGNI, N. (1983). Effects of polyamines on fruit-set of apple. Hortscience 18, 59-61.

- DAI, Y.-R., KAUR-SAWHNEY, R. and GALSTON, A.W. (1982). Promotion by gibberellic acid of polyamine biosynthesis in internodes of light-grown dwarf peas. Plant Physiology 69, 103-105.
- DANZIN, C., CASARA, P., CLAVERIE, N., METCALF, B.W. and JUNG, M. (1983). (2R, 5R)-6-heptyne-2,5-diamine, an extremely potent inhibitor of mammalian ornithine decarboxylase. Biochemical and Biophysical Research Communications 116, 237-243.
- DANZIN, C. and MAMONT, P.S. (1987). Polyamine inhibition in vivo and in organ growth and repairs. In: Inhibition of Polyamine Metabolism. Eds P.P. McCann, A.E. Pegg and A. Sjoerdsma, pp 141-164. Academic Press.
- DAVIS, R.H. and RISTOW, J.L. (1988). Polyamine transport in Neurospora crassa. Archives of Biochemistry and Biophysics 267, 479-489.
- DAVIS, R.H. and RISTOW, J.L (1989). Uptake, intracellular binding and excretion of polyamines during growth of Neurospora crassa. Archives of Biochemistry and Biophysics 271, 315-322.
- DE GEE, A.L.W., CARSTENS, P.H.B., McCANN, P.P. and MANSFIELD, J.M. (1984). Morphological changes in Trypanosoma brucei rhodesiense following inhibition of polyamine biosynthesis in vivo. Tissue Cell 16, 731-738.

- DE WAARD, M.A. and VAN NISTELROOY, J.G.M. (1979). Mechanism of resistance to Fenarimol in Aspergillus nidulans. Pesticide Biochemistry and Physiology 10, 219-229.
- DION, A.S. and COHEN, S.S. (1972). Polyamine stimulation of nucleic acid synthesis in an uninfected and phage-infected polyamine auxotroph of Escherichia coli K12. Proceedings of the National Academy of Sciences of the United States of America 69, 213-217.
- DUMORTIER, F.M., FLORES, H.E., SHEKHAWAT, N.S. and GALSTON, A.W. (1983). Gradients of polyamines and their biosynthetic enzymes in coleoptiles and roots of corn. Plant Physiology 72, 915-918.
- ELO, H., MUTIKAINEN, I., ALHONEN-HONGISTO, L., LAINE, R., JANNE, J. and LUMME, P. (1986). Biochemical properties and crystal structure of ethylmethylglyoxal bis (guanylhydrazone) sulfate - an extremely powerful novel inhibitor of adenosylmethionine decarboxylase. Zeitschrift fur Naturforschung 41c, 851-855.
- ERWIN, B.G. and PEGG, A.E. (1982). Uptake of  $\alpha$ -difluoromethylornithine by mouse fibroblasts. Biochemical Pharmacology 31, 2820-2823.
- FAIRLAMB, A.H., BLACKBURN, P., ULRICH, P., CHAIT, B.T. and CERAMI, A. (1985). Trypanothione: a novel bis (glutathionyl) spermidine cofactor for glutathione reductase in trypanosomatids. Science 227, 1485-1487.



- FEHRMANN, H. (1988). Fusarium culmorum (W.G. Sm.) Sacc. In: European Handbook of Plant Disease. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 287-289. Blackwell Scientific Publications.
- FEVERSTEIN, B., PORTER, C.W. and DAVE, C. (1979). A selective effect of methylglyoxal bis (guanyldrazone) on the synthesis of mitochondrial DNA in cultures of L1210 leukemia cells. Cancer Research 39, 4130-4137.
- FIENBERG, A.A., CHOI, J.H., LUBICH, W.P. and SUNG, Z.R. (1984). Developmental regulation of polyamine metabolism in growth and differentiation of carrot culture. Planta 162, 532-539.
- FLORES, H.E. and GALSTON, A.W. (1982). Polyamines and plant stress: activation of putrescine biosynthesis by osmotic shock. Science 217, 1259-1261.
- FOZARD, J.R., PART, M.L., PRAKASH, N.J., GROVE, J., SCHECHTER, P.J., SJOERDSMA, A. and KOCH-WESER, J. (1980). L-ornithine decarboxylase: an essential role in early mammalian embryogenesis. Science 208, 505-508.
- FRIEDMAN, R., LEVIN, N. and ALTMAN, A. (1986). Presence and identification of polyamines in xylem and phloem exudates of plants. Plant Physiology 82, 1154-1157.
- GALSTON, A.W. (1983). Polyamines as modulators of plant development. BioScience 33, 382-388.

- GIFFIN, B.F., McCANN, P.P. and BACCHI, C.J. (1986). Effects of putrescine on the respiration of Trypanosoma brucei brucei. Molecular and Biochemical Parasitology 20, 165-171.
- GIFFIN, B.F., McCANN, P.P., BITONTI, A.J. and BACCHI, C.J. (1986). Polyamine depletion following exposure to DL- $\alpha$ -difluoromethylornithine both in vivo and in vitro initiates morphological alterations and mitochondrial activation in a monomorphic strain of Trypanosoma brucei brucei. Journal of Protozoology 33, 238-243.
- GOLDEN, J.A., SJOERDSMA, A. and SANTI, D.V. (1984). Pneumocystis carinii pneumonia treated with  $\alpha$ -difluoromethylornithine - a prospective study among patients with the acquired immunodeficiency syndrome (clinical investigation). Western Journal of Medicine 141, 613-623.
- GUARINO, L.A. and COHEN, S.S. (1979). Uptake and accumulation of putrescine and its lethality in Anacystis nidulans. Proceedings of the National Academy of Sciences of the United States of America 76, 3184-3188.
- GUHA, S.K. and JANNE, J. (1976). The synthesis and accumulation of polyamines in reproductive organs of the rat during pregnancy. Biochimica et Biophysica Acta 437, 244-252.
- GUYE, M.G., VIGH, L. and WILSON, J.M. (1986). Polyamine titre in relation to chill-sensitivity in Phaseolus sp. Journal of Experimental Botany 37, 1036-1043.

- HANNONEN, P., JANNE, J. and RAINA, A. (1972). Partial purification and characterization of spermine synthase from rat brain. Biochimica et Biophysica Acta 289, 225-231.
- HELLER, J.S., CANELLAKIS, E.S., BUSSOLOTTI, D.L. and COWARD, J.K. (1975). Stable multisubstrate adducts as enzyme inhibitors: potent inhibition of ornithine decarboxylase by N-(5'-phosphopyridoxyl)-ornithine. Biochimica et Biophysica Acta 403, 197-207.
- HERBST, E.J. and SNELL, E.E. (1948). Putrescine as a growth factor for Haemophilus parainfluenza. Journal of Biological Chemistry 176, 989-990.
- HOLM, I., PERSSON, L. and STJERNBORG, L. (1989). Feedback control of ornithine decarboxylase expression by polyamines. Biochemical Journal 258, 343-350.
- HOLTTA, E., HANONNEN, P., PISPA, J. and JANNE, J. (1973). Effect of methylglyoxal bis (guanylhydrazone) on polyamine metabolism in normal and regenerating rat-liver and rat thymus. Biochemical Journal 136, 669-676.
- HOLTTA, E., JANNE, J. and HOVI, T. (1979). Suppression of the formation of polyamines and macromolecules by DL- $\alpha$ -difluoromethylornithine and methylglyoxal bis (guanylhydrazone) in phytohaemagglutinin-activated human lymphocytes. Biochemical Journal 178, 109-117.

- HOLTTA, E., KORPELA, H. and HOVI, T. (1981). Several inhibitors of ornithine and adenosylmethionine decarboxylases may also have anti-proliferative effects unrelated to polyamine depletion. Biochimica et Biophysica Acta 677, 90-102.
- HUNG, D.T., MARTON, L.J., DEEN, D.F. and SHAFER, R.H. (1983). Depletion of intracellular polyamines may alter DNA conformation in 9L rat-brain tumour cells. Science 221, 268-270.
- INOUE, H., KATO, Y., TAKIGAWA, M., ADACHI, H. and TAKEDA, Y. (1975). Effect of DL-alpha-hydrazino-delta-aminovaleric acid, an inhibitor of ornithine decarboxylase, on polyamine metabolism in isoproterenol-stimulated mouse parotid glands. Journal of Biochemistry 77, 879-893.
- ITO, H., HIBASAMI, H., SHIMURA, K., NAGAI, J. and HIDAKA, H. (1982). Tumor effect of dicyclohexylammonium sulfate, a potent inhibitor of spermidine synthase against P388 leukemia. Cancer Letters 15, 229-235.
- JANNE, J., ALHONEN-HONGISTO, L., NIKULA, P. and ELO, H. (1985). S-adenosylmethionine decarboxylase as target of chemotherapy. Advances in Enzyme Regulation 24, 125-139.
- JANNE, J., ALHONEN-HONGISTO, L., SEPPANEN, P. and HOLTTA, E. (1981). Cellular compensatory mechanisms during chemically induced polyamine deprivation. Advances in Polyamine Research 3, 85-95.

- JANNE, J., WILLIAMS-ASHMAN, H.G. and SCHENONE, A. (1971). Spermidine synthesizing enzymes in bakers-yeast. Biochemical and Biophysical Research Communications 43, 1362-1365.
- JARVIS, B.C., YASMIN, S. and COLEMAN, M.T. (1985). RNA and protein metabolism during adventitious root formation in stem cuttings of Phaseolus aureus cultivar berkin. Physiologia Plantarum 64, 53-59.
- KALLIO, A., McCANN, P.P. and BEY, P. (1981). DL-2-(difluoromethyl) arginine: a potent enzyme-activated irreversible inhibitor of bacterial arginine decarboxylase. Biochemistry 20, 3163-3166.
- KAMEJI, T., MURAKAMI, Y. and HAYASHI, S. (1979). Effect of diamino-butene and diaminopropane on diet-stimulated polyamine synthesis and cell-proliferation in rat liver. Journal of Biochemistry 86, 191-197.
- KASHIWAGI, K., KOBAYASHI, H. and IGARASHI, K. (1986). Apparently uni-directional polyamine transport by proton motive force in polyamine-deficient Escherichia coli. Journal of Bacteriology 165, 972-977.
- KAUR-SAWHNEY, R. and GALSTON, A.W. (1979). Interaction of polyamines and light on biochemical processes involved in leaf senescence. Plant, Cell Environment 2, 189-196.
- KELLN, R.A. and WARREN, R.A.J. (1973). Studies on biosynthesis of alpha-putrescinyllthymine in bacteriophage ØW-14-infected Pseudomonas acidovorans. Journal of Virology 12, 1427-1433.

- LEMAIRE, J.M. (1988). Gaeumannomyces graminis (Sacc.) v. Arx and H. Olivier. In: European Handbook of Plant Diseases. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 323-324. Blackwell Scientific Publications.
- LIN, P.P.C., EGLI, D.B., LI, G.M. and MECKEL, L. (1984). Polyamine titre in the embryonic axis and cotyledons of Glycine max (L.) during seed growth and maturation. Plant Physiology 76, 366-371.
- LINDBERG, L. (1980). Kinetic studies of a ( $\text{Na}^+ + \text{K}^+ + \text{Mg}^{2+}$ ) ATPase in sugar beet roots. III. A proposed model for the ( $\text{Na}^+ + \text{K}^+$ ) activation and its significance for field properties. Physiologia Plantarum 48, 65-70.
- LITTLE, J.R. (1962). Inhibition of reticulocyte lysis by alkyl polyamines. Proceedings of the Society for Experimental Biology and Medicine 109, 910-912.
- LUK, G.D. and BAYLIN, S.B. (1984). Ornithine decarboxylase as a biological marker in familial colonic polyposis. New England Journal of Medicine 311, 80-83.
- MAGER, J., BENEDICT, M. and ARTMAN, M. (1962). Common site of action for polyamines and streptomycin. Biochimica et Biophysica Acta 62, 202-204.

- MAMONT, P.S., DANZIN, C., KOLB, M., GERHART, F., BEY, P. and SJOERDSMA, A. (1986). Marked and prolonged inhibition of mammalian ornithine decarboxylase in vivo by esters of (E)-2-(fluoromethyl) dehydro-ornithine. Biochemical Pharmacology 35, 159-165.
- MAMONT, P.S., SIAT, M., JODER-OHLENBUSCH, A.-M., BERNHARDT, A. and CASARA, P. (1984). Effects of (2R, 5R)-6-heptyne-2, 5-diamine, a potent inhibitor of L-ornithine decarboxylase, on rat hepatoma cells cultured in vitro. European Journal of Biochemistry 142, 457-463.
- MARCHANT, R. (1966). Wall structure and spore germination in Fusarium culmorum. Annals of Botany 30, 821-830.
- MARTON, L.J. and MORRIS, D.R. (1987). Molecular and cellular functions of the polyamines. In: Inhibition of Polyamine Metabolism. Eds P.P. McCann, A.E. Pegg and A. Sjoerdsma, pp 79-105. Academic Press.
- McDONALD, W.C. (1967). Variability and the inheritance of morphological mutants in Pyrenophora teres. Phytopathology 57, 747-755.
- MEIJER, E.G.M and SIMMONDS, J. (1988). Polyamine levels in relation to growth and somatic embryogenesis in tissue cultures of Meidcago sativa L. Journal of Experimental Botany 203, 787-794.
- MELVIN, M.A.L. and KEIR, H.M. (1978). Diminished excretion of polyamines from BHK-21-C13 cells exposed to methylglyoxal bis (guanyldrazóne). Biochemical Journal 174, 349-352.

- METCALF, B.W., BEY, P., DANZIN, C., JUNG, M.J., CASARA, P. and VEVERT, J.P. (1978). Catalytic irreversible inhibition of mammalian ornithine decarboxylase (EC 4.1.1.17) by substrate and product analogues. Journal of the American Chemical Society 100, 2551-2553.
- McCANN, P.P., BACCHI, C.J., CLARKSON, A.B., BEY, P., SJOERDSMA, A., SCHECHTER, P.J., WALZER, P.D. and BARLOW, J.L.R. (1986). Inhibition of polyamine biosynthesis by  $\alpha$ -difluoromethylornithine in African trypanosomes and Pneumocystis carinii as a basis of chemotherapy: biochemical and clinical aspects. American Journal of Tropical Medicine and Hygiene 35, 1153-1156.
- MITCHELL, J.L.A and CARTER, D.D. (1977). Physical and kinetic distinction of 2 ornithine decarboxylase forms in Physarum. Biochimica et Biophysica Acta 483, 425-434.
- MITCHELL, J.L.A., CARTER, D.D. and RYBSKI, J.A. (1978). Control of ornithine decarboxylase activity in Physarum by polyamines. European Journal of Biochemistry 92, 325-331.
- MITCHELL, J.L.A. and KOTTAS, G.E. (1979). Osmotically induced modification of ornithine decarboxylase in Physarum. FEBS Letters 102, 265-268.
- MONTAGUE, M.J., KOPPENBRINK, J.W. and JAWORSKI, E.G. (1978). Polyamine metabolism in embryonic cells of Daucus carota. I. Changes in intracellular content and rates of synthesis. Plant Physiology 62, 430-433.



- MORRIS, D.R. and FILLINGHAM, H. (1974). Regulation of amino-acid decarboxylation. Annual Review of Biochemistry 43, 303-326.
- MORRIS, D.R. and JORSTAD, C.M. (1970). Isolation of conditionally putrescine-deficient mutants of Escherichia coli. Journal of Bacteriology 101, 731-737.
- MORRIS, D.R., JORSTAD, C.M. and SEYFRIED, C.E. (1977). Inhibition of synthesis of polyamines and DNA in activated lymphocytes by a combination of alpha-methylornithine and methylglyoxal bis (guanylhydrazone). Cancer Research 37, 3169-3172.
- MUNRO, G.F., BELL, C.A. and LEDERMAN, M. (1974). Multiple transport components for putrescine in Escherichia coli. Journal of Bacteriology 118, 952-963.
- MUSSELL, H., OSMELOSKI, J., WETTLAUFER, S.H. and WEINSTEIN, L. (1987). Suppression of Verticillium wilt of tomato by difluoromethylornithine, a suicidal inhibitor of polyamine biosynthesis. Plant Disease 71, 313-316.
- NADLER, S.G. and TAKAHASHI, M.T. (1985). Putrescine transport in human platelets. Biochimica et Biophysica Acta 812, 345-352.
- NAIK, B.I. and SRIVASTAVA, S.K. (1978). Effect of polyamines on tissue permeability. Phytochemistry 17, 1885-1887.

- NATHAN, R., ALTMAN, A. and MONSELISE, S.P. (1984). Changes in activity of polyamine biosynthetic enzymes and in polyamine contents in developing fruit tissues of 'Murcott' mandarin. Scientia Horticultura 22, 359-364.
- NICKERSON, K.W., DUNKLE, L.D. and VAN ETEN, J.L. (1977). Absence of spermine in filamentous fungi. Journal of Bacteriology 129, 173-176.
- NIKULA, P., ALHONEN-HONGISTO, L., SEPPANEN, P. and JANNE, J. (1984). Inhibition of long-chain fatty acid oxidation by methylglyoxal bis (guanylhydrazone). Biochemical and Biophysical Research Communications 120, 9-14.
- NORTH, M.J. and TURNER, R. (1977). Diamine content of cellular slime mold Dictyostelium discoideum - presence of 1,3-diaminopropane and putrescine. Microbios Letters 4, 221-228.
- NOTHIG-LASLO, V., WEYGAND-DURASAVIC, I. and KUCAN, Z. (1985). Structural changes of yeast transfer RNA TYZ caused by the binding of divalent ions in the presence of spermine. Journal of Biomolecular Structure and Dynamics 5, 941-951.
- PALAVAN, N. and GALSTON, A.W. (1982). Polyamine biosynthesis and titer during various developmental stages of Phaseolus vulgaris. Physiologia Plantarum 55, 438-444.
- PALL, M.L. (1970). Amino acids in Neurospora crassa. III. Acidic amino acid transport. Biochimica et Biophysica Acta 211, 513-520.

- PARKER, R.E. (1983). Introductory Statistics for Biology. Studies in Biology number 43. Edward Arnold.
- PEGG, A.E. (1979). Investigation of the turnover of rat liver S-adenosylmethionine decarboxylase using a specific antibody. Journal of Biological Chemistry 254, 3249-3253.
- PEGG, A.E. (1984). S-adenosylmethionine decarboxylase - a brief review. Cell Biochemistry and Function 2, 11-15.
- PEGG, A.E. and JACOBS, G. (1983). Comparison of inhibitors of S-adenosylmethionine decarboxylase from different species. Biochemical Journal 213, 495-502.
- PEGG, A.E., MATSUI, I., SEELY, J.E., PRITCHARD, M.L. and POSO, H. (1981). Formation of putrescine in rat liver. Medical Biology 59, 327-333.
- PEGG, A.E. and McCANN, P.P. (1982). Polyamine metabolism and function. American Journal of Physiology 243, 212-221.
- PEGG, A.E. and McCANN, P.P. (1988). Polyamine metabolism and function in mammalian cells and protozoans. ISI Atlas of Science: Biochemistry, 11-18.
- PEGG, A.E. and MCGILL, S.M. (1979). Decarboxylation of ornithine and lysine in rat tissues. Biochimica et Biophysica Acta 568, 416-427.

- PEGG, A.E., POSO, H., SHUTTLEWORTH, K. and BENNETT, R.A. (1982). Effect of inhibition of polyamine synthesis on the content of decarboxylated S-adenosylmethionine. Biochemical Journal 202, 519-526.
- PERA, P.J., KRAMER, D.L., SUFRIN, J.R. and PORTER, C.W. (1986). Comparison of the biological effect of four irreversible inhibitors of ornithine decarboxylase in two Murine lymphocytic leukemia cell lines. Cancer Research 46, 1148-1154.
- PERERA, R.G. and WHEELER, B.E.J. (1975). Effect of water droplets on the development of Sphaerotheca pannosa on rose leaves. Transaction of the British Mycological Society 64, 313-319.
- PERSSON, L., KHOMUTOV, A.R. and KHOMUTOV, R.M. (1989). Feedback regulation of S-adenosylmethionine decarboxylase synthesis. Biochemical Journal 257, 929-931.
- PFALLER, M.A., GERARDEN, T. and RILEY, J. (1987). Growth and inhibition of pathogenic yeast isolates by  $\alpha$ -difluoromethylornithine: an inhibition of ornithine decarboxylase. Mycopathologia 98, 3-8.
- PHILLIPS, R., PRESS, M.C., BINGHAM, L. and GRIMMER, C. (1988). Polyamines in cultured artichoke explants: effects are primarily on xylogenesis rather than cell division. Journal of Experimental Botany 39, 473-480.

- PHILLIPS, M.A. and WANG, C.C. (1987). A Trypanosoma brucei mutant resistant to  $\alpha$ -difluoromethylornithine. Molecular and Biochemical Parasitology 22, 9-17.
- PISTOCCHI, R., BAGNI, N. and CREUS, J.A. (1986). Polyamine uptake, kinetics, and competition among polyamines and between polyamines and inorganic cations. Plant Physiology 80, 556-560.
- PISTOCCHI, R., BAGNI, N. and CREUS, J.A. (1987). Polyamine uptake in carrot cell culture. Plant Physiology 84, 374-380.
- PISTOCCHI, R., KELLER, F., BAGNI, N. and MATILE, P. (1988). Transport and subcellular localization of polyamines in carrot protoplasts and vacuoles. Plant Physiology 87, 514-518.
- PORTER, C.W., BERGERON, R.J. and STOLOWICH, N.J. (1982). Biological properties of N<sup>4</sup>-spermidine derivatives and their potential in anticancer chemotherapy. Cancer Research 42, 4072-4078.
- PORTER, C.W., MILLER, J. and BERGERON, R.J. (1984). Aliphatic chain length specificity of the polyamine transport system in ascites L1210 leukemia cells. Cancer Research 44, 126-128.
- PORTER, C.W. and SUFRIN, J.R. (1986). Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. AntiCancer Research 6, 525-542.

- POSO, H., SINERVIRTA, R. and JANNE, J. (1975). S-adenosylmethionine decarboxylase from bakers-yeast. Biochemical Journal 151, 67-73.
- PRAKASH, N.J., SCHECHTER, P.J., GROVE, J. and KOCH-WESER, J. (1978). Effect of  $\alpha$ -difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on L1210 leukemia in mice. Cancer Research 38, 3059-3062.
- PRIEBE, A., KLEIN, H. and JAGER, H.-J. (1978). Role of polyamines in SO<sub>2</sub>-polluted pea plants. Journal of Experimental Botany 29, 1045-1050.
- RAHIALA, A.E.-L., KEKOMAKI, M., JANNE, J., RAINA, A. and RAIHA, N.C. (1971). Inhibition of pyridoxal enzymes by L-canaline. Biochimica et Biophysica Acta 227, 337-343.
- RAINA, A., HYVONEN, T., ELORANTA, T., VOUTILAINEN, M., SAMEJIM, K. and YAMANOHA, B. (1984). Polyamine synthesis in mammalian tissues - isolation and characterization of spermidine synthase from bovine brain. Biochemical Journal 219, 991-1000.
- RAJAM, M.V. and GALSTON, A.W. (1985). The effect of some polyamine biosynthetic inhibitors on growth and morphology of phytopathogenic fungi. Plant Cell Physiology 26, 683-692.
- RAJAM, M.V., WEINSTEIN, L.H. and GALSTON, A.W. (1985). Prevention of a plant disease by specific inhibition of fungal polyamine biosynthesis. Proceedings of the National Academy of Sciences of the United States of America 82, 6874-6878.

- RAJAM, M.V., WEINSTEIN, L.H. and GALSTON, A.W. (1986). Kinetic studies on the control of the bean rust fungus (Uromyces phaseoli L.) by an inhibitor of polyamine biosynthesis. Plant Physiology 82, 485-487.
- RAMAKRISHNA, S. and ADIGA, P.R. (1975). Amine levels in Lathyrus sativus seedlings during development. Phytochemistry 14, 63-68.
- RICH, A.E. (1983). Potato Diseases. Academic Press, New York.
- ROTEM, J., BASHI, E. and KRANZ, J. (1983). Studies on crop loss in potato blight caused by Phytophthora infestans. Plant Pathology 32, 117-122.
- RUSSELL, D.H. (1977). Clinical relevance of polyamines as biochemical markers of tumor kinetics. Clinical Chemistry 23, 22-27.
- RUSSELL, D.H., LEVY, C.C., SCHIMPF, S.C. and HAWK, I.A. (1971). Urinary polyamines in cancer patients. Cancer Research 31, 1555-1558.
- SANDERSON, F.R. and SCOTT, P.R. (1988). Leptosphaeria nodorum E. Muller. In: European Handbook of Plant Diseases. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 370-371. Blackwell Scientific Publications.

- SCALABRINO, G., POSO, H., HOLTTA, E., HANNONEN, P., KALLIO, A. and JANNE, J. (1978). Synthesis and accumulation of polyamines in rat liver during chemical carcinogenesis. International Journal of Cancer 21, 239-245.
- SCHINDLER, M., KOPPEL, D.E. and SHEETZ, M.P. (1980). Modulation of membrane protein lateral mobility by polyphosphates and polyamines. Proceedings of the National Academy of Sciences of the United States of America - Biological Sciences 77, 1457-1461.
- SCHWARZBACH, E. and SMITH, P.M. (1988). Erysiphe graminis DC. In: European Handbook of Plant Diseases. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 259-261. Blackwell Scientific Publications.
- SHATTOCK, R. (1988). Phytophthora infestans (Mont.) de Bary. In: European Handbook of Plant Disease. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 202-204. Blackwell Scientific Publications.
- SHEARD, J. and FARRAR, J.F. (1987). Transport of sugar in Phytophthora palmivora (Butl.) Butl. New Phytologist 105, 265-272.
- SHIPTON, W.A., BOYD, W.R.J., ROSIELLA, A.A. and SHEARER, B.I. (1971). The common Septoria diseases of wheat. The Botanical Review 37, 231-262.
- SIKORA, L. and McDOUGALL, K.J. (1978). Regulation of ornithine decarboxylase in Neurospora crassa. Experimental Mycology 2, 352-358.



- SJOERDSMA, A. and SCHECHTER, P.J. (1984). Chemotherapeutic implications of polyamine biosynthesis inhibition. Clinical Pharmacology and Therapeutics 35, 287-300.
- SLOCUM, R.D. and GALSTON, A.W. (1985a). In vivo inhibition of polyamine biosynthesis and growth in tobacco ovary tissues. Plant Cell Physiology 26, 1519-1526.
- SLOCUM, R.D. and GALSTON, A.W. (1985b). Arginase-mediated hydrolysis of DFMA to DFMO in vivo. Plant Physiology 77 (supplement), 45-47.
- SLOCUM, R.D. and GALSTON, A.W. (1985c). Changes in polyamine biosynthesis associated with post-fertilization growth and development in tobacco ovary tissues. Plant Physiology 79, 336-343.
- SLOCUM, R.D. and GALSTON, A.W. (1987). Inhibition of polyamine biosynthesis in plants and plant pathogenic fungi. In: Inhibition of Polyamine Metabolism. Eds P.P. McCann, A.E. Pegg and A. Sjoerdsma, pp 305-315. Academic Press.
- SLOCUM, R.D., KAUR-SAWHNEY, R. and GALSTON, A.W. (1984). The physiology and biochemistry of polyamines in plants. Archives of Biochemistry and Biophysics 235, 283-303.
- SMEDEGAARD-PETERSON, F. (1988). Pyrenophora teres Drechsler. In: European Handbook of Plant Diseases. Eds I.M. Smith, J. Dunez, R.A. Lelliott, D.H. Phillips and S.A. Archer, pp 379-382. Blackwell Scientific Publications.

- SMITH, T.A. (1970). Putrescine, spermidine and spermine in higher plants. Phytochemistry 9, 1479-1489.
- SMITH, T.A. (1971). Occurrence, metabolism and functions of amines in plants. Biological Reviews 46, 201-212.
- SMITH, T.A. (1972). The physiology of the polyamines and related compounds. Endeavour 31, 22-28.
- SMITH, T.A. (1985). Polyamines. Annual Review of Plant Physiology 36, 117-143.
- SPATHAS, D.H., PATEMAN, J.A. and CLUTTERBUCK, A.J. (1982). Polyamine transport in Aspergillus nidulans. Journal of General Microbiology 128, 557-563.
- STEVENS, L., MCKINNON, I.M. and WINTHER, M. (1976). Polyamine and ornithine metabolism during the germination of conidia of Aspergillus nidulans. Biochemical Journal 158, 235-241.
- STEVENS, L. and STEVENS, E. (1981). Regulation of ornithine decarboxylase activity during the germination of conidia of Aspergillus nidulans. FEMS Microbiology Letters 11, 229-232.
- STEVENS, L. and WINTHER, M.D. (1979). Spermine, spermidine and putrescine in fungal development. Advances in Microbial Physiology 19, 63-148.
- STRYER, L. (1981). Biochemistry. Freeman and Company, New York.

- SUNKARA, P.S. and PRAKASH, N.J. (1984). Inhibitors of polyamine biosynthesis as antitumor and antimetastatic agents. In: Novel Approaches to Cancer Therapy. Ed. P.S. Sunkara, pp 93-126. Academic Press, New York.
- SUNKARA, P.S., PRAKASH, N.J., CHANG, C.C. and SJOERDSMA, A. (1983). Cytotoxicity of methylglyoxal bis (guanylhydrazone) in combination with alpha-difluoromethylornithine against helacells and mouse L1210 leukemia. Journal of the National Cancer Institute 70, 505-509.
- SUNKARA, P.S., PRAKASH, N.J., MAYER, G.D. and SJOERDSMA, A. (1983). Tumor suppression with a combination of alpha-difluoromethylornithine and interferon. Science 219, 851-853.
- SURESH, M.R., RAMAKRISHNA, S. and ADIGA, P.R. (1978). Regulation of arginine decarboxylase and putrescine levels in Cucumis sativus cotyledons. Phytochemistry 17, 57-63.
- TABOR, C.W. (1962). Stabilization of protoplasts and spheroplasts by spermine and other polyamines. Journal of Bacteriology 83, 1101-1111.
- TABOR, C.W. (1981). Mutants of Saccharomyces cerevisiae deficient in polyamine biosynthesis: studies on the regulation of ornithine decarboxylase. Medical Biology 59, 272-278.

TABOR, C.W. and DOBBS, L.G. (1970). Metabolism of 1,4-diaminobutane and spermidine in Escherichia coli: the effects of low temperature during storage and harvesting of cultures. Journal of Biological Chemistry 245, 2086-2091.

TABOR, C.W. and TABOR, H. (1966). Transport systems for 1,4-diaminobutane, spermidine and spermine in Escherichia coli. Journal of Biological Chemistry 241, 3714-3723.

TABOR, C.W. and TABOR, H. (1976). 1,4-diaminobutane (putrescine), spermidine and spermine. Annual Review of Biochemistry 45, 285-306.

TABOR, C.W. and TABOR, H. (1984). Methionine adenosyltransferase (S-adenosylmethionine synthase) and S-adenosylmethionine decarboxylase. Review. Advances in Enzymology and Related Areas of Molecular Biology 56, 251-282.

TABOR, C.W. and TABOR, H. (1985). Polyamines in microorganisms. Microbiological Reviews 49, 81-99.

TABOR, H. and TABOR, C.W. (1972). Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine, and related amines. Advances in Enzymology 36, 203-211.

TABOR, H. and TABOR, C.W. (1975). Isolation, characterization and turnover of glutathionylspermidine from Escherichia coli. Journal of Biological Chemistry 250, 2648-2654.

- TAKEMOTO, T., NAGAMATSU, Y. and OKA, T. (1983). The study of spermidine stimulated polypeptide synthesis in cell-free translation of messenger RNA from lactating mouse mammary gland. Biochimica et Biophysica Acta 740, 73-79.
- TANG, K.-C., PEGG, A.E. and COWARD, J.K. (1980). Specific and potent inhibition of spermidine synthase by the transition-state analog, S-adenosyl-3-thio-1,8-diaminooctane. Biochemical and Biophysical Research Communications 96, 1371-1377.
- TEITEL, D.C., COHEN, E., ARAD, S.M., BIRNBAUM, E. and MIZRAHI, Y. (1985). The possible involvement of polyamines in the development of tomato fruits in vitro. Plant Growth Regulation 3, 309-317.
- TORRIGIANI, P., SERAFINI-FRACASSINI, D., BIONDI, S. and BAGNI, N. (1986). Evidence for the subcellular localization of polyamines and their biosynthetic enzymes in plant cells. Journal of Plant Physiology 124, 23-29.
- TROPP, J.S. and REDFIELD, A.G. (1983). Proton exchange rates in transfer RNA as a function of spermidine and magnesium. Nucleic Acids Research 11, 2121-2134.
- TURNER, L.B. and STEWART, G.R. (1988). Factors affecting polyamine accumulation in barley (Hordeum vulgare L.) leaf sections during osmotic stress. Journal of Experimental Botany 39, 311-316.

- VOLZ, R.K. and KNIGHT, J.N. (1986). The use of growth regulators to increase precocity in apple trees. Journal of Horticultural Science 61, 181-199.
- WALTERS, D.R. (1986). The effects of a polyamine biosynthesis inhibitor on infection of Vicia faba L. by the rust fungus, Uromyces viciae-fabae (Pers.) Schroet. New Phytologist 104, 613-619.
- WALTERS, D.R. (1989). Polyamines and plant disease. Plants Today, January-February, 22-26.
- WALTERS, D.R. and KINGHAM, G. (1989). Uptake and translocation of the polyamine biosynthesis inhibitor  $\alpha$ -difluoromethylornithine by barley seedlings: effects on mildew infection. New Phytologist, in press.
- WALTERS, D.R. and WYLIE, M.A. (1986). Polyamines in discrete regions of barley leaves infected with the powdery mildew fungus, Erysiphe graminis. Physiologia Plantarum 67, 630-633.
- WEINSTEIN, L.H., OSMELOSKI, J.F., WETTLAUFER, S.H. and GALSTON, A.W. (1987). Protection of wheat against leaf and stem rust and powdery mildew disease by inhibition of polyamine metabolism. Plant Science 51, 311-316.
- WHAUN, J.M and BROWN, N.D. (1985). Ornithine decarboxylase inhibition and the Malaria-infected red blood cell: a model for polyamine metabolism and growth. Journal of Pharmacology and Experimental Therapeutics 233, 507-511.

WILLIAMS-ASHMAN, H.G., COPPOC, G.L. and WEBER, G. (1972). Imbalance in ornithine metabolism in hepatomas of different growth rates as expressed in formation of putrescine, spermidine and spermine. Cancer Research 32, 1924-1932.

WILLIAMS-ASHMAN, H.G. and PEGG, A.E. (1981). In: Polyamines in Biology and Medicine. Eds D.R. Morris and C.J. Marton, pp 43-73. Dekker, New York.

WILLIAMS-ASHMAN, H.G. and SCHENONE, A. (1972). Methyl glyoxal bis (guanylhydrazone) as a potent inhibitor of mammalian and yeast S-adenosylmethionine decarboxylases. Biochemical and Biophysical Research Communications 46, 288-295.

WINER, L. and APELBAUM, A. (1986). Involvement of polyamines in the development and ripening of Avocado fruits. Journal of Plant Physiology 126, 223-233.

YAMADA, H., ISOBE, K. and TANI, Y. (1980). Oxidation of polyamines by fungal enzymes. Agricultural and Biological Chemistry 44, 2469-2476.

YAMANOHA, B. and COHEN, S.S. (1985). S-adenosylmethionine decarboxylase and spermidine synthase from Chinese cabbage. Plant Physiology 78, 784-790.

YOUNG, N.D. and GALSTON, A.W. (1983). Are polyamines transported in etiolated peas? Plant Physiology 73, 912-914.

APPENDIX I



TABLE I. Effect of ODC inhibitors on growth of *P. teres* (grey form). Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 3	DAY 4	DAY 6
<b>DFMO:</b>			
1.0	88.8 $\pm$ 4.2	93.9 $\pm$ 5.8	96.8 $\pm$ 4.8
2.0	62.4 $\pm$ 8.3 <sup>c</sup>	66.1 $\pm$ 9.2 <sup>c</sup>	61.8 $\pm$ 9.8 <sup>c</sup>
4.0	23.2 $\pm$ 2.4 <sup>c</sup>	22.4 $\pm$ 2.7 <sup>c</sup>	20.4 $\pm$ 5.3 <sup>c</sup>
<b><math>\Delta</math>-MFMO:</b>			
0.5	78.4 $\pm$ 4.1 <sup>b</sup>	85.5 $\pm$ 2.8 <sup>b</sup>	94.3 $\pm$ 3.0
1.0	70.4 $\pm$ 4.5 <sup>c</sup>	72.1 $\pm$ 6.7 <sup>c</sup>	79.6 $\pm$ 5.4 <sup>b</sup>
<b><math>\Delta</math>-MFMO.Me:</b>			
0.5	88.0 $\pm$ 1.8 <sup>a</sup>	95.2 $\pm$ 3.2	103.6 $\pm$ 2.4
1.0	76.8 $\pm$ 4.2 <sup>b</sup>	80.0 $\pm$ 4.5 <sup>b</sup>	92.1 $\pm$ 1.2 <sup>a</sup>
<b>RR-MAP:</b>			
0.5	92.8 $\pm$ 1.7	100.0 $\pm$ 1.2	110.4 $\pm$ 1.3 <sup>b</sup>
1.0	83.2 $\pm$ 1.9 <sup>b</sup>	91.5 $\pm$ 2.6 <sup>a</sup>	101.8 $\pm$ 3.2

TABLE II. Effect of ODC inhibitors on growth of *P. teres* (orange form). Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c. \* indicates that mycelium had reached the edge of the Petri dish.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>DFMO:</b>			
1.0	114.3 $\pm$ 2.1 <sup>c</sup>	114.2 $\pm$ 0.8 <sup>c</sup>	*
2.0	107.1 $\pm$ 1.1 <sup>b</sup>	107.8 $\pm$ 0.8 <sup>b</sup>	*
4.0	91.7 $\pm$ 2.6 <sup>b</sup>	92.2 $\pm$ 1.0 <sup>c</sup>	85.6 $\pm$ 0.7 <sup>c</sup>
<b><math>\Delta</math>-MFMO:</b>			
0.5	82.1 $\pm$ 5.8 <sup>b</sup>	85.8 $\pm$ 2.7 <sup>c</sup>	87.0 $\pm$ 1.5 <sup>c</sup>
1.0	98.8 $\pm$ 2.4	92.2 $\pm$ 1.5 <sup>b</sup>	92.1 $\pm$ 0.8 <sup>c</sup>
<b><math>\Delta</math>-MFMO.Me:</b>			
0.5	89.3 $\pm$ 6.7	91.3 $\pm$ 4.5 <sup>a</sup>	93.0 $\pm$ 1.3 <sup>b</sup>
1.0	92.9 $\pm$ 1.3 <sup>a</sup>	94.5 $\pm$ 1.0 <sup>a</sup>	94.9 $\pm$ 0.6 <sup>b</sup>
<b>RR-MAP:</b>			
0.5	107.1 $\pm$ 3.3 <sup>a</sup>	99.5 $\pm$ 0.5	98.6 $\pm$ 1.1
1.0	106.0 $\pm$ 2.2	99.1 $\pm$ 1.4	94.9 $\pm$ 0.7 <sup>b</sup>

TABLE III. Effect of ODC inhibitors on growth *G. graminis*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>DFMO:</b>			
1.0	63.2 $\pm$ 6.7 <sup>c</sup>	76.8 $\pm$ 3.0 <sup>c</sup>	86.0 $\pm$ 1.3 <sup>c</sup>
2.0	46.3 $\pm$ 6.8 <sup>c</sup>	61.4 $\pm$ 4.4 <sup>c</sup>	72.4 $\pm$ 2.4 <sup>c</sup>
4.0	40.0 $\pm$ 5.3 <sup>c</sup>	60.0 $\pm$ 1.5 <sup>c</sup>	69.6 $\pm$ 0.6 <sup>c</sup>
<b><math>\Delta</math>-MFMO:</b>			
0.5	64.2 $\pm$ 6.6 <sup>c</sup>	77.3 $\pm$ 3.5 <sup>c</sup>	79.2 $\pm$ 2.8 <sup>c</sup>
1.0	69.5 $\pm$ 4.5 <sup>c</sup>	86.8 $\pm$ 1.0 <sup>c</sup>	92.5 $\pm$ 1.5 <sup>b</sup>
<b><math>\Delta</math>-MFMO.Me:</b>			
0.5	89.5 $\pm$ 2.4 <sup>b</sup>	95.0 $\pm$ 1.9 <sup>a</sup>	94.7 $\pm$ 1.6 <sup>a</sup>
1.0	88.4 $\pm$ 4.8 <sup>a</sup>	89.5 $\pm$ 5.1 <sup>a</sup>	96.0 $\pm$ 2.9
<b>RR-MAP:</b>			
0.5	89.5 $\pm$ 5.9 <sup>a</sup>	95.5 $\pm$ 3.3	95.3 $\pm$ 2.2 <sup>a</sup>
1.0	81.1 $\pm$ 3.9 <sup>c</sup>	91.8 $\pm$ 2.5 <sup>b</sup>	93.5 $\pm$ 1.2 <sup>b</sup>

TABLE IV. Effect of ODC inhibitors on growth of *S. nodorum*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>DFMO:</b>			
1.0	93.8 $\pm$ 1.1	96.9 $\pm$ 0.9	100.6 $\pm$ 0.5
2.0	92.8 $\pm$ 2.2	100.9 $\pm$ 1.3	105.9 $\pm$ 0.3 <sup>b</sup>
4.0	115.5 $\pm$ 2.7 <sup>b</sup>	104.0 $\pm$ 1.7	98.2 $\pm$ 1.1
<b><math>\Delta</math>-MFMO:</b>			
0.5	110.3 $\pm$ 2.8 <sup>a</sup>	106.7 $\pm$ 1.3 <sup>b</sup>	103.5 $\pm$ 0.7 <sup>a</sup>
1.0	112.4 $\pm$ 1.8 <sup>b</sup>	103.1 $\pm$ 0.9	97.9 $\pm$ 0.5
<b><math>\Delta</math>-MFMO.Me:</b>			
0.5	112.4 $\pm$ 1.8 <sup>b</sup>	103.6 $\pm$ 2.1	105.9 $\pm$ 1.3 <sup>b</sup>
1.0	103.1 $\pm$ 2.0	96.4 $\pm$ 0.9 <sup>a</sup>	99.1 $\pm$ 0.6
<b>RR-MAP:</b>			
0.5	100.0 $\pm$ 3.1	100.4 $\pm$ 2.2	101.8 $\pm$ 1.1
1.0	95.9 $\pm$ 3.2	99.1 $\pm$ 0.9	98.2 $\pm$ 0.6

TABLE V. Effect of ODC inhibitors on growth of F. culmorum. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH	
	DAY 2	DAY 3
<b>DFMO:</b>		
1.0	108.8 $\pm$ 2.9	98.7 $\pm$ 1.6
2.0	105.6 $\pm$ 2.3	96.4 $\pm$ 1.7
4.0	122.4 $\pm$ 4.6 <sup>b</sup>	78.9 $\pm$ 1.7 <sup>c</sup>
<b><math>\Delta</math>-MFMO:</b>		
0.5	98.4 $\pm$ 4.9	76.0 $\pm$ 2.9 <sup>c</sup>
1.0	106.4 $\pm$ 4.5	84.7 $\pm$ 3.1 <sup>c</sup>
<b><math>\Delta</math>-MFMO.Me:</b>		
0.5	108.8 $\pm$ 3.7	89.3 $\pm$ 2.9 <sup>b</sup>
1.0	97.6 $\pm$ 3.3	76.6 $\pm$ 0.9 <sup>c</sup>
<b>RR-MAP:</b>		
0.5	116.0 $\pm$ 2.1 <sup>b</sup>	90.6 $\pm$ 2.4 <sup>b</sup>
1.0	106.4 $\pm$ 3.8	89.9 $\pm$ 5.2 <sup>a</sup>

TABLE VI. Effect of inhibitors of polyamine biosynthesis on growth of *P. infestans*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 9	DAY 12	DAY 15
<b>DFMO:</b>			
1.0	78.7 $\pm$ 10.8 <sup>a</sup>	71.3 $\pm$ 5.6 <sup>b</sup>	65.2 $\pm$ 3.1 <sup>a</sup>
<b>DFMA:</b>			
1.0	134.0 $\pm$ 4.8 <sup>a</sup>	126.7 $\pm$ 4.7 <sup>b</sup>	119.1 $\pm$ 3.7 <sup>b</sup>
<b>DFMO + DFMA:</b>			
1.0 + 1.0	83.0 $\pm$ 5.1	64.4 $\pm$ 4.6 <sup>c</sup>	56.7 $\pm$ 2.7 <sup>c</sup>

TABLE VII. Effect of inhibitors of polyamine biosynthesis on growth of *P. ultimum*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c. \* indicates that mycelium had reached the edge of the Petri dish.

TREATMENT (mM)	PER CENT GROWTH	
	DAY 1	DAY 2
DFMO:		
1.0	99.3 $\pm$ 1.5	99.2 $\pm$ 0.8
DFMA:		
1.0	102.9 $\pm$ 2.7	*
DFMO + DFMA:		
1.0 + 1.0	105.8 $\pm$ 1.2 <sup>a</sup>	*

TABLE VIII. Effect of inhibitors of polyamine biosynthesis on growth of *U. maydis*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 7	DAY 14	DAY 21
<b>DFMO:</b>			
1.0	74.0 $\pm$ 10.8 <sup>a</sup>	82.3 $\pm$ 7.6	84.7 $\pm$ 1.6 <sup>b</sup>
4.0	66.0 $\pm$ 6.1 <sup>b</sup>	80.2 $\pm$ 3.9 <sup>a</sup>	79.2 $\pm$ 1.8 <sup>b</sup>
<b>MGBG:</b>			
1.0	72.0 $\pm$ 8.3 <sup>a</sup>	83.3 $\pm$ 5.0	82.6 $\pm$ 3.4 <sup>b</sup>
2.0	56.0 $\pm$ 10.7 <sup>b</sup>	72.9 $\pm$ 1.4 <sup>b</sup>	74.3 $\pm$ 2.2 <sup>c</sup>
<b>DFMO + MGBG:</b>			
0.5 + 0.5	62.0 $\pm$ 6.5 <sup>b</sup>	77.1 $\pm$ 4.1 <sup>a</sup>	80.6 $\pm$ 2.7 <sup>b</sup>
1.0 + 1.0	62.0 $\pm$ 9.7 <sup>b</sup>	68.8 $\pm$ 4.5 <sup>b</sup>	72.9 $\pm$ 2.4 <sup>c</sup>



TABLE IX. Effect of inhibitors of polyamine biosynthesis on growth of *P. teres* (grey form). Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 3	DAY 4	DAY 6
<b>MGBG:</b>			
0.5	81.6 $\pm$ 1.0 <sup>b</sup>	86.1 $\pm$ 1.4 <sup>b</sup>	98.9 $\pm$ 1.1
1.0	78.4 $\pm$ 1.0 <sup>b</sup>	83.6 $\pm$ 1.4 <sup>c</sup>	95.0 $\pm$ 1.5
2.0	24.8 $\pm$ 7.1 <sup>c</sup>	26.7 $\pm$ 6.8 <sup>c</sup>	20.7 $\pm$ 3.4 <sup>c</sup>
<b>DFMO + MGBG:</b>			
0.5 + 0.5	23.2 $\pm$ 7.6 <sup>c</sup>	28.5 $\pm$ 4.3 <sup>c</sup>	25.4 $\pm$ 2.8 <sup>c</sup>
1.0 + 1.0	19.2 $\pm$ 5.0 <sup>c</sup>	22.4 $\pm$ 5.4 <sup>c</sup>	21.1 $\pm$ 3.4 <sup>c</sup>
<b>CHA:</b>			
0.5	100.0 $\pm$ 2.5	121.2 $\pm$ 2.5 <sup>c</sup>	105.4 $\pm$ 2.0
1.0	96.8 $\pm$ 4.4	112.7 $\pm$ 1.6 <sup>b</sup>	102.1 $\pm$ 1.0
2.0	97.6 $\pm$ 2.0	112.1 $\pm$ 1.1 <sup>b</sup>	103.6 $\pm$ 1.4
4.0	93.6 $\pm$ 6.6	103.0 $\pm$ 7.1	83.6 $\pm$ 7.7 <sup>a</sup>

TABLE X. Effect of inhibitors of polyamine biosynthesis on growth of *P. teres* (orange form). Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>MGBG:</b>			
0.5	82.1 $\pm$ 2.9 <sup>c</sup>	90.0 $\pm$ 1.0 <sup>c</sup>	87.6 $\pm$ 0.5 <sup>c</sup>
1.0	78.6 $\pm$ 1.5 <sup>c</sup>	86.3 $\pm$ 0.5 <sup>c</sup>	83.7 $\pm$ 0.5 <sup>c</sup>
2.0	59.5 $\pm$ 2.0 <sup>c</sup>	72.6 $\pm$ 0.6 <sup>c</sup>	74.4 $\pm$ 0.4 <sup>c</sup>
<b>DFMO + MGBG:</b>			
0.5 + 0.5	83.3 $\pm$ 2.9 <sup>c</sup>	90.0 $\pm$ 0.5 <sup>c</sup>	84.8 $\pm$ 0.7 <sup>c</sup>
1.0 + 1.0	72.6 $\pm$ 1.6 <sup>c</sup>	82.2 $\pm$ 0.6 <sup>c</sup>	80.0 $\pm$ 0.3 <sup>c</sup>
<b>CHA:</b>			
0.5	90.5 $\pm$ 1.3 <sup>b</sup>	92.7 $\pm$ 1.5 <sup>b</sup>	92.7 $\pm$ 0.8 <sup>c</sup>
1.0	86.9 $\pm$ 1.4 <sup>c</sup>	91.8 $\pm$ 1.5 <sup>b</sup>	91.5 $\pm$ 0.7 <sup>c</sup>
2.0	92.9 $\pm$ 2.6 <sup>a</sup>	91.3 $\pm$ 1.5 <sup>b</sup>	89.3 $\pm$ 1.2 <sup>c</sup>
4.0	83.3 $\pm$ 4.3 <sup>c</sup>	84.5 $\pm$ 1.6 <sup>c</sup>	83.4 $\pm$ 2.3 <sup>c</sup>

TABLE XI. Effect of inhibitors of polyamine biosynthesis on growth of *G. graminis*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>MGBG:</b>			
0.5	46.3 $\pm$ 4.5 <sup>c</sup>	44.1 $\pm$ 3.1 <sup>c</sup>	53.4 $\pm$ 2.2 <sup>c</sup>
1.0	7.4 $\pm$ 5.7 <sup>c</sup>	12.3 $\pm$ 7.4 <sup>c</sup>	11.5 $\pm$ 5.7 <sup>c</sup>
2.0	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.0 <sup>c</sup>
<b>DFMO + MGBG:</b>			
0.5 + 0.5	35.8 $\pm$ 5.9 <sup>c</sup>	44.1 $\pm$ 2.1 <sup>c</sup>	53.1 $\pm$ 1.0 <sup>c</sup>
1.0 + 1.0	11.6 $\pm$ 9.1 <sup>c</sup>	12.3 $\pm$ 3.7 <sup>c</sup>	23.0 $\pm$ 4.9 <sup>c</sup>
<b>CHA:</b>			
0.5	80.0 $\pm$ 5.3 <sup>b</sup>	87.7 $\pm$ 2.1 <sup>c</sup>	88.5 $\pm$ 2.0 <sup>c</sup>
1.0	72.6 $\pm$ 4.3 <sup>c</sup>	82.7 $\pm$ 3.8 <sup>c</sup>	88.2 $\pm$ 2.0 <sup>c</sup>
2.0	53.7 $\pm$ 7.8 <sup>c</sup>	51.4 $\pm$ 7.1 <sup>c</sup>	49.7 $\pm$ 6.9 <sup>c</sup>
4.0	26.3 $\pm$ 4.0 <sup>c</sup>	21.8 $\pm$ 4.2 <sup>c</sup>	20.8 $\pm$ 5.5 <sup>c</sup>

TABLE XII. Effect of inhibitors of polyamine biosynthesis on growth of *S. nodorum*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>MGBG:</b>			
0.5	95.9 $\pm$ 3.2	82.7 $\pm$ 1.1 <sup>c</sup>	80.3 $\pm$ 1.1 <sup>c</sup>
1.0	83.5 $\pm$ 2.5	68.9 $\pm$ 1.3 <sup>c</sup>	65.9 $\pm$ 1.3 <sup>c</sup>
2.0	70.1 $\pm$ 5.9	52.0 $\pm$ 2.6 <sup>c</sup>	50.0 $\pm$ 2.4 <sup>c</sup>
<b>DFMO + MGBG:</b>			
0.5 + 0.5	92.8 $\pm$ 2.2	83.1 $\pm$ 1.6 <sup>c</sup>	81.8 $\pm$ 1.1 <sup>c</sup>
1.0 + 1.0	82.5 $\pm$ 2.5 <sup>c</sup>	73.8 $\pm$ 3.6 <sup>c</sup>	71.8 $\pm$ 1.6 <sup>c</sup>
<b>CHA:</b>			
0.5	96.9 $\pm$ 3.2	94.2 $\pm$ 1.4 <sup>b</sup>	100.3 $\pm$ 0.9
1.0	108.0 $\pm$ 1.0 <sup>a</sup>	100.4 $\pm$ 0.9	101.5 $\pm$ 1.9
2.0	95.9 $\pm$ 3.2	97.3 $\pm$ 0.9	102.1 $\pm$ 1.1
4.0	88.7 $\pm$ 2.3 <sup>a</sup>	91.6 $\pm$ 1.0 <sup>c</sup>	98.8 $\pm$ 1.1

TABLE XIII. Effect of inhibitors of polyamine biosynthesis on growth of *F. culmorum*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH	
	DAY 2	DAY 3
<b>MGBG:</b>		
0.5	100.8 $\pm$ 1.6	90.9 $\pm$ 1.0 <sup>b</sup>
1.0	88.8 $\pm$ 7.2	84.4 $\pm$ 3.6 <sup>b</sup>
2.0	90.4 $\pm$ 6.2	58.8 $\pm$ 5.5 <sup>c</sup>
<b>DFMO + MGBG:</b>		
0.5 + 0.5	117.6 $\pm$ 1.4 <sup>b</sup>	81.5 $\pm$ 2.0 <sup>c</sup>
1.0 + 1.0	108.0 $\pm$ 4.4	79.5 $\pm$ 2.0 <sup>c</sup>
<b>CHA:</b>		
0.5	127.2 $\pm$ 1.3 <sup>c</sup>	104.2 $\pm$ 0.6
1.0	121.6 $\pm$ 2.0 <sup>c</sup>	103.2 $\pm$ 1.4
2.0	124.0 $\pm$ 1.9 <sup>c</sup>	97.4 $\pm$ 1.4
4.0	120.8 $\pm$ 5.3 <sup>a</sup>	94.2 $\pm$ 3.1

TABLE XIV. Effect of polyamines and polyamines plus DFMO on growth of *P. teres* (grey form). Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>PUT:</b>			
0.5	94.4 $\pm$ 4.3	109.1 $\pm$ 3.3	105.4 $\pm$ 2.1
1.0	107.2 $\pm$ 5.4	129.7 $\pm$ 4.7 <sup>c</sup>	115.7 $\pm$ 3.2 <sup>b</sup>
<b>SPD:</b>			
0.5	93.6 $\pm$ 6.8	106.1 $\pm$ 7.4	103.9 $\pm$ 5.9
1.0	116.0 $\pm$ 5.3 <sup>a</sup>	121.8 $\pm$ 4.5 <sup>b</sup>	113.9 $\pm$ 2.7 <sup>b</sup>
<b>DFMO + PUT:</b>			
4.0 + 1.0	102.4 $\pm$ 8.4	109.1 $\pm$ 7.8	110.4 $\pm$ 6.9
<b>DFMO + SPD:</b>			
4.0 + 1.0	110.4 $\pm$ 5.8	124.8 $\pm$ 5.8 <sup>b</sup>	116.1 $\pm$ 5.2 <sup>a</sup>

TABLE XV. Effect of polyamines and polyamines plus DFMO on growth of *P. teres* (orange form). Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
PUT:			
0.5	110.7 $\pm$ 2.2 <sup>b</sup>	101.8 $\pm$ 1.3	99.2 $\pm$ 0.6
1.0	111.9 $\pm$ 2.1 <sup>b</sup>	103.7 $\pm$ 0.4 <sup>a</sup>	102.0 $\pm$ 0.2
SPD:			
0.5	102.4 $\pm$ 1.2	100.5 $\pm$ 0.9	98.9 $\pm$ 0.7
1.0	95.2 $\pm$ 2.5	93.2 $\pm$ 2.5 <sup>a</sup>	94.1 $\pm$ 1.2 <sup>b</sup>
DFMO + PUT:			
4.0 + 1.0	107.1 $\pm$ 3.3	99.5 $\pm$ 0.9	95.2 $\pm$ 0.9 <sup>b</sup>
DFMO + SPD:			
4.0 + 1.0	107.1 $\pm$ 1.1 <sup>a</sup>	97.3 $\pm$ 0.9	95.8 $\pm$ 0.5 <sup>b</sup>

TABLE XVI. Effect of polyamines and polyamines plus DFMO on growth of *G. graminis*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
<b>PUT:</b>			
0.5	98.9 $\pm$ 4.3	95.9 $\pm$ 2.4	99.4 $\pm$ 2.1
1.0	100.0 $\pm$ 3.2	98.6 $\pm$ 1.8	102.2 $\pm$ 1.2
<b>SPD:</b>			
0.5	101.1 $\pm$ 1.0	105.9 $\pm$ 0.9 <sup>a</sup>	105.3 $\pm$ 1.2 <sup>b</sup>
1.0	102.1 $\pm$ 4.1	105.0 $\pm$ 3.0	107.8 $\pm$ 2.5 <sup>a</sup>
<b>DFMO + PUT:</b>			
4.0 + 1.0	82.3 $\pm$ 3.7 <sup>b</sup>	90.5 $\pm$ 2.5 <sup>b</sup>	89.4 $\pm$ 2.4 <sup>b</sup>
<b>DFMO + SPD:</b>			
4.0 + 1.0	92.6 $\pm$ 5.7	96.8 $\pm$ 2.8	96.3 $\pm$ 2.2



TABLE XVII. Effect of polyamines and polyamines plus DFMO on growth of *S. nodorum*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH		
	DAY 2	DAY 4	DAY 6
PUT:			
0.5	96.9 $\pm$ 2.1	95.1 $\pm$ 0.9 <sup>a</sup>	98.5 $\pm$ 0.5
1.0	99.0 $\pm$ 2.1	96.4 $\pm$ 0.5 <sup>a</sup>	97.1 $\pm$ 0.8
SPD:			
0.5	110.3 $\pm$ 2.8 <sup>a</sup>	106.2 $\pm$ 0.8 <sup>b</sup>	104.7 $\pm$ 0.6 <sup>b</sup>
1.0	104.1 $\pm$ 2.0	94.7 $\pm$ 0.5 <sup>b</sup>	93.2 $\pm$ 0.7 <sup>b</sup>
DFMO + PUT:			
4.0 + 1.0	99.0 $\pm$ 3.1	96.4 $\pm$ 1.4	97.1 $\pm$ 0.5
DFMO + SPD:			
4.0 + 1.0	101.0 $\pm$ 3.1	96.9 $\pm$ 1.4	99.1 $\pm$ 1.3

TABLE XVIII. Effect of polyamines and polyamines plus DFMO on growth of *F. culmorum*. Results are expressed as percentage of control value (= 100%)  $\pm$  the standard error of the mean expressed as a percentage of the mean. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	PER CENT GROWTH	
	DAY 2	DAY 3
<b>PUT:</b>		
0.5	139.2 $\pm$ 2.9 <sup>c</sup>	111.7 $\pm$ 1.5 <sup>b</sup>
1.0	144.8 $\pm$ 2.8 <sup>c</sup>	114.6 $\pm$ 1.3 <sup>c</sup>
<b>SPD:</b>		
0.5	136.8 $\pm$ 3.5 <sup>c</sup>	109.7 $\pm$ 1.8 <sup>b</sup>
1.0	142.4 $\pm$ 1.7 <sup>c</sup>	111.4 $\pm$ 0.9 <sup>c</sup>
<b>DFMO + PUT:</b>		
4.0 + 1.0	125.6 $\pm$ 6.4 <sup>b</sup>	106.8 $\pm$ 2.8
<b>DFMO + SPD:</b>		
4.0 + 1.0	144.8 $\pm$ 4.4 <sup>c</sup>	110.4 $\pm$ 1.5 <sup>b</sup>

TABLE XIX. Effect of ODC inhibitors on cell diameter of P. teres, G. graminis, S. nodorum and F. culmorum on the sixth day after inoculation  $\pm$  the standard error of the mean. Results for grey and orange forms of P. teres are pooled. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b; P = 0.002 - 0.001 c.

TREATMENT (mM)	CELL DIAMETER ( $\mu\text{m}$ )			
	<u>P. TERES</u>	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
Control	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
DFMO:				
1.0	4.2 $\pm$ 0.3	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.8 $\pm$ 0.5 <sup>a</sup>
4.0	3.9 $\pm$ 0.2	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	7.0 $\pm$ 1.1 <sup>b</sup>
$\Delta$ -MFMO:				
0.5	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	-
1.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.8 $\pm$ 0.3 <sup>b</sup>
$\Delta$ -MFMO.Me:				
0.5	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.0 $\pm$ 0.2	-
1.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	5.9 $\pm$ 0.5 <sup>c</sup>
RR-MAP:				
0.5	-	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	-
1.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	5.1 $\pm$ 0.6 <sup>a</sup>

TABLE XX. Effect of inhibitors of polyamine biosynthesis on cell diameter of P. teres, G. graminis, S. nodorum and F. culmorum on the sixth day after inoculation  $\pm$  the standard error of the mean. Results for grey and orange forms of P. teres are pooled. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b.

TREATMENT (mM)	CELL DIAMETER ( $\mu$ m)			
	<u>P. TERES</u>	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
Control	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
MGBG:				
0.5	-	3.8 $\pm$ 0.0	-	-
1.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.4 $\pm$ 0.4
2.0	4.0 $\pm$ 0.2	-	3.8 $\pm$ 0.0	4.6 $\pm$ 0.5
CHA:				
1.0	-	-	-	5.7 $\pm$ 0.7 <sup>b</sup>
2.0	4.1 $\pm$ 0.2 <sup>a</sup>	-	3.8 $\pm$ 0.0	4.0 $\pm$ 0.2
4.0	4.0 $\pm$ 0.1	-	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
DFMO + MGBG:				
0.5 + 0.5	4.2 $\pm$ 0.3	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
1.0 + 1.0	4.0 $\pm$ 0.1	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.6 $\pm$ 0.5

TABLE XXI. Effect of polyamines and polyamines plus DFMO on cell diameter of P. teres, G. graminis, S. nodorum and F. culmorum on the sixth day after inoculation  $\pm$  the standard error of the mean. Results for grey and orange forms of P. teres are pooled. Significant differences are shown at P = 0.1 - 0.05 a; P = 0.02 - 0.01 b.

TREATMENT (mM)	CELL DIAMETER ( $\mu$ m)			
	<u>P. TERES</u>	<u>G. GRAMINIS</u>	<u>S. NODORUM</u>	<u>F. CULMORUM</u>
Control	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
PUT:				
0.5	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.4 $\pm$ 0.4
1.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.6 $\pm$ 0.8
SPD:				
0.5	4.7 $\pm$ 0.3 <sup>b</sup>	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	4.2 $\pm$ 0.4
1.0	4.1 $\pm$ 0.2 <sup>a</sup>	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
DFMO + PUT:				
4.0 + 1.0	3.9 $\pm$ 0.1	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0	3.8 $\pm$ 0.0
DFMO + SPD:				
4.0 + 1.0	3.9 $\pm$ 0.1	3.8 $\pm$ 0.0	4.0 $\pm$ 0.2	4.0 $\pm$ 0.2

TABLE XXII. Standard error of the means relating to effect of pH on uptake of polyamines, precursors and DFMO by *F. culmorum*. Results are expressed as  $\mu\text{M/g/h}$ .

pH	UPTAKE ( $\mu\text{M/g/h}$ )					
	ORN	ARG	DFMO	PUT	SPD	SPM
4.0	0.64	2.16	0.71	0.13	0.95	0.45
5.0	0.18	5.69	0.28	1.58	1.31	1.16
6.0	0.66	0.33	0.57	2.98	0.46	0.95
7.0	0.21	0.85	0.33	0.22	0.31	0.53
8.0	0.62	0.88	0.13	1.39	2.31	1.41
9.0	0.27	1.78	0.03	0.72	1.14	1.06

TABLE XXIII. Standard error of the means relating to the time course of polyamine, precursor and DFMO uptake by *F. culmorum*. Results are expressed as  $\mu\text{M/g/h}$ .

TIME (Mins)	UPTAKE ( $\mu\text{M/g/h}$ )					
	ORN	ARG	DFMO	PUT	SPD	SPM
10	2.83	1.86	1.56	6.19	3.41	4.32
20	6.70	7.66	5.84	1.94	1.14	2.61
30	1.82	7.55	2.78	6.08	6.59	0.48
40	9.39	0.66	1.56	1.43	2.06	1.28
50	6.25	2.85	1.07	4.49	5.11	0.69
60	8.57	5.86	1.23	10.50	9.24	0.91
120	19.15	18.93	0.81	17.88	4.23	1.05

TABLE XXIV. Standard error of the means relating to uptake of polyamines, precursors and DFMO by *F. culmorum* as a function of substrate concentration in the external medium. Results are expressed as  $\mu\text{M/g/h}$ .

[S] mM	UPTAKE ( $\mu\text{M/g/h}$ )					
	ORN	ARG	DFMO	PUT	SPD	SPM
0.006	0.02	0.03	0.006	0.05	0.03	0.05
0.013	0.03	0.08	0.004	0.21	0.08	0.12
0.025	0.10	0.27	0.02	0.12	0.06	0.09
0.05	0.36	0.45	0.07	0.09	0.08	1.67
0.1	0.31	2.09	0.05	0.57	-	0.22
0.25	1.70	0.73	0.37	1.59	0.42	0.74
0.5	0.20	3.38	0.41	2.17	4.83	11.81
1.0	9.34	17.32	1.18	7.02	4.09	4.18
10	56.47	12.86	28.66	18.70	8.23	20.07
25	202.05	60.83	22.89	74.12	30.17	21.80
50	82.55	149.56	209.40	78.87	25.73	131.55



TABLE XXV. Standard error of the means relating to uptake of polyamines, precursors and DFMO by *G. graminis* as a function of substrate concentration in the external medium. Results are expressed as  $\mu\text{M/g/h}$ .

[S] mM	UPTAKE ( $\mu\text{M/g/h}$ )				
	ORN	ARG	DFMO	PUT	SPM
0.1	0.11	0.31	0.10	0.43	0.71
0.25	-	1.16	0.38	1.04	1.36
0.5	0.74	2.22	0.39	1.62	1.34
1.0	0.89	2.93	3.78	1.33	1.81
10	6.45	42.98	46.98	28.59	7.38
25	34.52	51.35	166.77	25.43	5.16

TABLE XXVI. Standard error of the means relating to uptake of spermidine by G. graminis as a function of substrate concentration in the external medium. Results are expressed as  $\mu\text{m/g/h}$ .

[S] mM	$\mu\text{M/g/h}$ SPD
0.4	0.39
0.6	0.69
2.0	1.19
8.5	3.26
21.0	3.84

TABLE XXVII. Standard error of the means relating to uptake of polyamines and DFMO by *F. culmorum* when subjected to competition by other non-labelled substrates. Results are expressed as  $\mu\text{M/g/h}$ .

UNLABELLED SUBSTRATE	LABELLED SUBSTRATE		
	PUT	SPD	DFMO
PUT	3.55	26.21	2.88
SPD	3.55	0.94	2.88
ORN	49.73	16.85	3.84
DFMO	23.09	44.93	3.84

TABLE XXVIII. Standard error of the means relating to the effect of growing *G. graminis* in the presence of DFMO and MGBG on the uptake of polyamines by the fungus. Results are expressed as  $\mu\text{M/g/h}$ .

TREATMENT (mM)	UPTAKE ( $\mu\text{M/g/h}$ )		
	PUT	SPD	SPM
Control	13.38	6.22	0.67
DFMO:			
1.0	21.31	2.07	0.55
4.0	32.96	5.48	0.43
MGBG:			
0.5	5.48	1.15	0.80

TABLE XXIX. Standard error of the means relating to the effect of the presence of cations within the growth medium on uptake of polyamines by F. culmorum and G. graminis. Results are expressed as  $\mu\text{M/g/h}$ .

TREATMENT	<u>F. CULMORUM</u>			<u>G. GRAMINIS</u>		
	PUT	SPD	SPM	PUT	SPD	SPM
Control (PA)	11.15	2.09	1.67	5.06	2.11	2.93
+ Na <sup>+</sup>	4.08	6.16	7.40	1.96	2.51	4.56
+ K <sup>+</sup>	0.96	3.54	18.10	1.02	1.47	3.00
+ Mg <sup>2+</sup>	5.89	1.20	0.58	0.17	2.39	1.56

APPENDIX II

## CONVERSION OF RADIOACTIVE COUNTS TO MOLES

Counts obtained from the LKB 1215 Rackbeta liquid scintillation counter were in the form of disintegrations per minute (dpm). The dpm obtained for the control treatments were subtracted from counts for treatments containing radiolabel. Thus, the background level of radiation was taken into account prior to carrying out further analysis of the data. In performing the experiments on uptake as a function of substrate concentration in the external medium, the amount of radiolabelled substrate added remained constant, whereas the concentration of cold substrate was altered. This results in a dilution of the radiolabelled compound as the concentration of cold substrate was increased. This shows itself as a reduction in radiolabelled substrate at progressively higher concentrations of cold substrate. To calculate the 'true' uptake of substrate (that is, of both labelled and cold), this dilution effect had to be accounted for and a multiplication factor calculated. A multiplication factor was also calculated for other experiments where the quantity of cold substrate remained constant as the ratio of hot to cold substrate still had to be accounted for. The calculations carried out were as follows:

$$1 \mu\text{Ci} = 2.2 \times 10^6 \text{ dpm}$$

(information given by Amersham International PLC)

$$20 \mu\text{l} \text{ contains } 1 \mu\text{Ci}, \text{ then } \frac{12.5}{20} = 0.625 \mu\text{Ci}$$

Specific Activity: S.A. of the compounds varied (section 2.3.9). An example of 54  $\mu\text{Ci}/\mu\text{M}$  will be used here

$$\text{Therefore, } 1 \mu\text{Ci} = \frac{1}{54} = 0.0185 \mu\text{M}$$

Assume a count of 200 dpm, then

$$\frac{200}{2.2 \times 10^6} = 9.091 \times 10^{-5}$$

Therefore,  $\mu\text{M}$  of labelled substrate in the sample =

$$9.091 \times 10^{-5} \times 0.0185 = 1.682 \times 10^{-6} \text{ mM}$$

$$\text{then, } \frac{0.625}{54} = 0.01 \mu\text{M} = 0.00001 \mu\text{M}$$

Therefore,  $\frac{\text{cold [S]}}{0.00001} = \text{multiplication factor}$

then,  $1.682 \times 10^{-6} \mu\text{M} \times \text{multiplication factor} = x \mu\text{M}$  of substrate taken up by the fungus. The final figure was multiplied by 2 in order that the results could be expressed as  $\mu\text{M/g/h}$ .



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