THE EFFECT OF HERBICIDES ON SOIL MICRO-ORGANISMS

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The effect of herbicides on soil micro-organisms

SUMMARY

The w-substituted phenoxyacetic and γ-phenoxybutyric acid herbicides (e.g. MCPA, 2,4-D, MCPB, 2,4-DB, etc.) are widely used as selective weedkillers. During crop treatment these chemicals can reach the soil in some quantity and therefore it is of interest to consider the effect, if any, they may have on the microbiological activity of the soil environment.

Investigations carried out in vitro with a selection of micro-organisms (fungi, yeasts and green algae) agreed with published reports that such herbicides were harmless at concentrations (<5 ppm) used in agricultural practice. However, it was also found that, at higher levels (>100 ppm), these chemicals showed a consistent and striking difference in effect between the acetic and γ-butyric acid forms, the latter being toxic whilst the former had no effect on growth. This was the reverse of the effect on higher plants where the plant growth regulating activity of the γ-butyric acid is dependent on its β-oxidative breakdown by the plant to the active acetic acid homologue.

The influence of chemical structure on the fungitoxicity of the γ-butyric acid form of the w-substituted phenoxycarboxylic acid herbicides was investigated using homologous series of these herbicides from the acetic to the valeric acid side chain forms, and in related chemicals variously substituted in the side chain and ring of the basic phenoxycarboxylic acid. It appeared that activity against micro-organisms was largely a function of the length of the side chain rather than that of the total molecular weight of the side chain plus substituted groups. The activity of the γ-butyric acids and higher members of the homologous series was further shown not to be dependent on their breakdown to simpler forms by β-oxidation.

The toxicity and growth inhibition in fungi was paralleled by inhibition by MCPB of endogenous and mitochondrial respiration.

The effect of the w-substituted γ-phenoxybutyric acids at higher concentrations is therefore concluded to be a direct toxicity equivalent to the burning of tissues found in higher plants when treated with excessive concentrations of herbicides.

Jean L. Shennan, 1971.

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SUMMARY

The w-substituted phenoxyacetic and γ-phenoxybutyric acid herbicides (4-chloro-2-methylphenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, etc., and their γ-butyric homologues) are widely used as selective weedkillers. During crop treatment these chemicals can reach the soil in some quantity and therefore it is of interest to consider the effect they may have on the microbiological activity of the soil environment.

Results of investigations on in vitro culture with a selection of micro-organisms (fungi, yeasts and green algae) agreed with published reports that such herbicides were harmless at concentrations (< 5 ppm) used in agricultural practice. However, it was also found that, at higher levels (>100 ppm), these herbicides showed a consistent and striking difference in effect between the acetic and γ-butyric acid forms at equivalent concentrations, the latter being toxic whilst the former had little or no effect on growth of fungi. This was a reversal of the effect in higher plants where plant growth regulating activity of the γ-butyric acid form is dependent on its β-oxidative breakdown by the plant to the active acetic acid homologue.

The influence of chemical structure on the fungitoxicity of the γ-butyric acid form of the w-substituted phenoxycarboxylic acid herbicides exemplified by γ-MCPB (γ-4-chloro-2-methylphenoxybutyric acid) was investigated in homologous series of these chemicals from the acetic to the valeric acid forms, and in other phenoxycarboxylic acids variously substituted in the side chain and the ring structure of the molecule. It appeared that activity toward micro-organisms was largely a function of the length of the side chain rather than

its total molecular weight and was enhanced by increase in the number of substituted groups on the ring. Furthermore the activity of the γ-butyric acids and higher members of the homologous series was shown not to be dependent on their breakdown to simpler forms by β-oxidation.

The toxicity and growth inhibition in Penicillium notatum by γ-MCPB was paralleled by inhibition of endogenous and mitochondrial respiration.

It is concluded that γ-MCPB at relatively high concentrations has an intrinsic toxicity towards micro-organisms in addition to its hormonal effect on higher plants when converted to MCPA by β-oxidation.

I. INTRODUCTION

I. INTRODUCTION

The experimental work described in this thesis concerns the *in vitro* effect on certain species of micro-organisms of w-substituted phenoxycarboxylic acid herbicides in common usage. The literature on plant growth regulating herbicides includes the many varied aspects of research which have resulted from interest in these compounds. Some of the relevant topics more closely related to the content of the present thesis are discussed below by way of introduction.

The major developments in weed control in the United Kingdom using auxin herbicides began with the publication of investigations into the selective action of 4-chloro-2-methylphenoxyacetic acid (MCPA) by Slade, Templeman and Sexton (1945) and of 2,4-dichlorophenoxyacetic acid (2,4-D) by Nutman, Thorton and Quastel (1945) together with systematic field trials carried out by Blackman (1945).

These early publications stimulated research on selective and non-selective herbicides and spraying for weed control has now become a significant feature of routine farm management in both hemispheres, millions of acres being treated annually with auxin herbicides.

The mechanisms of herbicidal activity of many auxin herbicides are still unresolved although attempts have been made to characterise the primary site of action of both synthetic and natural plant growth regulators through theoretical explanations compatible with the wealth of experimental data on these compounds. Research on the mode of action of herbicides has been reviewed by many authors including Galston and Purves (1960), Crafts (1961), Pilet (1961), Hilton, Jansen and Hull (1963) and Audus (1964).

More recent theories on regulation of growth processes by auxins, reviewed by Osborne (1965), suggest that the primary action may be a stimulation of the synthesis of nucleic acids, proteins and hence enzymes in the cell, Key and Shannon (1964), for instance, showing that incorporation of adenosine diphosphate into ribonucleic acid was increased two-fold in the presence of growth stimulatory concentrations of indoleacetic acid and 2,4-D.

The auxin receptor in the plant cell has generally been assumed to be a protein as the concentrations of auxin effective for growth regulation are so low as to make its action probably that of a coenzyme or enzyme activator. In reviews of enzymic responses to herbicides (Woodford, Holly & McCready, 1958; Wort, 1962) a considerable variation in the extent and direction of response is revealed. For instance, in most cases where 2,4-D is applied to cell-free filtrates or to purified enzymes with substrate, inhibition of activity results. In such work the importance of 2,4-D concentration is evident, Freed, Reithel and Remmert (1961) showing that with several enzyme systems 2,4-D stimulation in activity at low concentrations (40 - 100 ppm) and inhibition at higher levels (1000 ppm) can occur.

The most recent hypotheses on RNA involvement in auxin action, reviewed by Thimann (1970), conclude that auxin functions by activating a messenger-type RNA and thus induces synthesis of specific enzymes. These would bring about the insertion of new materials into the cell wall causing its extension. Enlargement of the cell then follows. As auxin action in the plant is very rapid, and induces multiple responses such as increased respiration and accelerated protoplasmic streaming in addition to cell wall modification, auxin action may well involve soluble RNA.

The economic importance of the auxin herbicides and the search for new chemicals with improved selective activity have given rise to considerable interest in the relationship between the chemical structure of herbicides and their plant growth regulating and , therefore, herbicidal properties.

One of the more important groups of these herbicides are the w-substituted phenoxycarboxylic acids, e.g MCPA, 2,4-D and their γ-butyric acid analogues. They are selective translocated weed-killers depending for this selectivity on some difference in enzymic constitution of weed and crop connected with the entry of the herbicides and, in some cases, their breakdown within the plant tissues by β-oxidation. This is a process whereby two carbon atoms are removed from the side chain at each oxidative step, forming an acid with two fewer carbon atoms, hence only even numbered carbon chain acids will give rise to the herbicidally active acetic acid form.

Early evidence that the breakdown of the higher members of w-substituted phenoxycarboxylic acid series was due to β-oxidation was given in 1937 when Grace discovered that the growth promoting activity of w-substituted aryl- or aryloxyalkylcarboxylic acids was not directly correlated with side chain length since an alternation in activity occurred as the homologous series were ascended. Later, Thompson, Swanson and Newman (1946) and Synerholm and Zimmermann (1947) reported alternation in growth promoting activity through the 2,4-D homologous series from the highly active acetic acid derivative to the heptanoic acid. Members of the series with even numbers of carbon atoms in the side chain, including the carbonyl atom, were all active; those acids with odd numbers were completely inactive. Consequently, the periodicity of cell growth promotion for members of such homologous series could not be explained by a gradual increase in molecular weight as the number of carbon atoms

increased. Members of the series higher than the acetic acid were considered by these authors to be inactive *per se* but were converted by the plant either to the active acetic acid homologue or to an inactive metabolic product.

The possibility of selective herbicidal action in the w-substituted phenoxycarboxylic series of acids arose from the discovery that the β-oxidation of the aliphatic side chain was not a general phenomenon. Whereas regular alternation with a wheat test was shown with 4-chloro-, 2,4-dichloro- and 2,4,5-trichloro- series, only the first two series gave any alternation with pea and tomato tests, all homologues higher than the acetic acid being inactive in the 2,4,5-T series (Wain & Wightman, 1953, 1954). From further experimentation (Wain, 1955) it was concluded that, either certain plant species do not possess the appropriate enzyme system for break-down, or that, if β-oxidation enzymes are present, whether or not the plant is capable of degrading the side chain of specific w-phenoxy-carboxylic acids would depend on the nature and position of the nuclear substituents.

The products and intermediates in β-oxidative breakdown in plants have been described (Fawcett, Pascal, Pybus, Taylor, Wain & Wightman, 1959; Taylor & Wain, 1962). In most cases a greater or lesser proportion of the herbicidal chemical was shown to have been degraded but a substantial amount of the original compound remained in solution in the case of the n-propionic, n-butyric and n-valeric acid forms of the phenoxycarboxylic acid series investigated. Most workers, however, assumed any plant growth regulating activity to be due to the ultimate degradation product of β-oxidation.

In whatever fashion they are used, whether as foliar or ground sprays or in fallow treatment, herbicidal chemicals sooner or later reach the soil. An important aspect for consideration in

the use of herbicides has been, therefore, the interaction between these compounds and the soil microflora. As with their herbicidal activity, the fate and persistence of herbicides in the soil depends to a great extent on their chemical structure.

The processes involved in the microbiological breakdown of herbicides have been reviewed by several authors including Bollen (1961), Audus (1964), Fletcher (1966) and Wright (1971). Alexander (1965) considered that five conditions should be met for a pesticide to be subject to biodegradation: organisms effective in metabolising the compound must either exist in the soil or be capable of developing therein; the compound must be in a degradable form; it must reach the organisms and must induce the formation of enzymes concerned in the degradation, few of which are likely to be constitutive; lastly environmental conditions must favour proliferation of the organisms and operation of the enzymes.

Proof of the dominant role of micro-organisms in the degradation of herbicides comes with the isolation of effective strains and their growth in pure culture, using the herbicide as sole carbon source, together with the demonstration of their ability to inactivate the chemical when perfused with it through soil. This was first successfully carried out by Audus (1949, 1950) with a soil organism identified as Bacterium globiforme, on medium containing 0.1% 2,4-D as sole carbon source. Representatives of all the major microbial groups have been found capable of at least certain degradative steps in the decomposition of many diverse herbicides, but bacteria appear to be the most effective in this role (Jensen & Petersen, 1952; Evans & Smith, 1954; Stapp & Spicher, 1954; Walker & Newman, 1956; Rogoff & Reid, 1956; Steenson & Walker, 1958; Bell, 1957; Gaunt & Evans, 1961).

Metabolic pathways for the degradation of the w-substituted phenoxycarboxylic acid group of herbicides have been reviewed by

several authors (Audus, 1964; Elsdon & Peel, 1958; Ray, 1958; Rogoff, 1961) but only limited success has been attained in the study of these mechanisms using bacterial isolates in pure culture.

As soil micro-organisms vary in their ability to metabolise herbicides it is implicit that herbicides should exert a selective effect on micro-organisms and may in consequence bring about qualitative changes in the soil population and a malfunctioning of the various biological cycles essential for plant growth and the maintenance of soil fertility.

Several reports, however, have shown 2,4-D and MCPA to be only weak inhibitors of nitrite production by Nitrosomonas under pure culture conditions and in soil experiments and therefore could have no effect on soil nitrification at field rates of application (Jensen & Petersen, 1952; Jones, 1948; Flieg & Pfaff, 1951; Koike & Gainey, 1952; Goarin & St. Armand, 1957; Teater, Mortensen & Pratt, 1958). Heavier doses (25 - 100 ppm) in certain soil and soil percolation experiments were reported to show an initial reduction in nitrifying and nitrate-forming bacteria but this was followed quickly by recovery to normal (Smith, Dawson & Wenzel, 1945; Slepecky & Beck, 1950; Rogoff & Reid, 1952). Azotobacter was unaffected in soil by field rates of 2,4-D (Alencar, 1956) but higher concentrations of up to 500 ppm (Lenhard, 1959) or "100-fold" rates (van Schreven,Lindenbergh & Koridon, 1970) were inhibitory. Rhizobium was relatively susceptible to 2,4-D in pure culture as compared with aerobic Gram negative organisms (Worth & McCabe, 1948; Jensen & Petersen, 1952). The results from these specific studies agree generally with reports on the investigation of the overall microbial activity of the soil as measured by plate counts or carbon dioxide evolution methods in that the w-substituted phenoxyacetic acids at normal field rates and, in some cases, considerably higher doses, have no deleterious effect

(Kratochvil, 1951; Duda & Pedziwilk, 1952; Gamble, Mayhew & Chappell, 1952; Hoover & Colmer, 1953, Fletcher, 1960, Shklyar, Voevodin & Beshanov, 1961, Audus, 1970).

The initial objective of the work programme reported in this thesis was the examination *in vitro* of the effect of herbicidal chemicals of the w-substituted phenoxycarboxylic acid type on selected common filamentous fungi. Although references had been found in the literature on the effect of the acetic acid forms of these herbicides, i.e. 4-chloro-2-methylphenoxyacetic acid (MCPA) , 2,4-dichlorophenoxy-acetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), there was little or no record of any observations on the α-propionic and γ-butyric acid forms of these compounds, themselves available commercially as herbicidal chemicals.

Following initial findings of marked differences in fungitoxic effect toward *Penicillium notatum* between the acetic and α-propionic acid homologues of the above herbicides on the one hand and the γ-butyric acid forms on the other, several other micro-organisms were tested with these w-substituted phenoxycarboxylic acids, in many cases in screening experiments, to verify the extent of the effects illustrated with *P. notatum*.

The experimental programme was then developed to investigate the effect of a range of other w-substituted phenoxycarboxylic acids supplied by May and Baker, Ltd., the sponsors of the author's scholarship. These chemicals, both with and without herbicidal properties, were tested on filamentous fungi, including *P. notatum*. The chemicals included members of the w-substituted phenoxycarboxylic acid homologous series, putative intermediates in the β-oxidative breakdown of the γ-butyric acid forms of the above herbicides to the acetic acid forms, and variously substituted phenoxyacetic and

phenoxybutyric acids. These chemicals were investigated with the object of studying to some extent the effect of chemical structure on fungitoxicity in this group of compounds. The influence of structure on plant growth regulating activity has been a much discussed topic in the elucidation of the selective herbicidal activity of these and similar herbicides.

Finally the effect of the fungitoxicity discovered in γ-4-chloro-2-methylphenoxybutyric acid (γ-MCPB) was investigated at the subcellular level on the respiration of mycelial preparations of Penicillium notatum.

II. METHODS

II METHODS

A. MATERIALS

1. Cultures

The majority of the organisms used in this investigation were obtained from the following collections;

The Commonwealth Mycological Institute, Kew. (C.M.I.)
The National Collection of Yeast Cultures, Nutfield. (N.C.Y.C.)
The Culture Collection of Algae and Protozoa, Cambridge. (C.C.A.P.)

Certain other cultures were obtained from colleagues in the West of Scotland Agricultural College and the Medical Mycology Department of the University of Glasgow. All the organisms used are listed in Appendix A together with their sources.

For the duration of the investigation the cultures were maintained on agar slopes of the media recommended in each case ; potato dextrose agar or malt agar (fungi and yeasts), glucose peptone agar (dermatophytes) and peptone mineral agar (algae). The cultures were stored at $4^{\circ}C$ and were subcultured every four months.

2. Chemicals

The test chemicals were obtained from May & Baker, Ltd. of Dagenham. The chemical names, formulae, molecular weights,etc., of these compounds are listed in Appendix B.

As the chemicals were supplied in the acid form (insoluble) it was necessary to prepare solutions of the soluble sodium salts by reaction with either sodium bicarbonate or sodium hydroxide. The latter reagent was generally used. The required quantity of chemical was weighed out and transferred to a 100 ml volumetric flask.

The molecular equivalent of a normal standard volumetric solution of NaOH was added and the mixture shaken until the reaction was complete. The volume was made up with distilled water. This concentrated solution was then filtered through a sintered glass filter and diluted aseptically as required. The chemicals were added to the melted sterile agar media or to liquid media to obtain the desired final concentration of inhibitor.

In all cases the preparation of solutions was made in chemically clean glassware, care being taken to avoid chemical contamination of the test compounds.

All mineral salts, sugars, etc., used in the preparation of media were 'Analar' grade. 'Oxoid' or 'Difco' prepared media, agar and media ingredients were also used.

The enzymes and cofactors used were obtained from Koch-Light Laboratories Ltd, Colnbrook, Bucks.

3. Media

The various media employed during the investigation are listed in Appendix C, together with details of their composition and preparation.

B. EXPERIMENTAL TECHNIQUES

Except where stated otherwise all culture media were prepared at pH 7.0. The incubation temperature for experiments was 23°C with certain exceptions.

The following paragraphs detail the various culture techniques used.

1. Minimum Inhibitory Concentration (M.I.C.)

Filamentous fungi were grown on small test tube slopes (3" x $\frac{1}{2}$") of the appropriate agar medium incorporating the test chemical; yeasts were streaked on to the surface of agar plates. There were three replicates per treatment, a concentration range from 10,000 ppm to 100 ppm being tested.

Development on test tube slopes and plates was compared with controls (no addition) for presence or absence of growth after seven days incubation.

2. Petri Dish Culture

The appropriate agar medium was distributed in 15 ml amounts in 100 x 15 mm petri dishes. The plates were inoculated centrally with agar discs 3mm in diameter, containing mycelium taken from the growing margin of a young colony. There were five replicates to each treatment. The plates were incubated at 23°C for four to ten days, depending on the species. The growth of each colony was estimated as the mean of two diameters at right angles to each other measured at the end of the incubation period.

3. Liquid Culture of Fungi

The medium, potato dextrose broth, was distributed in 50 ml aliquots in 250 ml Erlenmeyer flasks. These were then autoclaved and the required test chemical added aseptically prior to inoculation with a spore suspension. The flasks were either incubated static or were placed on a reciprocal shaker for the duration of the growth period.

The mycelial mat (static culture) or mycelial pellets (shaken culture) were harvested by suction filtration on a tared filter paper and washed with distilled water. The samples were

then dried in an oven to constant weight.

4. Culture of Algae

The medium (Bristol's solution) was dispensed in 9" x 1½" test tubes in either 50 ml aliquots for liquid culture or, solidified with agar, in 30 ml quantities for agar slope culture. The chemicals were sterilised by filtration and the appropriate concentration added to each test tube after autoclaving to give the desired amount of inhibitor.

The tubes were then inoculated with a suspension of the alga under examination and placed in racks of ten upright in a water bath maintained at 25°C, illuminated evenly from below by a bank of fluorescent lights. With liquid culture the tubes were gently agitated by hand each day to resuspend the contents. The total growth period varied from two to four weeks, depending on the species; cell yield was assessed as follows;

Dry weight: the cell suspension from agar slopes was washed off on to tared filter papers and dried in an oven to constant weight.

Coulter Counter method: the normal mode of operation of this instrument was followed (Toennies, Izzard, Rogers & Shockman, 1961). Samples of liquid cultures were diluted by a factor of ten with a 1% solution of NaCl as electrolyte. The operating conditions were as follows - threshold 15; aperture 280µ; sample 2 ml. Five counts were made per replicate; five replicates per treatment.

5. Spore germination

Spores obtained from seven day old cultures of fungi were suspended in distilled water with 0.1% orange juice added as germination stimulant. Three replicate drops from a 1 ml pipette

of this spore suspension were placed on each slide. After twenty-four hours incubation in a moist chamber at 23°C the germination of 100 spores was recorded in two groups of 50 from different parts of each drop. A further replication in time was made by repeating the experiment using spores from a different original culture.

6. Manometric techniques

Standard respirometric techniques were employed (Umbreit, Burris & Stauffer, 1957) using a Braun Warburg Respirometer.

(a) Respiration of intact mycelium

Penicillium notatum was grown on potato dextrose medium in Roux bottles. The mycelial mat was picked off after five days, washed and blotted dry. 8 mm discs were cut from the tissue with a cork borer to a total of 50 mg per flask. These quantities were then teased out and placed in each Warburg flask. 5 ml of phosphate buffer (0.01M PO_4 at pH 6.0) were added together with the chosen inhibitor. The mycelium and inhibitor were incubated together at 30°C for 30 minutes prior to measuring the respiration rate.

(b) Preparation of cell-free extracts

Mycelium for enzyme studies was grown in static flask culture on glucose peptone medium at 25°C from a heavy inoculum of spores. 72 hour cultures gave mycelium with a high oxidative capacity. The culture medium was removed by filtration and the tissue rinsed with tap and distilled water and blotted dry. Succeeding operations were carried out at 4°C using precooled materials.

A weighed quantity of mycelium was ground in a mortar with acid-washed sand, using a ratio of 1 g mycelium : 2 g sand : 4 ml citrate-sucrose buffer (0.4M sucrose; 0.2M Tris - tri(hydroxymethyl) aminoethane; 0.005M EDTA; 0.01M K_2HPO_4; 0.02M sodium citrate, pH 7.5).

Normally 25 to 50 g mycelium was used prepare the extract. Enough buffer was added to the sand and mycelium to obtain a thick paste; the mixture was ground until smooth and sticky, usually for five minutes. The remainder of the buffer was then added. The resulting homogenate was passed through several layers of muslin and clarified by centrifugation at 1500 g for ten minutes in a MSE 'High Speed 17' refrigerated centrifuge to remove coarse wall debris, nuclei and sand. The remaining particulate fraction was then sedimented from the supernatant fluid by centrifugation at 20,000 g for 30 minutes. This sediment was resuspended in buffer and centrifuged again at 20,000 g for 30 minutes. The deposit was taken up in a small volume of 0.6M sucrose in 0.08M phosphate buffer at pH 7.3.

(c) Measurement of respiratory activity of isolated particles.

Conventional manometric techniques were used to determine oxygen uptake. All reactions took place at 30°C. Ten minutes were allowed for equilibration and the respiration was followed for 100 minutes. All values represent the mean of duplicate flasks.

The observations were made in a system buffer of 0.6M sucrose in 0.08M phosphate at pH 7.3. All reaction vessels contained the following cofactors (millimoles): $MgSO_4$, 1.5; cytochrome c, 0.12; adenosine triphosphate (ATP), 1.2. In experiments with tricarboxylic acid cycle substrates further cofactors were required (micromoles): coenzyme A, 1.3; cocarboxylase, 13.0; nicotinamide adenine nucleotide (NAD), 13.0.

C. STATISTICAL TREATMENT AND PRESENTATION OF EXPERIMENTAL RESULTS.

The principal method used to obtain the results presented in Part III was a petri dish plating technique. The effects of chemicals incorporated in the agar medium at various concentrations on growth of fungi inoculated on to the centre of the plate were assessed by measurement of the colony diameter (Part II, B,2.)

The raw experimental data were derived, in most cases, from single experiments consisting of a control (no addition) and a range of concentrations of the test compound, all replicated five times, performed as and when the chemicals became available for test. Usually it was possible to handle only two experiments simultaneously, i.e. testing the effect of one chemical on two organisms.

Due partly to these impediments of lack of laboratory facilities and piecemeal receipt of test chemicals from the research laboratories of May and Baker, Ltd., the data grouped in any particular table or section for discussion are not necessarily contemporaneous.

All the results for each organism are compiled in Appendix E. In the text comparison of reactions of chemicals or fungi are built up from these appended tables. The data are expressed as the means of the colony measurements for the five replicates in each treatment together with the standard deviation of the mean for each treatment, (≡ standard error). The order of variation in the measurements is thus obtained.

Turning to statistical treatment of the results for comparison of various chemicals and organisms as presented in the tables given in the text, the following possibilities were considered.

With several chemicals each tested at several concentration levels an analysis of variance to obtain a single error appropriate to all observations would appear to be the most obvious analysis even

with some sets of data missing as occurs occasionally. This treatment was not, however, considered suitable for the following reasons. For analysis of variance all observations should have the same variance, but in the present context, different doses could be expected to have different variances. For instance, a completely toxic dose gives zero measurements with no error. In an experiment comparing chemicals which have completely different patterns of activity, different groups of compounds might well be expected to have totally different variances. In addition, the available information on replicate error is appropriate only to comparisons within a set of observations obtained simultaneously; as indicated above this could only be applied to comparisons within a single experiment of one chemical and one or two organisms.

In order to analyse these experiments further, a suitable transformation of the dose scale and the response scale was sought, such that the transformed dose-response relationship would be linear. The more usual transformations involve plotting either the logarithm of the dose or the square root of the dose against response. Neither method was suitable for all the results. In the majority of experiments, however, a reasonably linear dose-response relationship was obtained when colony diameter was plotted against the square root of the dose.

For those chemicals giving inhibition of growth, toxicity can best be measured by the ED (effective dose) 50. Since formal statistical comparisons between chemicals using an error estimated from within a chemical are invalid there was little to be gained from estimating accurately the error of the ED 50. In practice the ED 50 was obtained by fitting a straight line by eye through the three experimental values on either side of the point of 50% reduction in diameter on the diameter: square root of dose scale. In view of

the pattern of the observed dose-response curves, and the difference in the controls, it was decided unwise to estimate the ED 50 from any assumed form for the whole response curve.

Those experimental results which did not transform to a linear relationship by this method (forming a quadratic instead) were mostly among the more toxic of the compounds tested. Thus estimations of the ED 50 values in such cases were made on that part of the quadratics which approximated most closely to a straight line. This treatment gave the same result as plotting such experimental data on a logarithm dose-response scale.

Where little or no inhibition of growth was observed over the concentration ranges tested, and where a trend of slight toxicity was difficult to convert to a straight line by eye-fit, the linear regression of response against the square root of the dose was tested. ED 50s so derived are indicated in the tables by the suffix (R) and regression statistics are given in Appendix D, 8.

Difference in the controls for any group of experiments compared, due to their separate dates of execution, made direct comparisons of different chemicals difficult. Comparisons between organisms on this point were also difficult to make accurately due to the different rates and forms of growth on the agar surface of petri dish cultures. For instance, Penicillium notatum and Aspergillus niger gave even and compact growth whereas Pythium debaryanum grew with rapid extension of a thin mat of mycelium. Pyrenophora avenae was considerably variable in growth rate from one experiment to another and this was reflected in the higher standard errors often seen among replicate means of this fungus.

Where increase in growth in the presence of a test chemical was observed, the stimulant effect was tested by a t-test of responses

at the test dose against responses at no dose. Any tendency toward stimulation at low doses did, of course, result in further difficulties in attempting to fit standard types of dose-response curves to full sets of data.

III. RESULTS

III RESULTS

A. AGAR PLATE CULTURE TREATMENTS.

1. The effect of w-substituted phenoxycarboxylic acid herbicides on Penicillium notatum and other filamentous fungi.

The first exploratory experiments to find the effect, if any, of the acetic, α-propionic and ϒ-butyric substituted phenoxycarboxylic acid herbicides on micro-organisms were carried out in agar plate culture using a common mould species, Penicillium notatum, Westling.

The technique used is described in Part II, B,2.

The following herbicides were tested. They will be referred to henceforth by the abbreviations shown:

4-chloro-2-methylphenoxyacetic acid	MCPA
α-4-chloro-2-methylphenoxypropionic acid	α-MCPP
ϒ-4-chloro-2-methylphenoxybutyric acid	ϒ-MCPB
2,4-dichlorophenoxyacetic acid	2,4-D
α-2,4-dichlorophenoxypropionic acid	α-2,4-DP
ϒ-2,4-dichlorophenoxybutyric acid	ϒ-2,4-DB
2,4,5-trichlorophenoxyacetic acid	2,4,5-T
α-2,4,5-trichlorophenoxypropionic acid	α-2,4,5-TP
ϒ-2,4,5-trichlorophenoxybutyric acid	ϒ-2,4,5-TB

The concentrations tested were from 5 ppm to 1000 ppm, that is, ranging upwards from the levels that such herbicides are expected to reach when applied to the soil at the recommended rates for weed-killing purposes (between 0.25 and 2 lb per acre; 2 to 6 lb per acre for 2,4,5-T used for killing woody plants).

The results are given in Table 1 and the dose response lines illustrated in Figure 1.

A marked difference in effect was shown between the acetic, α-propionic and ϒ-butyric acid forms of these herbicides. The acetic acids, MCPA, 2,4-D and 2,4,5-T, were only slightly inhibitory giving 9% to 15% reduction in growth at the highest concentrations

TABLE 1

THE EFFECT OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACID HERBICIDES ON PENICILLIUM NOTATUM

Results expressed as mean colony diameter in mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.3 ± 0.58	17030 (R)
α-4-chloro-2-methylphenoxypropionic acid	18.9 ± 0.52	24.1 ± 0.73	22.0 ± 0.50	26.4 ± 0.33	26.9 ± 0.26	25.3 ± 0.46	5700 (R)
γ-4-chloro-2-methylphenoxybutyric acid	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
2, 4-dichlorophenoxyacetic acid	19.5 ± 0.76	19.8 ± 0.30	19.6 ± 0.22	20.8 ± 0.34	19.5 ± 0.45	21.3 ± 0.33	28560 (R)
α-2, 4-dichlorophenoxypropionic acid	12.7 ± 0.43	16.7 ± 0.35	19.3 ± 0.26	19.2 ± 0.38	23.8 ± 0.26	17.4 ± 0.38	2088
γ-2, 4-dichlorophenoxybutyric acid	1.9 ± 0.19	3.3 ± 0.30	12.0 ± 0.36	14.0 ± 0.73	22.8 ± 0.14	25.5 ± 0.64	80
2, 4, 5-trichlorophenoxyacetic acid	23.3 ± 0.25	23.1 ± 0.08	23.4 ± 0.22	24.5 ± 0.63	24.6 ± 0.22	26.3 ± 0.08	24650 (R)
α-2, 4, 5-trichlorophenoxypropionic acid	(no test)	20.0 ± 0.10	27.9 ± 0.45	29.5 ± 0.65	31.0 ± 1.02	32.4 ± 0.48	936
γ-2, 4, 5-trichlorophenoxybutyric acid	(no test)	2.2 ± 0.10	8.2 ± 0.09	10.5 ± 0.32	19.4 ± 0.22	29.7 ± 0.17	25

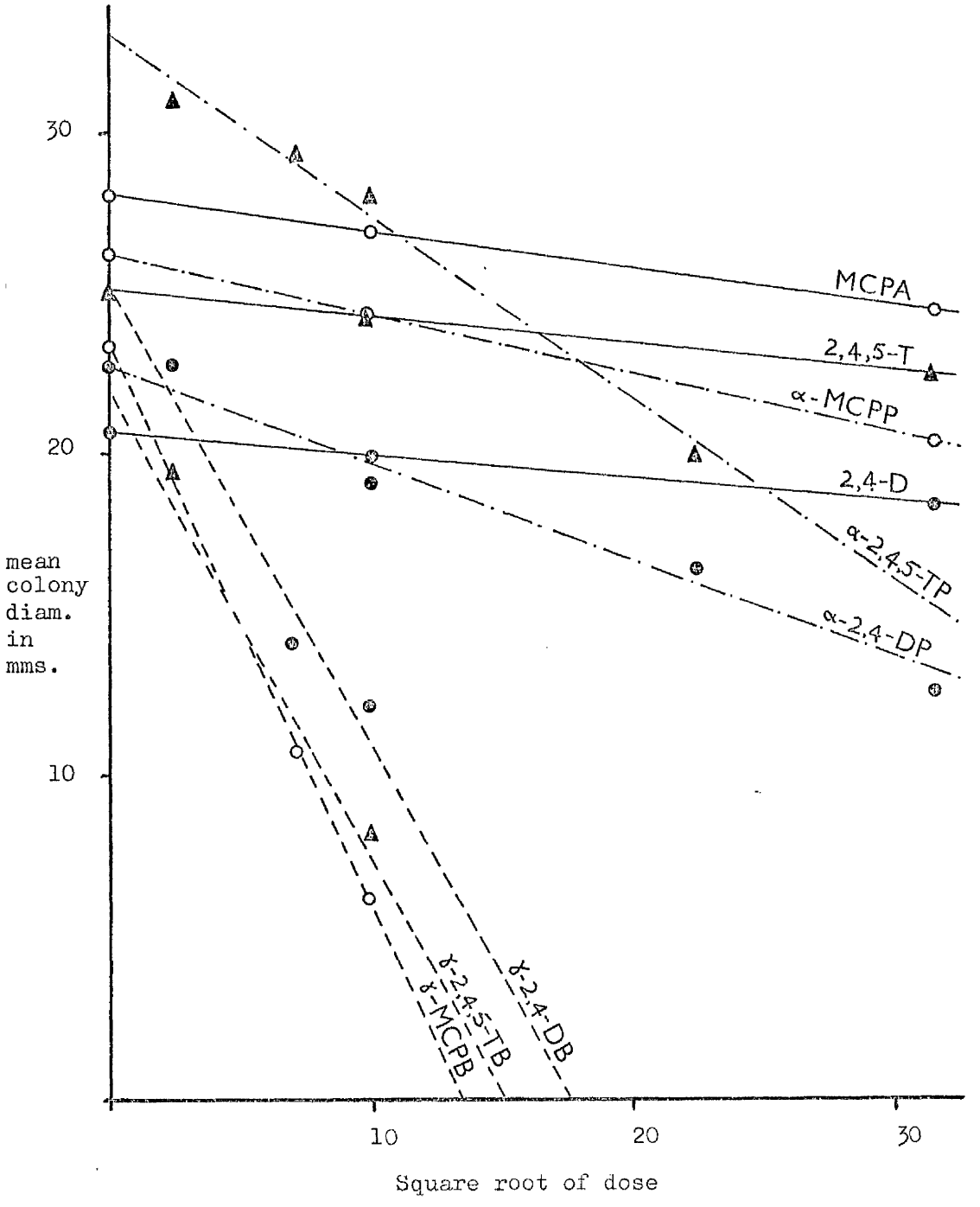


Figure 1

Effect of w-substituted phenoxycarboxylic acid herbicides on growth of Penicillium notatum in petri dish culture - response vs. square root of dose lines.

MCPA - 4-chloro-2-methylphenoxyacetic acid : α-MCPP - α-4-chloro-2-methylphenoxypropionic acid : γ-MCPB - γ-4-chloro-2-methylphenoxybutyric acid : 2,4-D - 2,4-dichlorophenoxyacetic acid : α-2,4-DP - α-2,4-dichlorophenoxypropionic acid : γ-2,4-DB - γ-2,4-dichlorophenoxybutyric acid : 2,4,5-T - 2,4,5-trichlorophenoxyacetic acid : α-2,4,5-TP - α-2,4,5-trichlorophenoxypropionic acid : γ-2,4,5-TB - γ-2,4,5-trichlorophenoxybutyric acid.

tested (1000 ppm). ED 50s of 17030 ppm, 28560 ppm and 24650 ppm respectively were estimated from regression lines of response against the square root of the dose (regression coefficients: MCPA, b= -0.1007; 2,4-D, b= -0.0618; 2,4,5-T, b= -0.0764). Although extrapolation of the dose-response lines in this way is inaccurate it serves to quantify the slight inhibition observed.

Of the α-propionic acids, α-MCPP was least toxic with an ED 50 of 5700 ppm, whilst the ED 50s of α-2,4-DP and α-2,4,5-TP were 2088 ppm and 936 ppm. The γ-butyric acid forms in each series were considerably more toxic, even at a level of 50 ppm, the ED 50s being 52 ppm (γ-MCPB), 80 ppm (γ-2,4-DB) and 25 ppm (γ-2,4,5-TB). In the case of γ-2,4,5-TB, 5 ppm reduced growth by 30 % as compared with the control.

During the course of the total working period, experiments on the effect of MCPA and γ-MCPB were repeated and in Table 2 results spread over three years are gathered together. Similar reactions for Penicillium notatum growing in agar culture were recorded on the three occasions tested. An attempt was made to analyse this replication in time by statistical treatment of the results. The two sets of data, for three experiments each with MCPA and γ-MCPB, are set out in Appendix D together with appropriate analyses of variance and linear regression statistics.

For MCPA, the individual analyses of variance show that at each date of test the variance between concentrations (groups) was very highly significant ($P < 0.001$) when tested against error. This established a relationship between dose and response. As the underlying relationship for any one chemical would be expected to be of a similar form on all occasions tested, the linearity of

TABLE 2

THE EFECT OF ω-SUBSTITUTED PHENOXYCARBOXYLIC ACID HERBICIDES ON PENICILLIUM NOTATUM

(a) Agar culture Comparison of results from three experiments

Results expressed as mean colony diameter in m.m

Chemical	Date Tested	Concentration (p pm) 1000	500	100	50	5	Control No addition	ED 50 (p pm)
MCPA	May 1960	22.2 ± 0.74	21.8 ± 1.20	25.1 ± 0.30	26.2 ± 0.15	25.3 ± 0.86	25.3 ± 0.96	10610 (R)
	Mar 1961	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.2 ± 0.58	17030 (R)
	Feb 1962	24.1 ± 0.36	24.3 ± 0.34	25.1 ± 0.48	25.5 ± 0.53	26.9 ± 0.10	26.3 ± 0.25	25600 (R)
	Average % control	88.5	88.5	94.0	96.2	99.0		
γ- MCPB	May 1960	1.8 ± 0.08	4.1 ± 0.17	7.7 ± 0.37	8.3 ± 0.32	15.5 ± 0.90	20.7 ± 0.49	47
	Mar 1961	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
	Feb 1962	5.0 ± 0.47	12.4 ± 0.27	14.5 ± 0.18	16.9 ± 0.10	27.8 ± 0.24	31.8 ± 0.46	72
	Average % control	11.7	26.6	39.1	46.8	86.8		

regression was tested against non-linear terms (with one and four degrees of freedom) and found to be marginally significant ($P < 0.05$) on two occasions and not significant on a third. The inconsistency of the terms for deviation from linear regression between occasions (significant at $P < 0.05$ in one test, not significant in the other two experiments) can be regarded as potential random variability.

The regression coefficients, computed on the means of treatments, were as follows;

MCPA, May 1960 -0.1303 ± 0.0356 95% c.l. $L_1 = 0.2291$; $L_2 = 0.0351$
MCPA, Mar 1961 -0.1007 ± 0.0384 95% c.l. $L_1 = 0.2069$; $L_2 = 0.0055$
MCPA, Feb 1962 -0.0806 ± 0.0180 95% c.l. $L_1 = 0.1306$; $L_2 = 0.0306$

When the overall results were compounded in an analysis of variance for comparison of regression coefficients for MCPA, the variance ratio of the mean square of regression at three occasions and the mean square of non-linearity of regression at three occasions was not significant, and it is concluded that the three groups were sampled from populations of equal slopes. Likewise the variance ratio of the deviations from regression and the total error was not significant (four and 72 degrees of freedom).

However, when the three experiments were plotted individually against the square root of the dose (Appendix D,7.) it appeared that different patterns could be distinguished. Compared with the averaged means for the pooled experiment, it was apparent that no one pattern of response on dose would apply in each case.

Apart from these inconsistencies the overall effect was concluded to be one of linear reduction up to the 500 ppm level approximately, with no further reduction at higher concentrations. The non-linearity of regression would therefore appear to be caused by this change in response pattern above a certain level rather than by continually changing responses.

The results for three trials of the effect of Y-MCPB on Penicillium notatum were similarly treated. When regression of response was plotted against the square root of the dose the result was a quadratic, not a linear, relationship. Plotting responses against the logarithm of the dose, however, gave a better approximation to a linear relationship for the effect of this chemical on P. notatum. Calculations of the analyses of variance with regression, testing responses against the logarithm of the dose are given in Appendix D,4-6.

Although the terms for linearity in Y-MCPB were very highly significant ($P < 0.001$), the deviations from linearity of regression were large in the individual experiments and thus made it impossible to compare regressions. In the overall analysis of variance (Appendix D, 6.) although the linear term differed between times its size was so significant that it swamped the deviations from regression. Hence the variance ratio of the mean squares of linear regression on three occasions and the mean square of deviations from regression on three occasions was not significant and therefore, as with MCPA, the three groups were concluded to have been sampled from populations of equal slopes. However, the difference between deviations at three times tested against error (72 degrees of freedom) was found to be highly significant ($P < 0.001$), further complicating the interpretation of the overall analysis of variance. The conclusion was made that, in the case of Y-MCPB, the data were not amenable to full statistical analysis. The differences between the pattern for Y-MCPB and MCPA were 'obvious' and needed no statistical 'proof'. Hence the eye-fit estimations of ED 50 were valid for the more toxic of these two compounds.

In subsequent tables where results for MCPA and Y-MCPB feature, the 1961 experimental results from Table 2 are presented.

The 4-chloro-2-methylphenoxycarboxylic acid herbicides were also tested in flask culture using the techniques described in Part II, B,3. Both static culture, resulting in a surface mat of sporing mycelium, and shaken culture, in which the fungus grew in pellet form, were attempted. The results, of dry weight yields, expressed as grams per litre, are shown in Table 3.

In shaken culture MCPA was non-toxic to Penicillium notatum over the concentration range tested, there being no significant difference between treatments. Analyses of variance with regression for these and other results quoted in succeeding pages are to be found in Appendix D, 8. In static culture there was a significant difference between concentrations but regression of response on dose was not significant (Appendix D , 8 (a) and (b)). This herbicide was therefore less toxic at the higher levels in liquid than in agar culture.

The results for α-MCPP in static culture likewise showed no toxicity towards Penicillium notatum, whereas in agar culture, 1000 ppm for instance, reduced growth by 25% (Table 1). Although the results were erratic, significant stimulation tested by a t-test was present at 100 ppm and 1000 ppm with α-MCPP in flask culture.

The three experiments on the effect of Υ-MCPB on Penicillium notatum in liquid medium were all carried out in shaken flasks. The resulting ED 50s, ranging between 50 ppm and 58 ppm, were similar to those for agar culture.

Therefore, in flask culture at the concentrations tested, the acetic and α-propionic forms of the 4-chloro-2-methyl- substituted phenoxycarboxylic acid herbicides were more or less non-toxic to

TABLE 3

THE EFFECT OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACID HERBICIDES ON PENICILLIUM NOTATUM

(b) Flask culture

Results expressed as g/l dry weight

Chemical	Culture	Concentration (p pm) 1000	500	100	50	5	Control No addition	ED 50 p pm
MCPA	Static	0.62 ± 0.022	0.70 ± 0.017	0.72 ± 0.022	0.67 ± 0.014	0.73 ± 0.018	0.69 ± 0.021	non-toxic (R)
	Shaken	1.95 ± 0.068	1.89 ± 0.027	2.09 ± 0.012	1.96 ± 0.089	2.12 ± 0.074	1.99 ± 0.064	non-toxic (R)
	Average % control	93.9	97.9	104.5	97.7	106.2		
α-MCPP	Static	1.39 ± 0.011	1.30 ± 0.049	1.44 ± 0.019	1.26 ± 0.014	1.28 ± 0.051	1.26 ± 0.036	non-toxic
	Average % control	110.4	103.0	114.4	100.0	102.0		
ɣ-MCPB	Shaken	0.50 ± 0.030	0.36 ± 0.042	0.87 ± 0.102	1.55 ± 0.127	2.36 ± 0.098	2.82 ± 0.054	55
	Shaken	(no test)	(no test)	0.87 ± 0.044	0.94 ± 0.109	1.87 ± 0.072	2.01 ± 0.253	58
	Shaken	(no test)	(no test)	0.47 ± 0.043	0.66 ± 0.049	1.20 ± 0.076	1.44 ± 0.010	50
	Average % control	1.8	12.6	35.5	49.1	86.5		

Penicillium notatum whereas in agar culture the higher concentrations showed some inhibition of growth. This difference may reflect a cumulative effect in agar culture of the inhibitor and staling products of metabolism, more readily diffused away in the liquid medium of flask culture. On the other hand, the yields of flask culture controls were relatively low and this technique may not, perhaps, have expressed the effects of MCPA and α-MCPP to their full extent. However, the toxicity of the γ-butyric acid (γ-MCPB) was of the same order in both forms of culture.

The effect of the 4-chloro-2-methyl- and the 2,4-dichloro-substituted phenoxycarboxylic acid herbicides was tested on a second fungal species, Aspergillus niger van Tieghem. Both agar plate and static flask culture methods were used with MCPA, α-MCPP and γ-MCPB; agar culture only with 2,4-D, α-2,4-DP and γ-2,4-DB. The results are given in Table 4.

Comparing the effect of these herbicides tested on Aspergillus niger with the equivalent results given for Penicillium notatum, (Tables 1 and 3) the former was found to be much more susceptible to the acetic and α-propionic acid forms of both groups of herbicides, the ED 50s ranging between 230 ppm and 1210 ppm.

The γ-butyric acid analogues of these herbicides were, in each case, more toxic; ED 50s for γ-MCPB were similar to those of P. notatum but γ-2,4-DB was somewhat less toxic to A. niger.

MCPA, α-MCPP and γ-MCPB were also tested in agar culture with seven other filamentous fungi, Ascochyta pisi Lib., Corticium solani (Prill & Delacr.) Bourd & Galz., Gibberella zeae (Schw.) Petch., Microsporum canis Bodin, Mucor hiemalis Wehmer, Pyrenophora avenae Ito & Kuribay and Pythium debaryanum Hesse. The results are given in Table 5 and regression statistics in Appendix D, 8.

TABLE 4

THE EFFECT OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACID HERBICIDES ON ASPERGILLUS NIGER

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
(a) Agar culture							
4-chloro-2-methylphenoxyacetic acid	11.2 ± 0.89	17.8 ± 0.58	22.9 ± 0.50	38.5 ± 0.73	45.2 ± 0.85	42.2 ± 0.56	230
α-4-chloro-2-methylphenoxypropionic acid	30.7 ± 1.04	28.7 ± 0.71	34.0 ± 0.34	39.4 ± 0.54	46.9 ± 1.55	48.8 ± 0.60	713
ɣ-4-chloro-2-methylphenoxybutyric acid	0.0	4.9 ± 0.19	13.7 ± 0.28	22.8 ± 0.59	37.0 ± 0.64	40.4 ± 0.17	64
2, 4-dichlorophenoxyacetic acid	24.0 ± 0.72	26.9 ± 0.23	38.7 ± 0.72	(no test)	(no test)	41.6 ± 0.49	1210
α-2, 4-dichlorophenoxypropionic acid	9.8 ± 0.49	12.8 ± 0.93	35.5 ± 0.43	47.7 ± 0.56	(no test)	40.7 ± 0.62	324
ɣ-2, 4-dichlorophenoxybutyric acid	1.3 ± 0.09	2.0 ± 0.31	26.2 ± 0.75	35.5 ± 0.67	(no test)	41.6 ± 0.49	190
(b) Flask culture							
4-chloro-2-methylphenoxybutyric acid	0.40 ± 0.074	0.57 ± 0.067	0.93 ± 0.045	0.90 ± 0.098	0.98 ± 0.017	0.96 ± 0.017	767
α-4-chloro-2-methylphenoxypropionic acid	0.37 ± 0.032	0.62 ± 0.072	0.86 ± 0.022	0.90 ± 0.010	0.94 ± 0.022	1.03 ± 0.014	660
ɣ-4-chloro-2-methylphenoxybutyric acid	0.03 ± 0.004	0.09 ± 0.010	0.61 ± 0.022	0.76 ± 0.033	1.85 ± 0.014	1.96 ± 0.014	48

(a) results expressed as mean colony diameter in mm.

(b) results expressed as g/l dry weight

TABLE 5

COMPARISON OF THE EFFECT OF THE HERBICIDES MCPA, ∝-MCPP and ϒ-MCPB ON SEVEN FILAMENTOUS FUNGI

Results expressed as mean colony diameter in mm.

Organism	Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
Ascochyta pisi	MCPA	46.5 ± 1.43	45.2 ± 1.48	44.7 ± 1.30	44.8 ± 0.85	42.6 ± 1.63	39.6 ± 1.35	non-toxic (R)
	∝- MCPP	38.5 ± 1.45	38.5 ± 1.50	41.6 ± 1.35	37.8 ± 1.48	37.1 ± 0.82	40.0 ± 0.65	non-toxic (R)
	ϒ- MCPB	0.0	0.0	7.3 ± 0.26	20.1 ± 1.21	29.8 ± 0.81	36.5 ± 1.15	46
Corticium solani	MCPA	68.5 ± 0.98	70.1 ± 0.39	80.9 ± 0.93	76.8 ± 0.49	76.8 ± 0.78	77.6 ± 0.51	16900 (R)
	∝- MCPP	63.6 ± 0.83	63.7 ± 0.74	64.5 ± 0.57	64.6 ± 0.51	68.0 ± 0.96	66.3 ± 0.62	non-toxic (R)
	ϒ- MCPB	0.0	0.0	28.5 ± 0.50	36.8 ± 1.23	58.5 ± 0.81	58.1 ± 0.64	94
Gibberella zeae	MCPA	51.7 ± 0.77	64.3 ± 0.58	66.8 ± 0.86	66.9 ± 0.78	76.7 ± 0.49	76.4 ± 0.40	3250
	∝- MCPP	55.7 ± 1.12	62.9 ± 1.00	74.7 ± 0.80	76.0 ± 1.23	79.6 ± 1.33	81.0 ± 0.84	2600
	ϒ- MCPB	0.0	0.0	28.1 ± 0.39	37.7 ± 0.44	57.8 ± 0.47	64.5 ± 0.78	77
Microsporum canis	MCPA	42.6 ± 0.53	41.2 ± 0.68	42.6 ± 0.24	40.9 ± 0.51	40.7 ± 0.73	37.9 ± 0.43	non-toxic (R)
	∝- MCPP	30.1 ± 0.50	30.0 ± 0.69	28.5 ± 0.45	29.4 ± 0.56	30.3 ± 0.66	31.1 ± 1.27	non-toxic (R)
	ϒ- MCPB	0.0	0.0	24.8 ± 0.43	32.8 ± 0.20	33.8 ± 0.51	44.3 ± 1.16	121
Mucor hiemalis	MCPA	51.4 ± 0.25	51.3 ± 0.25	52.7 ± 0.46	50.5 ± 0.35	52.3 ± 0.26	52.9 ± 0.60	non-toxic (R)
	∝- MCPP	38.9 ± 0.43	35.0 ± 1.18	37.9 ± 0.41	39.7 ± 0.37	43.8 ± 0.68	44.1 ± 0.55	12276 (R)
	ϒ- MCPB	0.0	0.0	10.5 ± 0.42	22.6 ± 2.19	40.1 ± 0.29	41.6 ± 0.40	55
Pyrenophora avenae	MCPA	9.6 ± 0.62	24.1 ± 0.89	28.9 ± 0.24	34.3 ± 1.26	42.1 ± 3.42	29.7 ± 0.62	767
	∝- MCPP	39.6 ± 1.30	38.4 ± 1.50	50.9 ± 2.08	51.5 ± 1.27	69.8 ± 2.03	66.9 ± 1.04	1037
	ϒ- MCPB	0.0	0.0	22.2 ± 0.49	36.8 ± 1.31	79.7 ± 0.51	79.5 ± 0.47	55
Pythium debaryanum	MCPA	0.0	15.9 ± 0.54	44.5 ± 1.43	49.0 ± 0.74	53.9 ± 0.87	54.9 ± 0.62	300
	∝- MCPP	4.7 ± 0.20	19.6 ± 0.48	44.5 ± 0.65	47.7 ± 0.49	48.6 ± 0.75	48.5 ± 0.35	424
	ϒ- MCPB	0.0	0.0	15.3 ± 0.56	25.3 ± 0.82	42.5 ± 0.42	45.5 ± 1.52	61

The reactions of four of these fungi, Ascochyta pisi, Corticium solani, Microsporum canis and Mucor hiemalis were similar to those of Penicillium notatum (Table 1) in that MCPA and α-MCPP were considerably less toxic than γ-MCPB. MCPA gave a significantly positive regression of response on dose with A. pisi and M. canis. With M. hiemalis there was no significant regression and, for C. solani, the ED 50 was estimated by regression to be 16900 ppm. For A. pisi, C. solani and M.canis, α-MCPP was non-toxic over the concentration range tested, linear regression being not significant. An ED 50 of 12276 ppm was estimated for α-MCPP with M. hiemalis. Gibberella zeae had a lower tolerance for MCPA and α-MCPP (ED 50s of 3250 ppm and 2600 ppm respectively).

The remaining two species were much more susceptible to MCPA, Pyrenophora avenae having an ED 50 of 767 ppm whilst Pythium debaryanum (ED 50 of 300 ppm) reacted similarly to Aspergillus niger (Table 4). α-MCPP had a similar effect to MCPA in these fungi.

In all seven organisms tested in Table 5, γ-MCPB was considerably toxic at 50 ppm, the ED 50s ranging from 46 ppm to 121 ppm.

In attempting a summary of the effects of MCPA, α-MCPP and γ-MCPB on the nine fungi tested in agar culture, the responses were variable to the acetic and α-propionic acid forms of these herbicides (ED 50s of 230 ppm to more than 10,000 ppm or absence of effect) while γ-MCPB was always toxic with a much smaller spread of response (ED 50s of 46 ppm to 121 ppm).

Ascochyta pisi, Microsporum canis and Pyrenophora avenae showed an increase in growth on MCPA as compared with the control. This increase, of the order of 8 - 16%, occurred over the whole range of concentration tested in A. pisi and M. canis. Neither α-MCPP nor γ-MCPB had any stimulatory effect on any of the species tested.

In observing the growth of the species investigated, no sectoring in response to growth on the various compounds tested was seen, although there have been reports that 2,4-D induced sectoring in Helminthosporium sativum (Hsia & Christensen, 1951) and variants of Neurospora (Nysterakis, 1956) and Pestalotia (Naito, 1957).

Decreased pigmentation, observed by the present author in colonies of Gibberella zeae growing on γ-MCPB have also been reported as a reaction to 2,4-D, MCPA and 2,4,5-T treatment in Phycomyces and Neurospora (Richards, 1948).

The effect of MCPA and γ-MCPB on spore germination of Penicillium notatum was tested following the method described in Part II, B.5. The resulting percentage germination data are given in Table 6. As the experimental treatments were made in duplicate only, the data have not been treated further statistically.

Results for MCPA were erratic but showed evidence of some slight inhibition. γ-MCPB was effective in reducing germination at the higher concentrations tested (ED 50 of 3480 ppm). Although the level of γ-MCPB inhibition was much higher than that found in other methods of assessment, the contrast between the two herbicides was still apparent.

The effect of treatment with γ-MCPB on the subsequent germination of spores in water was also observed. The viability of Mucor hiemalis and Penicillium notatum spores was unaffected but there was a lag in the initiation of germination compared with a control. Repeated subculture on toxic medium gave, however, a marked loss of viability in Penicillium notatum, the germination in water after four transfers on 500 ppm γ-MCPB being only 14 % of the control.

TABLE 6

The effect of MCPA and γ-MCPB on spore germination of Penicillium notatum.

Results expressed as percentage germination compared with control (no addition).

Chemical	Concentration (ppm)						ED 50 (ppm)
	10000	5000	1000	500	100	50	
MCPA	83.3	no test	79.0	92.6	88.0	91.4	not determined
γ-MCPB	16.0	32.7	78.4	85.8	79.6	90.1	3480

Spores of Aspergillus niger grown for four weeks on 500 ppm γ-MCPB without subculturing showed a slight increase in total viability and a very marked stimulation in the speed of germination. On transfer to water the treated spores gave a 25% increase in germination in 24 hours compared with the control.

Such stimulation in water as a result of having been grown on 2,4-D has been attributed in Gibberella zeae (Nishikada & Inoue, 1955) to a weakening of the structure of the spore coat allowing a more rapid production of germ tubes. Naito and Tani (1950) reported that 5 ppm and 500 ppm 2,4-D stimulated conidia of Uromyces vignae and Gleosporium olivarum respectively although Peturson (1951) and Richards (1948) found no stimulation of germination due to 2,4-D with any of the species they investigated.

Returning to plate culture techniques, it was realised that hydrogen ion concentration would affect both the growth of the organism and the toxicity of the chemicals. The relationship between pH and toxicity was therefore investigated using Penicillium notatum as the test organism.

The pH of the agar medium was adjusted to give values of 7.0 and 4.4 after autoclaving and the subsequent addition of the filter sterilised MCPA and γ-MCPB solutions. The results are given in Table 7. It was found that, at the lower pH, the toxicity of these compounds was considerably enhanced with the dissociation of the acid.

Reports in the literature on the inhibition of fungi in culture vary as to the level at which 2,4-D (or MCPA) is inhibitory. pH variation as well as differences in media, incubation temperature etc., may explain such anomalies in toxic concentration as these reports often fail to delimit the effective conditions under which

TABLE 7

THE EFFECT OF pH ON THE TOXICITY OF MCPA AND ɣ-MCPB TO PENICILLIUM NOTATUM

Results expressed as mean colony diameter in mm.

Chemical	pH	Concentration (ppm) 1000	500	100	50	Control No addition	ED 50 (ppm)
MCPA	7.0	24.1 ± 0.36	24.3 ± 0.34	25.1 ± 0.48	25.5 ± 0.53	26.3 ± 0.25	25600
	4.4	15.9 ± 0.09	24.2 ± 0.33	25.1 ± 0.58	29.2 ± 0.48	28.1 ± 0.44	1444
ɣ-MCPB	7.0	5.0 ± 0.47	12.4 ± 0.27	14.5 ± 0.18	16.9 ± 0.10	31.8 ± 0.46	74
	4.4	2.8 ± 0.38	4.0 ± 0.33	7.1 ± 0.33	11.17 ± 0.57	32.9 ± 0.57	35

the inhibitor acted. This effect of pH in the context of the w-substituted phenoxyacetic acid herbicides has been noted by several authors (Dubos, 1946; Dubrova, 1956; Naito, 1957; Erickson, Dewolfe & Brannaman, 1958). Newman (1947), for instance, reported that 2,4-D, 2,4,5-T and MCPA were inhibitory to Cunninghamella sp., Trichoderma sp. and Aspergillus niger at 125 - 500 ppm at pH 4.6; at pH 7.1 there was little or no effect.

2. Minimum inhibitory concentrations of the w-substituted phenoxycarboxylic acid herbicides in fungi.

Before proceeding to more detailed investigations with the group of filamentous fungi already tested, seven of the w-substituted phenoxycarboxylic acid herbicides i.e. MCPA, α-MCPP, γ-MCPB, 2,4-D, γ-2,4-DB, 2,4,5-T and γ-2,4,5-TB were tested in screening experiments against a wider selection of fungi and also against six species of yeast. The relative toxicity was measured as the minimum inhibitory concentration (M.I.C.) at which no growth occurred, testing, in the case of filamentous fungi, presence of growth on agar slopes and, with the yeasts, growth on streak plates. These methods of culture are described in Part II, B.1. The herbicides were tested at the following concentrations: 100, 500, 1000, 1250, 2500, 5000 and 10000 ppm. The results are given in Table 8, and the main trends are summarised below.

The marked differences in degree of toxicity described in the previous section between the acetic acid forms of the herbicides, MCPA and 2,4-D and the α-propionic acid herbicide tested, α-MCPP, and their γ-butyric acid homologues was sustained in twenty-four of the twenty-seven species tested. In the three remaining atypical species, Armillaria mellea, Phytophthora parasitica and Pythium debaryanum, growth was prevented by 5000 ppm, 1250 ppm and 2500 ppm

TABLE 8

Minimum inhibitory concentrations (ppm) of w-substituted phenoxycarboxylic acid herbicides.

Organism	MCPA	α-MCPP	Υ-MCPB
Alternaria solani	(>10000)	10000	500
Armillaria mellea	5000	2500	500
Ascochyta pisi	(>10000)	(>10000)	100
Aspergillus niger	(>10000)	(>10000)	500
Botrytis cinerea	(>10000)	(>10000)	500
Corticium solani	10000	10000	500
Fusarium conglutinans	(>10000)	(>10000)	500
F. nivale	(>10000)	5000	500
F. oxysporum f. lycopersici	(>10000)	(>10000)	500
Gibberella zeae	(>10000)	(>10000)	500
Helminthosporium sativum	(>10000)	(>10000)	500
Microsporum canis	10000	10000	500
Mucor hiemalis	(>10000)	(>10000)	500
Neurospora sitophila	(>10000)	(>10000)	500
Penicillium chrysogenum	(>10000)	(>10000)	1250
P. notatum	(>10000)	(>10000)	500
Phytophthora parasitica	1250	1250	500
Pyrenophora avenae	10000	(>10000)	500
Pythium debaryanum	2500	1250	500
Rhizopus stolonifer	(>10000)	(>10000)	500
Trichoderma viride	(>10000)	(>10000)	1250
Trichophyton sulphureum	10000	(>10000)	500
Candida albicans	(>10000)	(>10000)	500
C. pulcherrima	(>10000)	(>10000)	500
Saccharomyces acidifaciens	(>10000)	(>10000)	500
S. cereviseae	(>10000)	(>10000)	1250
S. fragilis	(>10000)	(>10000)	500

TABLE 8 (cont'd)

Organism	2,4-D	Y-2,4-DB	2,4,5-T	Y-2,4,5-TB
Alternaria solani	(>10000)	500	10000	500
Armillaria mellea	(>10000)	500	no test	
Ascochyta pisi	(>10000)	100	10000	100
Aspergillus niger	(>10000)	1250	10000	1250
Botrytis cinerea	(>10000)	500	10000	1250
Corticium solani	(>10000)	500	5000	500
Fusarium conglutinans	(>10000)	500	(>10000)	500
F. nivale	(>10000)	500	(>10000)	500
F. oxysporum f. lycopersici	(>10000)	500	(>10000)	500
Gibberella zeae	(>10000)	500	5000	100
Helminthosporium sativum	(>10000)	500	10000	500
Microsporum canis	(>10000)	500	10000	500
Mucor hiemalis	(>10000)	500	(>10000)	100
Neurospora sitophila	(>10000)	500	5000	100
Penicillium chrysogenum	(>10000)	1250	(>10000)	1250
P. notatum	(>10000)	1250	10000	1250
Phytophthora parasitica	1250	500	1250	500
Pyrenophora avenae	(>10000)	500	5000	100
Pythium debaryanum	1250	500	1250	500
Rhizopus stolonifer	(>10000)	500	10000	500
Trichoderma viride	(>10000)	500	5000	500
Trichophyton sulphureum	(>10000)	100	no test	
Candida albicans	(>10000)	1250	(>10000)	2500
C.pulcherrima	(>10000)	500	(>10000)	500
Saccharomyces acidifaciens	(>10000)	500	10000	100
S. cereviseae	(>10000)	500	5000	500
S. fragilis	(>10000)	1250	(>10000)	500

respectively of MCPA. A. mellea was not affected by the highest concentration of 2,4-D tested, but 1250 ppm was the M.I.C. for Phytophthora parasitica and Pythium debaryanum for this herbicide. However, even in these more susceptible fungi, M.I.C. levels for the γ-butyric acid forms of the three herbicides were similar to those of the other fungi tested, i.e. 500 ppm.

With MCPA and 2,4-D, growth was still possible at a level of 10,000 ppm in twenty of the twenty-seven fungi and yeasts tested. α-MCPP gave, in most cases, the same result as MCPA. In Alternaria solani and Fusarium nivale α-MCPP was more toxic than MCPA; in Trichophyton sulphureum it was less toxic than the acetic acid form.

2,4,5-T was more toxic than the other two acetic acid herbicides tested. In only eight species was 10,000 ppm ineffective, the M.I.C. levels in the rest being more typically 5000 ppm or 10,000 ppm. Phytophthora parasitica and Pythium debaryanum again had a low M.I.C. of 1250 ppm for this acetic acid form. The γ-butyric acid, γ-2,4,5-TB, was, as before, much more toxic than 2,4,5-T but no more so than the other γ-butyric acid herbicides tested.

3. The effect of the w-substituted phenoxycarboxylic acid herbicides, MCPA, α-MCPP and ϒ-MCPB on unicellular green algae.

The investigation of the contrasting toxicity of the acetic, α-propionic and ϒ-butyric acid forms of the 4-chloro-2-methyl-substituted phenoxyacid herbicides was further extended to three unicellular green algae, namely Chlamydomonas subangulosa, Fritsch and John, Chlorella vulgaris Beijerinck and Dictyococcus terrestris (Chod. & Kol.) Vischer.

Chlorella vulgaris was cultured on agar slopes containing the herbicides in a range of concentration from 5 ppm to 100 ppm and growth estimated from the dry weight of the harvested cells; Chlamydomonas subangulosa and Dictyococcus terrestris were grown in liquid culture medium containing the herbicides (using the same concentrations) and the extent of growth after incubation estimated using a Coulter Counter. Details of cultivation and harvesting are given in Part II, B.4. The results are shown in Table 9.

Estimation of growth of C. vulgaris grown on agar slopes in the presence of MCPA, α-MCPP and ϒ-MCPB showed the differential effect of these chemicals to be less obvious than that on fungi and yeasts. MCPA was nontoxic except for the highest concentration of 1000 ppm but an ED 50 of 2540 ppm was obtained on constructing a dose-response line with this data; the α-propionic acid form was fairly toxic at this level resulting in an ED 50 of 586 ppm. ϒ-MCPB was algicidal at 1000 ppm and 500 ppm although C. vulgaris was more resistant to the lower concentrations of this chemical than most of the other micro-organisms investigated (ED 50 of 250 ppm).

Table 9 also shows the reaction of Chlamydomonas subangulosa and Dictyococcus terrestris to the acetic and ϒ-butyric acid herbicides,

TABLE 9

THE EFFECT OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACID HERBICIDES ON UNICELLULAR GREEN ALGAE

Organism	Chemical	Concentration (ppm) 1000	5000	1000	50	5	Control No addition	ED 50 (ppm)
(a) Chlorella Vulgaris	MCPA	15.1 ± 1.08	21.3 ± 1.19	22.5 ± 1.11	21.6 ± 1.21	20.4 ± 1.28	20.0 ± 1.80	2540
	α-MCPP	4.7 ± 1.98	12.8 ± 1.27	17.6 ± 0.84	20.9 ± 1.04	23.0 ± 2.17	20.0 ± 1.80	586
	ɣ-MCPB	0.0	0.0	17.4 ± 0.97	18.7 ± 1.49	26.5 ± 0.67	20.0 ± 1.80	250
(b) Chlamydomonas subangulosa	MCPA	24493 ± 44.94	35365 ± 57.05	37604 ± 60.77	38546 ± 46.95	40838 ± 26.90	43767 ± 14.70	1500
	ɣ-MCPB	6844 ± 7.94*	6942 ± 7.40*	17942 ± 20.92	29962 ± 61.33	30846 ± 43.25	43767 ± 14.70	114
Dictyococus terrestris	MCPA	38466 ± 24.18	41482 ± 17.49	42013 ± 1.18	41968 ± 9.48	42563 ± 8.75	43322 ± 34.69	25600
	ɣ-MCPB	5876 ± 6.27*	6547 ± 24.91*	5669 ± 20.70*	6425 ± 13.75*	43576 ± 8.50	43322 ± 34.69	25

* little or no turbidity in growth medium

(a) dry weight in mg
(b) cell count per 2 ml (Coulter counter)

MCPA and γ-MCPB, in liquid culture. Growth of C.subangulosa was affected to some extent by MCPA at most levels, the ED 50 being 1500 ppm, while D. terrestris was less susceptible to MCPA at all the concentrations tested. The γ-butyric acid was more toxic to D. terrestris than to C. subangulosa (ED 50s of 114 ppm and 25 ppm respectively) allowing little growth at even 50 ppm.

These results,in spite of the variation in response to MCPA for the three algae tested, are therefore not dissimilar to those obtained with the filamentous fungi tested previously. In all cases the toxicity of γ-MCPB was considerably greater than that of MCPA.

4. Relationships between chemical structure and fungitoxic properties of the w-substituted phenoxycarboxylic acids.

The structure of the w-substituted phenoxycarboxylic acids consists of a benzene ring bearing various substitutions joined to an aliphatic side chain by an oxygen bridge. Herbicidal properties vary with the nature, position and number of the ring substitutions and on the length and form of the side chain. It was thought possible that these factors might similarly affect the antifungal activity of the compounds, following the observations reported in the preceding sections of considerable differences between the levels at which the acetic and γ-butyric forms of these herbicides affected the growth of Penicillium notatum and several other fungi.

To this end a selection of chemicals provided by May and Baker, Ltd. of Dagenham, being substituted phenoxycarboxylic acids with various ring and side chain substitutions and of differing side chain length were investigated, with the object of elucidating the molecular structure conferring fungitoxic properties on such herbicides as γ-MCPB (γ-4-chloro-2-methylphenoxybutyric acid).

The following experiments were carried out as described in Part II, B.2, the procedure being identical to that from which the data in Table 1 were derived. In most cases the test organism was Penicillium notatum. In several experiments six other fungi were also investigated, namely, Aspergillus niger, Gibberella zeae, Microsporum canis, Mucor hiemalis, Pyrenophora avenae and Pythium debaryanum.

The chemicals were prepared as the sodium salts as described in Part II, A.2. The formulae of all the compounds mentioned appear in Appendix B.

(a) Side chain length and β-oxidative degradation of the w-substituted phenoxycarboxylic acids.

To explore the probability that members of homologous series of the herbicides tested were fungitoxic per se and not being broken down by β-oxidation to the appropriate acetic or propionic forms, higher homologues of these herbicides and other compounds intermediate in the β-oxidation sequence were tested against a selection of fungi.

Five consecutive homologues of the 2,4-D series, ranging from the 2-carbon side chain (acetic acid) form to the 6-carbon side chain (caproic acid) form,were investigated for toxicity to Penicillium notatum and Aspergillus niger. The results are shown in Table 10.

With P. notatum the acetic acid, 2,4-D, was more or less non-toxic over the concentration range tested (estimated ED 50 of 28560 ppm) while the other four homologues in the series were inhibitory to this fungus. The β-propionic acid, β-2,4-dichlorophenoxypropionic acid, was somewhat less toxic than the higher members of the series (ED 50 of 500 ppm) whereas the γ-butyric acid was exceedingly inhibitory (ED 50 of 80 ppm). The d-2,4-dichlorophenoxyvaleric acid (ED 50 of 160 ppm) and e-2,4-dichlorophenoxycaproic acid (ED 50 of 112 ppm) forms were also highly toxic to P. notatum.

Similar results were obtained with the acetic, γ-butyric and e-caproic acid forms of the 4-chloro-2-methyl- substituted series (ED 50s of 17030 ppm, 52 ppm and 117 ppm respectively).

With Aspergillus niger, the same general pattern of effect was found except that the acetic acid homologues in the two series

TABLE 10

THE EFFECT OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACIDS IN HOMOLOGOUS SERIES ON PENICILLIUM NOTATUM AND ASPERGILLUS NIGER

Results expressed as mean colony diameter in mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 ppm
Penicillium notatum							
2, 4-dichlorophenoxyacetic acid	19.5 ± 0.76	19.8 ± 0.30	19.6 ± 0.22	20.8 ± 0.34	19.5 ± 0.45	21.3 ± 0.33	28560 (R)
β -2, 4-dichlorophenoxypropionic acid	3,5 ± 0.24	12.1 ± 0.49	14.9 ± 0.38	16.4 ± 0.52	(no test)	23.6 ± 0.46	500
γ -2, 4-dichlorophenoxybutyric acid	1.9 ± 0.19	3.3 ± 0.30	12.0 ± 0.36	14.0 ± 0.73	22.8 ± 0.14	25.5 ± 0.64	80
δ -2, 4-dichlorophenoxyvaleric acid	0.0	0.0	22.1 ± 0.37	30.0 ± 0.32	34.8 ± 0.90	37.3 ± 0.72	160
ε -2, 4-dichlorophenoxycaproic acid	0.0	2.4 ± 0.38	10.5 ± 0.17	11.1 ± 0.37	11.9 ± 0.38	19.1 ± 0.33	112
4-chloro-2-methylphenoxyacetic acid	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.3 ± 0.58	17030 (R)
γ -4-chloro-2-methylphenoxybutyric acid	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
ε -4-chloro-2-methylphenoxycaproic acid	0.0	3.7 ± 0.47	14.3 ± 0.37	15.2 ± 0.40	20.6 ± 0.17	26.3 ± 0.21	117
Aspergillus niger							
2, 4-dichlorophenoxyacetic acid	24.0 ± 0.72	26.9 ± 0.23	38.7 ± 0.72	(no test)	(no test)	41.6 ± 0.49	1210
β -2, 4-dichlorophenoxypropionic acid	0.0	4.7 ± 0.35	22.0 ± 0.13	37.5 ± 0.22	(no test)	41.6 ± 0.49	140
γ -2, 4-dichlorophenoxybutyric acid	1.3 ± 0.09	2.0 ± 0.31	26.2 ± 0.75	35.5 ± 0.67	(no test)	41.6 ± 0.49	190
δ -2, 4-dichloropehoxyvaleric acid	0.0	1.7 ± 0.11	32.2 ± 0.40	39.4 ± 0.43	42.6 ± 0.25	51.0 ± 0.27	114
4-chloro-2-methylphenoxyacetic acid	11.2 ± 0.89	17.8 ± 0.58	22.9 ± 0.50	38.5 ± 0.73	45.2 ± 0.85	42.2 ± 0.56	230
γ -4-chloro-2-methylphenoxybutyric acid	0.0	4.9 ± 0.19	13.7 ± 0.28	22.8 ± 0.59	37.0 ± 0.64	40.4 ± 0.17	64
ε -4-chloro-2-methylphenoxycaproic acid	0.0	1.5 ± 0.26	26.8 ± 0.14	37.4 ± 0.34	49.4 ± 0.27	53.0 ± 0.16	112

tested, 2,4-D and MCPA, were considerably more toxic to this organism than to Penicillium notatum (cf. Table 4).

It was concluded, therefore, from the short series tested that there was no evidence of an alternation of effect between acids with odd and even numbers of carbon atoms in the side chain. It would appear, on the contrary, that at the 3- to 4- carbon side chain length (β-propionic, γ-butyric forms), the compounds reached a level of fungitoxicity which was maintained in the 5- and 6- carbon side chain. Heptanoic and octanoic homologues were not, unfortunately, available to confirm this pattern further.

The comparison of the three even numbered side chain acids, MCPA, γ-MCPB and e-4-chloro-2-methylphenoxycaproic acid, was extended to five other fungi with similar results, the γ-butyric and e-caproic forms being more or less equivalent in toxicity (Table 11), in contrast to the less toxic or non-toxic MCPA.

The effect of blocking β-oxidation of γ-4-chloro-2-methyl-phenoxybutyric acid by substitution of methyl groups on the carbon atomsof the side chain methyl groups was next investigated. The replacement of one of the alpha or beta hydrogen atoms in the n-butyric acid side chain should be sufficient to obstruct β-oxidation as the formation of the intermediate β-keto acid is then prevented. In herbicide testing (personal communication, May and Baker,Ltd.) it was found that the inactivity of the α-methyl-substituted γ-MCPB was consistent with this prediction and the β-methyl-substituted γ-MCPB was definitely reduced in activity by the partial operation of this impediment. The γ-methyl-substituted form, on the other hand, should be freely β-oxidised by the plant treated to an α-substituted acetic acid equivalent to α-MCPP. The relevant side chain formulae are given overleaf.

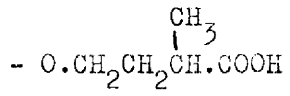
TABLE 11

THE EFFECT OF w-4-CHLORO-2-METHYL-SUBSTITUTED PHENOXYCARBOXYLIC ACID HOMOLOGUES ON FUNGI

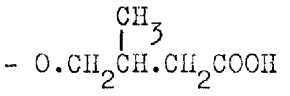
Results expressed as mean colony diameter in m.m.

Organism	Chemical	Concentration (p pm) 1000	500	100	50	5	Control No addition	ED 50 (p pm)
Gibberella zeae	MCPA*	51.7 ± 0.77	64.3 ± 0.58	66.8 ± 0.86	66.9 ± 0.78	76.7 ± 0.49	76.4 ± 0.40	3250
	γ- MCPB	0.0	0.0	28.1 ± 0.39	37.7 ± 0.44	57.8 ± 0.47	64.5 ± 0.78	77
	ε- MCPC	0.0	0.0	19.4 ± 1.09	22.9 ± 0.41	48.0 ± 0.72	53.2 ± 0.64	55
Microsporum canis	MCPA	42.6 ± 0.53	41.2 ± 0.68	42.6 ± 0.24	40.9 ± 0.51	40.7 ± 0.73	37.9 ± 0.43	non toxic (R)
	γ- MCPB	0.0	0.0	24.8 ± 0.43	32.8 ± 0.20	33.8 ± 0.51	44.3 ± 1.16	121
	ε- MCPC	0.0	0.0	0.0	25.7 ± 0.56	36.8 ± 1.07	39.9 ± 1.08	110
Mucor hiemalis	MCPA	51.4 ± 0.25	51.3 ± 0.25	52.7 ± 0.46	50.0 ± 0.35	52.3 ± 0.26	52.9 ± 0.60	non-toxic (R)
	γ- MCPB	0.0	0.0	10.5 ± 0.42	22.6 ± 2.19	40.1 ± 0.29	41.6 ± 0.40	55
	ε- MCPC	0.0	0.0	7.5 ± 0.55	17.8 ± 0.60	43.1 ± 1.18	50.6 ± 2.03	35
Pyrenophora avenae	MCPA	9.6 ± 0.62	24.1 ± 0.89	28.9 ± 0.24	34.3 ± 1.29	42.1 ± 3.42	29.7 ± 0.62	767
	γ-MCPB	0.0	0.0	22.2 ± 0.49	36.8 ± 1.31	79.7 ± 0.51	79.5 ± 0.47	55
	ε-MCPC	0.0	0.0	12.3 ± 0.27	15.6 ± 0.93	35.7 ± 1.48	38.0 ± 2.49	53
Pythium debaryanum	MCPA	0.0	15.9 ± 0.54	44.5 ± 1.43	49.0 ± 0.74	53.9 ± 0.87	54.9 ± 0.62	300
	γ-MCPB	0.0	0.0	15.3 ± 0.56	25.3 ± 0.82	42.5 ± 0.42	45.5 ± 1.52	61
	ε-MCPC	0.0	0.0	10.7 ± 0.60	33.9 ± 0.89	66.4 ± 1.31	68.5 ± 1.58	49

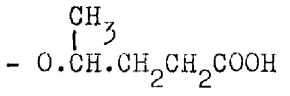
* MCPA 4-chloro-2-methylphenoxyacetic acid
γ - MCPB γ-4-chloro-2-methylphenoxybutyric acid
ε - MCPC ε-4-chloro-2-methylphenoxycaproic acid



α-methyl-γ-phenoxybutyric acid



β-methyl-γ-phenoxybutyric acid

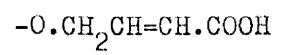


γ-methyl-γ-phenoxybutyric acid

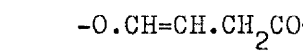
In testing several fungi with these substituted forms of γ-MCPB (results given in Table 12) no marked variation in effect was found in general between the α-, β- and γ- methyl substituted forms for any given organism, all three acids having an activity similar to that of γ-MCPB, although at the lowest concentration tested (5 ppm) results varied from stimulation (Mucor hiemalis; β-methyl-γ-MCPB) to considerable inhibition (Mucor hiemalis; γ-methyl-γ-MCPB). These results suggested again that the presence or absence of the β-oxidation mechanism has no relevance to the fungitoxic properties of the compounds.

As the pathway of breakdown in higher plants has been explored by the identification and isolation of intermediate compounds, three of these potential intermediates (synthetically prepared) between the γ-butyric and acetic acid forms of two series of w-substituted phenoxycarboxylic acids were investigated for fungitoxicity.

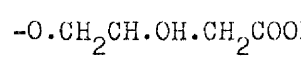
In Table 13 the effect on Penicillium notatum of breakdown products of both the 2,4-D and MCPA series is shown. The relevant side chain formulae are shown below.



γ-phenoxycrotonic acid



γ-phenoxyvinylacetic acid



β-hydroxy-γ-phenoxybutyric acid

TABLE 12

THE EFFECT OF SIDE CHAIN SUBSTITUTION ON THE FUNGI TOXICITY OF γ -4-CHLORO-2-METHYLPHENOXYBUTYRIC ACID

Results expressed as mean colony diameters in mm

Organism	Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
Aspergillus niger	γ-MCPB	0.0	4.9 ± 0.19	13.7 ± 0.28	22.8 ± 0.59	37.0 ± 0.64	40.4 ± 0.17	64
	α -methyl-γ -MCPB	0.0	4.3 ± 0.08	7.9 ± 0.46	16.8 ± 0.18	35.6 ± 0.64	36.6 ± 1.06	46
	β -methyl-γ -MCPB	(no test)	1.0 ± 0.22	7.1 ± 0.10	15.8 ± 0.32	32.7 ± 0.66	36.3 ± 0.73	49
	γ -methyl-γ-MCPB	2.1 ± 0.25	8.9 ± 0.32	9.9 ± 0.40	16.3 ± 0.26	32.4 ± 0.95	34.5 ± 0.59	55
Gibberella zeae	γ -MCPB	0.0	0.0	28.1 ± 0.39	37.7 ± 0.44	57.8 ± 0.47	64.5 ± 0.78	77
	α -methyl-γ -MCPB	1.1 ± 0.32	6.9 ± 0.20	28.9 ± 0.32	43.0 ± 1.44	61.8 ± 1.15	55.9 ± 0.98	150
	β -methyl-γ -MCPB	5.3 ± 0.41	21.4 ± 0.62	52.9 ± 0.56	59.8 ± 0.46	77.5 ± 1.37	85.0 ± 0.32	190
	γ -methyl-γ -MCPB	0.0	2.4 ± 0.22	42.0 ± 0.92	51.5 ± 1.77	69.7 ± 2.03	72.9 ± 2.27	137
Microsporum canis	γ -MCPB	0.0	0.0	24.8 ± 0.43	32.8 ± 0.20	33.8 ± 0.51	44.3 ± 1.16	121
	α -methyl-γ -MCPB	0.0	7.3 ± 0.51	37.2 ± 0.56	40.0 ± 0.76	46.9 ± 1.33	46.9 ± 0.58	231
	β -methyl-γ -MCPB	0.0	0.0	22.9 ± 0.23	29.2 ± 0.77	36.1 ± 1.17	43.4 ± 1.47	123
	γ -methyl-γ -MCPB	0.0	4.8 ± 0.25	28.1 ± 0.43	31.2 ± 0.77	36.2 ± 1.33	35.9 ± 0.98	177
Mucor hiemalis	γ -MCPB	0.0	0.0	10.5 ± 0.42	22.6 ± 2.19	40.1 ± 0.29	41.6 ± 0.40	55
	α -methyl-γ -MCPB	0.0	0.0	20.0 ± 0.27	30.1 ± 0.33	39.3 ± 0.80	40.3 ± 0.90	98
	β -methyl-γ -MCPB	0.0	0.0	5.3 ± 0.56	15.4 ± 1.28	40.7 ± 0.34	36.6 ± 0.53	38
	γ -methyl-γ -MCPB	0.0	0.0	3.8 ± 0.37	23.9 ± 0.45	35.8 ± 1.36	53.8 ± 1.45	21
Penicillium notatum	γ -MCPB	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
	α -methyl-γ -MCPB	0.9 ± 0.06	2.0 ± 0.21	6.0 ± 0.41	7.2 ± 0.10	15.3 ± 0.35	17.8 ± 0.73	46
	β -methyl-γ -MCPB	1.4 ± 0.17	5.6 ± 0.48	9.1 ± 0.22	10.2 ± 0.34	15.2 ± 0.46	16.8 ± 0.20	121
	γ -methyl-γ -MCPB	1.1 ± 0.09	1.2 ± 0.15	6.0 ± 0.21	9.0 ± 0.36	17.8 ± 0.48	23.9 ± 0.19	34
Pyrenophora avenae	γ -MCPB	0.0	0.0	22.2 ± 0.49	36.8 ± 1.31	79.7 ± 0.51	79.5 ± 0.47	55
	α -methyl-γ -MCPB	0.0	0.0	15.1 ± 0.51	24.3 ± 1.29	54.7 ± 1.77	53.5 ± 0.92	55
	β -methyl-γ -MCPB	0.0	0.0	6.0 ± 0.97	7.8 ± 1.25	23.4 ± 1.11	22.0 ± 0.52	52
	γ -methyl-γ -MCPB	0.0	0.0	13.2 ± 2.15	19.4 ± 1.53	45.0 ± 2.85	51.7 ± 1.99	40
Pythium debaryanum	γ -MCPB	0.0	0.0	15.3 ± 0.56	25.3 ± 0.82	42.5 ± 0.42	45.5 ± 1.52	61
	α -methyl-γ -MCPB	0.0	3.8 ± 0.20	27.4 ± 0.76	37.1 ± 1.04	54.5 ± 1.05	57.1 ± 0.48	92
	β -methyl-γ -MCPB	0.0	0.0	13.4 ± 0.93	28.4 ± 0.32	44.9 ± 1.65	48.4 ± 2.59	58
	γ -methyl-γ -MCPB	0.0	0.0	17.3 ± 1.08	28.0 ± 1.38	41.7 ± 0.68	43.7 ± 0.41	74

TABLE 13

THE FUNGI TOXIC EFFECTS OF INTERMEDIATES IN THE BREAKDOWN PATHWAY BETWEEN THE ɣ-BUTYRIC AND ACETIC HOMOLOGUES OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACIDS ON PENICILLIUM NOTATUM

Results expressed as mean colony diameter in mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
ɣ-2, 4-dichlorophenoxybutyric acid	1.9 ± 0.19	3.3 ± 0.30	12.0 ± 0.36	14.0 ± 0.73	22.8 ± 0.14	25.5 ± 0.64	80
ɣ-2, 4-dichlorophenoxycrotonic acid	3.5 ± 0.53	10.5 ± 0.35	11.8 ± 0.53	12.6 ± 0.54	22.6 ± 0.61	22.9 ± 0.74	177
ɣ-2, 4-dichlorophenoxyvinylacetic acid	1.3 ± 0.40	3.0 ± 0.33	10.2 ± 0.30	14.7 ± 0.46	26.5 ± 0.49	28.2 ± 0.38	14
β-hydroxy- ɣ -2, 4-dichlorophenoxy-butyric acid	18.5 ± 1.19	20.1 ± 0.19	21.1 ± 0.94	18.2 ± 0.34	20.0 ± 0.65	22.2 ± 0.40	non-toxic (R)
2, 4-dichlorophenoxyacetic acid	19.5 ± 0.76	19.8 ± 0.30	19.6 ± 0.22	20.8 ± 0.34	19.5 ± 0.45	21.3 ± 0.33	28560 (R)
ɣ-4-chloro-2-methylphenoxybutyric acid	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
ɣ-4-chloro-2-methylphenoxycrotonic acid	(no test)	9.1 ± 0.36	10.9 ± 1.00	13.4 ± 0.20	19.3 ± 0.26	22.7 ± 0.12	90
ɣ-4-chloro-2-methylphenoxyvinylacetic acid	6.5 ± 0.41	9.7 ± 0.52	10.5 ± 0.40	15.1 ± 0.43	20.0 ± 0.53	21.0 ± 0.69	110
β-hydroxy- ɣ -4-chloro-2-methylphenoxy-butyric acid	18.8 ± 0.22	19.1 ± 0.94	20.1 ± 0.46	20.2 ± 0.68	20.3 ± 1.34	21.2 ± 0.29	non-toxic (R)
4-chloro-2-methylphenoxyacetic acid	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.3 ± 0.58	17030 (R)

The γ-crotonic and γ-vinylacetic acid forms, the putative alternative derivatives from the dehydrogenation of the γ-butyric acid, were both toxic. However, the β-hydroxy-γ-phenoxybutyric acids were non-toxic, suggesting that hydroxylation of the butyric acid side chain rendered the acid inactive as a fungitoxic agent although these are still active as herbicides (personal communication, May and Baker, Ltd.).

Table 14 extends the investigation of two of these acids, the γ-4-chloro-2-methylphenoxycrotonic acid and the β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid, comparing their effect with that of γ-MCPB and MCPA on four other fungi, namely Gibberella zeae, Microsporum canis, Pyrenophora avenae and Pythium debaryanum. In all four species there was a distinct decrease in toxicity between the γ-butyric and γ-crotonic forms on the one hand and the β-hydroxy-γ-butyric and acetic forms on the other, confirming that a changeover from toxic to non-toxic properties or reduction in toxicity may be associated with hydroxylation of the side chain. The fungitoxicity of β-hydroxy-γ-MCPB varied, being in each case similar to that of the corresponding acetic acid. β-hydroxy-γ-MCPB was non-toxic (regression of response on dose not significant; Appendix D,8) to Microsporum canis, toxic (ED 50 of 888 ppm and 275 ppm respectively) to Pyrenophora avenae and Pythium debaryanum and showed an intermediate effect on Gibberella zeae (ED 50 of 2430 ppm).

TABLE 14

THE FUNGITOXIC EFFECTS OF INTERMEDIATES IN THE BREAKDOWN PATHWAY BETWEEN THE γ-BUTYRIC AND ACETIC ACID HOMOLOGUES OF 4-CHLORO-2-METHYL- SUBSTITUTED PHENOXYCARBOXYLIC ACIDS

Results expressed as mean colony diameter in mm.

Organism	Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
Gibberella zeae	γ- 4-chloro-2-methylphenoxybutyric acid	0.0	0.0	28.1 ± 0.39	37.7 ± 0.44	57.8 ± 0.47	64.5 ± 0.78	77
	γ- 4-chloro-2-methylphenoxycrotonic acid	(no test)	11.4 ± 0.64	30.7 ± 0.34	35.1 ± 0.53	47.3 ± 1.02	55.1 ± 0.60	144
	β-hydroxy- γ -4-chloro-2-methylphenoxybutyric acid	39.9 ± 1.62	50.2 ± 2.92	57.0 ± 1.19	61.2 ± 0.51	65.6 ± 0.99	53.6 ± 2.01	2430
	4-chloro-2-methylphenoxyacetic acid	51.7 ± 0.77	64.3 ± 0.58	66.8 ± 0.86	66.9 ± 0.78	76.7 ± 0.49	76.4 ± 0.40	3250
Microsporum canis	γ- 4-chloro-2-methylphenoxybutyric acid	0.0	0.0	24.8 ± 0.43	32.8 ± 0.20	33.8 ± 0.51	44.3 ± 1.16	121
	γ-4-chloro-2-methylphenoxycrotonic acid	0.0	11.1 ± 0.43	37.4 ± 0.24	38.7 ± 1.24	39.7 ± 1.02	38.9 ± 1.33	335
	β-hydroxy- γ -4-chloro-2-methylphenoxybutyric acid	35.3 ± 1.31	35.1 ± 1.04	32.9 ± 1.06	35.4 ± 1.06	38.2 ± 0.85	40.7 ± 0.46	non-toxic (R)
	4-chloro-2-methylphenoxyacetic acid	42.6 ± 0.68	41.2 ± 0.68	42.6 ± 0.24	40.9 ± 0.51	40.7 ± 0.73	37.9 ± 0.43	non-toxic (R)
Pyrenophora avenae	γ- 4-chloro-2-methylphenoxybutyric acid	0.0	0.0	22.2 ± 0.49	36.8 ± 1.31	79.7 ± 0.51	79.5 ± 0.47	55
	γ-4-chloro-2-methylphenoxycrotonic acid	(no test)	0.0	8.0 ± 0.27	11.8 ± 0.46	24.8 ± 2.01	26.1 ± 1.40	55
	β-hydroxy- γ -4-chloro-2-methylphenoxybutyric acid	20.3 ± 1.45	35.1 ± 1.83	39.9 ± 1.49	27.7 ± 1.69	42.4 ± 2.16	47.8 ± 1.66	888
	4-chloro-2-methylphenoxyacetic acid	9.6 ± 0.62	24.1 ± 0.89	28.9 ± 0.24	34.3 ± 1.26	42.1 ± 3.42	29.7 ± 0.62	767
Pythium debaryanum	γ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	15.3 ± 0.56	25.3 ± 0.82	42.5 ± 0.42	45.5 ± 1.52	61
	γ-4-chloro-2-methylphenoxycrotonic acid	(no test)	1.6 ± 0.51	28.4 ± 0.58	32.4 ± 1.44	59.1 ± 3.04	55.9 ± 1.58	93
	β-hydroxy- γ -4-chloro-2-methylphenoxybutyric acid	2.1 ± 0.52	16.7 ± 0.46	53.2 ± 2.21	60.3 ± 1.33	60.8 ± 1.37	66.7 ± 1.63	275
	4-chloro-2-methylphenoxyacetic acid	0.0	15.9 ± 0.54	44.5 ± 1.43	49.0 ± 0.74	53.9 ± 0.87	54.9 ± 0.62	300

(b) Substitution in the 2-carbon side chain (acetic acid form) of the 4-chloro-2-methylphenoxyacetic acids.

Comparison of results for the acetic and α-propionic forms of the w-substituted phenoxycarboxylic acid herbicides (Tables 1 and 5) showed that these two forms behaved somewhat similarly in most cases, being non-toxic or least toxic of the chemicals under test, depending on the organism tested. The α-propionic form of the side chain is equivalent to an α-substituted acetic acid, having the same chain length as the acetic acid. As a comparison, the effect of another form of substitution of greater molecular weight (C_2H_5) on the alpha carbon atom of the acetic acid side chain form of MCPA, to give α-4-chloro-2-methylphenoxybutyric acid (α-MCPB) was tested on several fungi, Aspergillus niger, Gibberella zeae, Microsporum canis, Mucor hiemalis, Pyrenophora avenae and Pythium debaryanum. The results, compared with MCPA and α-MCPP, appear in Table 15. These side chain forms are shown below.

$-O.CH_2COOH$	$-O.\underset{\displaystyle CH_3}{\underset{\vert}{CH}}.COOH$	$-O.\underset{\displaystyle C_2H_5}{\underset{\vert}{CH}}.COOH$
phenoxyacetic acid	α-phenoxypropionic acid	α-phenoxybutyric acid

The results were variable but followed the general pattern of sensitivity of the different fungi tested. With the exception of Aspergillus niger where the α-butyric acid was of equivalent toxicity to the acetic acid form (ED 50s of 270 ppm and 230 ppm respectively), α-MCPB was more toxic than MCPA or α-MCPP. In Microsporum canis where MCPA and α-MCPP were non-toxic, the ED 50 for α-MCPB, estimated from a regression test of response on dose (Appendix D, 8) was 11450 ppm.

With Pyrenophora avenae and Pythium debaryanum all three compounds were fairly toxic but the ED 50s for α-MCPB (121 ppm and 144 ppm) still showed it to be less fungitoxic than Y-MCPB (ED 50s

TABLE 15

THE EFFECT OF SUBSTITUTION IN THE 2-CARBON SIDE CHAIN OF THE 4-CHLORO-2-METHYLPHENOXYACETIC ACID ON ITS FUNGI TOXICITY

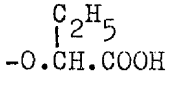
Results expressed as mean colony diameter in mm.

Organism	Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
Aspergillus niger	4-chloro-2-methylphenoxyacetic acid	11.2 ± 0.89	17.8 ± 0.58	22.9 ± 0.50	38.5 ± 0.73	45.2 ± 0.85	42.2 ± 0.56	230
	α-4-chloro-2-methylphenoxypropionic acid	30.7 ± 1.04	28.7 ± 0.71	34.0 ± 0.34	39.4 ± 0.54	46.9 ± 1.55	48.8 ± 0.60	713
	α-4-chloro-2-methylphenoxybutyric acid	9.7 ± 0.56	14.2 ± 0.46	24.8 ± 0.97	33.8 ± 0.37	46.9 ± 0.67	39.2 ± 0.75	270
Gibberella zeae	4-chloro-2-methylphenoxyacetic acid	51.7 ± 0.77	64.3 ± 0.58	66.8 ± 0.86	66.9 ± 0.78	76.7 ± 0.49	76.4 ± 0.40	3250
	α-4-chloro-2-methylphenoxypropionic acid	55.7 ± 1.12	62.9 ± 1.00	74.7 ± 0.80	76.0 ± 1.23	79.6 ± 1.33	81.0 ± 0.84	2600
	α-4-chloro-2-methylphenoxybutyric acid	40.5 ± 0.35	53.5 ± 0.42	62.6 ± 0.81	64.8 ± 0.90	60.2 ± 0.60	62.5 ± 0.50	1730
Microsporum canis	4-chloro-2-methylphenoxyacetic acid	42.6 ± 0.53	41.2 ± 0.68	42.6 ± 0.24	40.9 ± 0.51	40.7 ± 0.73	37.9 ± 0.43	non-toxic (R)
	α-4-chloro-2-methylphenoxypropionic acid	30.1 ± 0.50	30.0 ± 0.69	28.5 ± 0.45	29.4 ± 0.56	30.3 ± 0.66	31.1 ± 1.27	non-toxic (R)
	α-4-chloro-2-methylphenoxybutyric acid	32.0 ± 0.63	34.2 ± 0.75	35.6 ± 2.44	35.5 ± 0.71	37.5 ± 1.20	38.0 ± 0.65	11450 (R)
Mucor hiemalis	4-chloro-2-methylphenoxyacetic acid	51.4 ± 0.25	51.3 ± 0.25	52.7 ± 0.46	50.5 ± 0.35	52.3 ± 0.26	52.9 ± 0.60	non-toxic (R)
	α-4-chloro-2-methylphenoxypropionic acid	38.9 ± 0.43	35.0 ± 1.18	37.9 ± 0.41	39.7 ± 0.37	43.8 ± 0.68	44.1 ± 0.55	12276 (R)
	α-4-chloro-2-methylphenoxybutyric acid	23.0 ± 1.58	29.9 ± 0.93	40.3 ± 0.89	43.4 ± 0.62	42.5 ± 0.27	42.9 ± 0.51	1156
Pyrenophora avenae	4-chloro-2-methylphenoxyacetic acid	9.6 ± 0.62	24.1 ± 0.89	28.9 ± 0.24	34.3 ± 1.26	42.1 ± 3.42	29.7 ± 0.62	767
	α-4-chloro-2-methylphenoxypropionic acid	39.6 ± 1.30	38.4 ± 1.56	50.9 ± 2.08	51.5 ± 1.27	69.8 ± 2.03	66.9 ± 1.04	1037
	α-4-chloro-2-methylphenoxybutyric acid	5.7 ± 0.81	12.6 ± 2.12	19.7 ± 3.20	39.0 ± 3.90	46.8 ± 1.89	45.7 ± 1.04	121
Pythium debaryanum	4-chloro-2-methylphenoxyacetic acid	0.0	15.9 ± 0.54	44.5 ± 1.43	49.0 ± 0.74	53.9 ± 0.87	54.9 ± 0.62	300
	α-4-chloro-2-methylphenoxypropionic acid	4.7 ± 0.20	19.6 ± 0.48	44.5 ± 0.65	47.7 ± 0.49	48.6 ± 0.75	48.5 ± 0.35	424
	α-4-chloro-2-methylphenoxybutyric acid	(no test)	25.2 ± 1.02	38.3 ± 0.70	48.8 ± 1.93	64.5 ± 1.99	63.9 ± 1.24	144

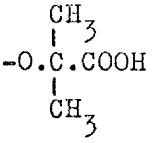
of 55 ppm and 61 ppm respectively). Gibberella zeae and Mucor hiemalis were affected to a lesser degree.

Table 16 presents results on the effect of a wider selection of α-substituted phenoxycarboxylic acids on the growth of Penicillium notatum. The unsubstituted form, phenoxyacetic acid, and its α-propionic homologue were non-toxic, stimulation in growth resulting at higher concentrations.

In the three 4-chloro-2-methyl- ring substituted acids tested, the α-propionic acid was intermediate in toxicity between the acetic acid and the α-butyric form, which latter, with an ED 50 of 960 ppm, was still much less toxic than the straight chain γ-butyric acid, γ-MCPB (ED 50 of 52 ppm). Of the 2,4-dichloro- series investigated the α-propionic acid form was, as above, intermediate in toxicity between the acetic and α-isobutyric acid forms. In this instance the configuration of the substitution in the α-butyric acid was altered, there being two methyl groups attached symmetrically to the α-carbon as shown below.



α-phenoxybutyric acid



α-phenoxyisobutyric acid

Both compounds had similar ED 50s, 960 ppm and 986 ppm. As before, the γ-butyric acid form of 2,4-D was more fungitoxic (ED 50 of 80 ppm).

The α-propionic form of 2,4,5-T was as toxic (ED 50 of 936 ppm) as the α-butyric acids discussed above. An α-butyric acid form of 2,4,5-T was not available for testing.

Comparing otherwise identical compounds with side chain substitution and an unsubstituted side chain of the same carbon

TABLE 16

THE EFFECT OF SUBSTITUTION IN THE 2-CARBON SIDE CHAIN OF w-SUBSTITUTED PHENOXYCARBOXYLIC ACIDS ON PENICILLIUM NOTATUM

Results expressed as mean colony diameter in mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
phenoxyacetic acid	23.6 ± 0.53	21.8 ± 0.53	20.5 ± 0.39	21.4 ± 0.56	20.3 ± 0.51	21.0 ± 0.26	non-toxic
α-phenoxypropionic acid	16.0 ± 0.93	13.4 ± 0.28	15.8 ± 0.52	14.5 ± 0.45	13.4 ± 0.29	14.3 ± 0.25	non-toxic
4-chloro-2-methylphenoxyacetic acid	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.3 ± 0.58	17030 (R)
α-4-chloro-2-methylphenoxypropionic acid	18.9 ± 0.52	24.1 ± 0.73	22.0 ± 0.50	26.4 ± 0.33	26.9 ± 0.26	25.3 ± 0.46	5700
α-4-chloro-2-methylphenoxybutyric acid	8.7 ± 0.26	10.6 ± 0.53	12.3 ± 0.58	13.8 ± 0.84	14.9 ± 0.39	17.8 ± 0.73	960
2, 4-dichlorophenoxyacetic acid	19.5 ± 0.76	19.8 ± 0.30	19.6 ± 0.22	20.8 ± 0.34	19.5 ± 0.45	21.3 ± 0.33	28560 (R)
α-2, 4-dichlorophenoxy propionic acid	12.7 ± 0.43	16.7 ± 0.35	19.3 ± 0.26	19.2 ± 0.38	23.8 ± 0.26	17.4 ± 0.38	2088
α-2, 4-dichlorophenoxyisobutyric acid	(no test)	12.1 ± 0.36*	12.2 ± 0.17	14.2 ± 0.84	15.1 ± 0.33	13.9 ± 0.06	986
2, 4, 5-trichlorophenoxyacetic acid	23.3 ± 0.25	23.1 ± 0.08	23.4 ± 0.22	24.5 ± 0.63	24.6 ± 0.22	26.3 ± 0.08	24650 (R)
α-2, 4, 5-trichlorophenoxypropionic acid	(no test)	20.0 ± 0.10	27.9 ± 0.45	29.5 ± 0.65	31.0 ± 1.02	32.4 ± 0.48	936

*200 ppm

number i.e. α-2,4-dichlorophenoxypropionic acid and β-2,4-dichlorophenoxypropionic acid (cf. Table 10), the appropriate ED 50s were 2088 ppm and 500 ppm. As these two compounds have the same molecular weight and the same ring substitution pattern, the difference must lie in the different configuration of the chemical, the presence of the side chain substitution decreasing toxicity.

(c) Variation in ring substitution in the phenoxycarboxylic acids.

The following section deals with the possible effects on fungitoxicity of the number, position and type of substituted groups attached to the benzene ring of the phenoxycarboxylic acid compounds available for testing.

In Table 16, discussed in the preceding paragraphs, results are given for four α-phenoxypropionic acids differing in nuclear substitution. Where the ring was unsubstituted (α-phenoxypropionic acid) the chemical had no toxic effect on Penicillium notatum at any of the concentrations tested. The addition of substituted groups in the nucleus to give α-MCPP, α-2,4-DP and α-2,4,5-TP increased toxicity progressively, the side chain structure of these compounds being identical. In α-MCPP (4-chloro-2-methyl- substitution) the highest concentration tested (1000 ppm) gave 25% inhibition of growth with an ED 50 of 5700 ppm. Substitution of two chlorine ions rather than one methyl group and one chlorine, to give α-2,4-dichlorophenoxypropionic acid resulted in a further increase in toxicity (ED 50 of 2088 ppm). Three chlorine substituents (2,4,5-trichloro-substitution) were even more inhibitory (ED 50 of 936 ppm). This increase in activity was presumably due to the increase in chlorine content or in some effect of molecular weight of the compounds investigated.

Results of several experiments on Penicillium notatum are condensed in Table 17 giving a comparison between the effect of absence of substitution and the addition of one, two or three substitutuents to the nucleus of the phenoxycarboxylic acids.

Considering first compounds with an acetic acid side chain, the unsubstituted phenoxyacetic acid had no toxicity whatsoever toward P. notatum as discussed previously. Disubstitution in the ring of the phenoxyacetic acid in the 2- and 4- positions, whether of chlorine or methyl groups to give 2,4-D or MCPA, had some slight effect on the toxicity of the acetic acid forms towards P. notatum (ED 50s of 28560 ppm and 17030 ppm respectively). However, inclusion of either form, chlorine or methyl group, in the 6-position to give 2,6- (di-ortho) substitution, did produce a greater inhibitory effect (ED 50s of 8280 ppm and 6400 ppm respectively). Three substitutions in the ring of the phenoxyacetic acid resulted in a further increase in toxicity depending on their position. Again when both ortho positions were filled (2,4,6-trichlorophenoxyacetic acid) toxicity was considerably increased (ED 50 of 900 ppm) but 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) with an ED 50 of 2450 ppm was similar in toxicity to 2,4-D and MCPA.

Turning to the γ-phenoxybutyric series in Table 17, the unsubstituted γ-phenoxybutyric acid was toxic at higher concentrations (ED 50 of 1436 ppm) reducing the growth of Penicillium notatum by 42 % and 32 % at 1000 ppm and 500 ppm respectively.

Of the two γ-phenoxybutyric acids with one substitution, the addition of a methyl group in the 2-position, γ-2-methylphenoxybutyric acid, did not increase its toxicity (ED 50 of 1253 ppm) to any great extent over that of the original γ-phenoxybutyric acid,

TABLE 17

THE INFLUENCE OF RING SUBSTITUTION ON FUNGITOXICITY OF THE w-SUBSTITUTED PHENOXY CARBOXYLIC ACIDS IN PENICILLIUM NOTATUM

Results expressed as mean colony diameter in mm.

Number of ring substituents	Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
	ACETIC ACIDS							
0	phenoxyacetic acid	23.6 ± 0.53	21.8 ± 0.53	20.5 ± 0.39	21.4 ± 0.56	20.3 ± 0.51	21.0 ± 0.26	non-toxic
2	2, 4-dichlorophenoxyacetic acid	19.5 ± 0.76	19.8 ± 0.30	19.6 ± 0.22	20.8 ± 0.34	19.5 ± 0.45	21.3 ± 0.33	28560 (R)
	2, 6-dichlorophenoxyacetic acid	22.1 ± 0.67	(no test)	25.0 ± 0.12	25.7 ± 0.15*	(no test)	27.1 ± 0.16	8280
	2, 6-dimethylphenoxyacetic acid	21.6 ± 0.05	(no test)	24.2 ± 0.22	25.6 ± 0.17*	(no test)	27.1 ± 0.16	6400
	4-chloro-2-methylphenoxyacetic acid	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.3 ± 0.58	17030 (R)
3	2, 4, 5-trichlorophenoxyacetic acid	23.3 ± 0.25	23.1 ± 0.08	23.4 ± 0.22	24.5 ± 0.63	24.6 ± 0.22	26.3 ± 0.08	24650 (R)
	2, 4, 6-trichlorophenoxyacetic acid	16.7 ± 0.37	(no test)	21.8 ± 0.18	24.3 ± 0.17 *	(no test)	27.1 ± 0.16	900
	BUTYRIC ACIDS							
0	ɣ - phenoxybutyric acid	10.3 ± 0.16	12.1 ± 0.23	14.7 ± 0.15	17.3 ± 0.29	19.3 ± 0.42	17.8 ± 0.98	1436
1	ǧ - 4-chlorophenoxybutyric acid	8.3 ± 0.56	(no test)	17.5 ± 0.20	24.3 ± 0.35*	(no test)	27.1 ± 0.16	365
	ɣ - 2-methylphenoxybutyric acid	10.7 ± 0.10	11.1 ± 0.34	13.4 ± 0.38	11.7 ± 0.27	14.4 ± 0.67	17.7 ± 0.15	1253
2	ɣ - 2, 4-dichlorophenoxy butyric acid	1.9 ± 0.19	3.3 ± 0.30	12.0 ± 0.36	14.0 ± 0.73	22.8 ± 0.14	25.5 ± 0.64	80
	ɣ - 4-chloro-2-methylphenoxy butyric acid	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
3	ɣ - 2, 4, 5-trichlorophenoxybutyric acid	(no test)	2.2 ± 0.10	8.2 ± 0.09	10.5 ± 0.32	19.4 ± 0.22	29.7 ± 0.17	25
	ǧ - 6-nitro-2, 4-dichlorophenoxybutyric acid	0.0	0.0	8.3 ± 0.97	12.6 ± 0.20	13.4 ± 0.64	16.2 ± 0.14	130
	ɣ - 2-methyl-4, 6-dichlorophenoxy butyric acid	1.7 ± 0.51	(no test)	10.7 ± 0.19	17.6 ± 0.39*	(no test)	27.1 ± 0.16	77

* 10 ppm

whereas the effect of the chlorine substitution in the 4- position rather than the 2- position (or to the replacement of chlorine by a methyl group) resulting in γ-4-chlorophenoxybutyric acid was to increase toxicity (ED 50 of 365 ppm).

The two disubstituted γ-butyric acids, γ-2,4-dichlorophenoxybutyric and γ-4-chloro-2-methylphenoxybutyric acids were, as discussed previously, highly toxic to Penicillium notatum resulting in ED 50 values of 52 ppm and 80 ppm respectively. Of the trisubstituted γ-phenoxybutyric acids, γ-2-methyl-4,6-dichlorophenoxybutyric acid was only slightly less toxic (ED 50 of 77 ppm) than its γ-2,4,5-trichloro- analogue (ED 50 of 25 ppm). Another trisubstituted form of γ-2,4-DB with an additional NO_3 group in the 6- position was also toxic to P. notatum (ED 50 of 130 ppm).

A shorter selection of these compounds was tested on Aspergillus niger and the results are presented in Table 18. This mould was much more sensitive than Penicillium notatum to the substituted phenoxyacetic acid herbicides, 2,4-D and MCPA (cf. Table 4). Comparing 2,4-D with 2,6-D, change of the second chlorine from the 4- position to the 6- position increased the ED 50 value from 1210 ppm to 6560 ppm. However, substitution of methyl groups for chlorine to give 2,6-dimethylphenoxyacetic acid removed toxicity altogether at the concentrations tested. Both these di-ortho compounds in fact considerably stimulated growth at 10 and 100 ppm.

As with Penicillium notatum, however, addition of a third chlorine to give 2,4,6-trichlorophenoxyacetic acid, increased toxicity (ED 50 of 818 ppm) to some degree over that of 2,4-D.

The reaction of Aspergillus niger to the γ-phenoxybutyric acids tested was similar to that of Penicillium notatum. The two mono-substituted acids, γ-4-chlorophenoxybutyric acid and

TABLE 18

THE INFLUENCE OF RING SUBSTITUTION OF FUNGITOXICITY OF THE w-SUBSTITUTED PHENOXYCARBOXYLIC ACIDS TO ASPERGILLUS NIGER

Results expressed as mean colony diameter in mm.

Number of ring substituents	Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
	ACETIC ACIDS							
2	2, 4-dichlorophenoxyacetic acid	24.0 ± 0.72	26.9 ± 0.23	38.7 ± 0.72	(no test)	(no test)	41.6 ± 0.49	1210
	2, 6-dichlorophenoxyacetic acid	27.0 ± 0.49	(no test)	43.9 ± 0.40	42.2 ± 0.66*	(no test)	33.9 ± 0.86	6560
	2, 6-dimethylphenoxyacetic acid	38.3 ± 0.98	(no test)	46.8 ± 0.26	43.8 ± 0.34*	(no test)	33.9 ± 0.86	non-toxic
	4-chloro-2-methylphenoxyacetic acid	11.2 ± 0.89	17.8 ± 0.58	22.9 ± 0.50	38.5 ± 0.73	45.2 ± 0.85	42.2 ± 0.56	230
3	2, 4, 6-trichlorophenoxyacetic acid	16.1 ± 0.62	(no test)	26.3 ± 0.49	34.0 ± 0.63	(no test)	33.9 ± 0.86	818
	BUTYRIC ACIDS							
1	ɣ- 4-chlorophenoxy butyric acid	1.5 ± 0.25	(no test)	20.2 ± 0.15	30.7 ± 0.29*	(no test)	33.9 ± 0.86	237
	ɣ- 2-methylphenoxybutyric acid	5.7 ± 0.37	12.0 ± 0.52	30.4 ± 0.38	33.7 ± 0.46	39.5 ± 0.42	39.8 ± 0.76	290
2	ɣ- 2, 4-dichlorophenoxy butyric acid	1.3 ± 0.09	2.0 ± 0.31	26.2 ± 0.75	35.5 ± 0.67	(no test)	41.6 ± 0.49	190
	ɣ- 4-chloro-2-methylphenoxybutyric acid	0.0	4.9 ± 0.19	13.7 ± 0.28	22.8 ± 0.59	37.0 ± 0.64	40.4 ± 0.17	64
3	ɣ- 2-methyl-4, 6-dichlorophenoxybutyric acid	0.0	(no test)	16.7 ± 0.19	25.3 ± 0.12*	(no test)	33.9 ± 0.86	94

* 10 ppm

γ-2-methylphenoxybutyric acid were somewhat less toxic (ED 50s of 237 ppm and 290 ppm respectively) than the disubstituted herbicides γ-2,4-DB and γ-MCPB. A compound with a second chlorine added to the ring of γ-MCPB, to give γ-2-methyl-4,6-dimethylphenoxybutyric acid gave a similar inhibition of growth in A. niger to that by γ-MCPB.

Therefore ring substitution was found to influence the fungitoxicity of the w-substituted phenoxycarboxylic acids, but whether the type or position of substituents increased or decreased activity did not appear to be fully consistent from one organism to another. Increase in the number of substitutions, probably combined with the concomitent increase in molecular weight, did enhance fungitoxicity.

B. MANOMETRIC INVESTIGATIONS.

The experimental work described in this section concerns manometric studies carried out in order to determine the inhibitory action of high levels of MCPA and Y-MCPB on the respiratory system of the fungus, Penicillium notatum.

The investigations followed techniques reported by Neiderpruem and Hackett (1961), Wessels (1959), Hilton and Smith (1959) and Slater (1949; 1949a) and details are given in full in Part II, B.6. As the experimental treatments were made in duplicate only, the data have not been treated further statistically.

1. The effect of MCPA and Y-MCPB on endogenous respiration of intact mycelium.

Mycelial discs from five day old agar plate cultures of Penicillium notatum were found to have a high endogenous respiration rate with a QO_2 (μl O_2/mg dry weight/hr) usually in the range 1.48 to 1.96. The nature of the endogenous enzyme system was examined with the aid of specific respiratory poisons, the results being illustrated in Figures 2 to 4. Sodium azide, which blocks the enzyme cytochrome oxidase, was inhibitory to the tissue preparation reducing respiration by 95 %. Fluoroacetic acid had no effect, although it is a specific inhibitor of the tricarboxylic acid cycle in mammalian systems. Malonic acid, an inhibitor of succinic dehydrogenase, was similarly ineffective against respiration of intact mycelium although, as reported below, it was inhibitory to succinate oxidation by a cell-free extract of P. notatum. These reactions were indicative of the presence of an active respiratory enzyme system in the mycelial tissue.

The effect of the w-substituted phenoxyacetic and phenoxy-butyric acid herbicides, MCPA and Y-MCPB, on the endogenous respiration

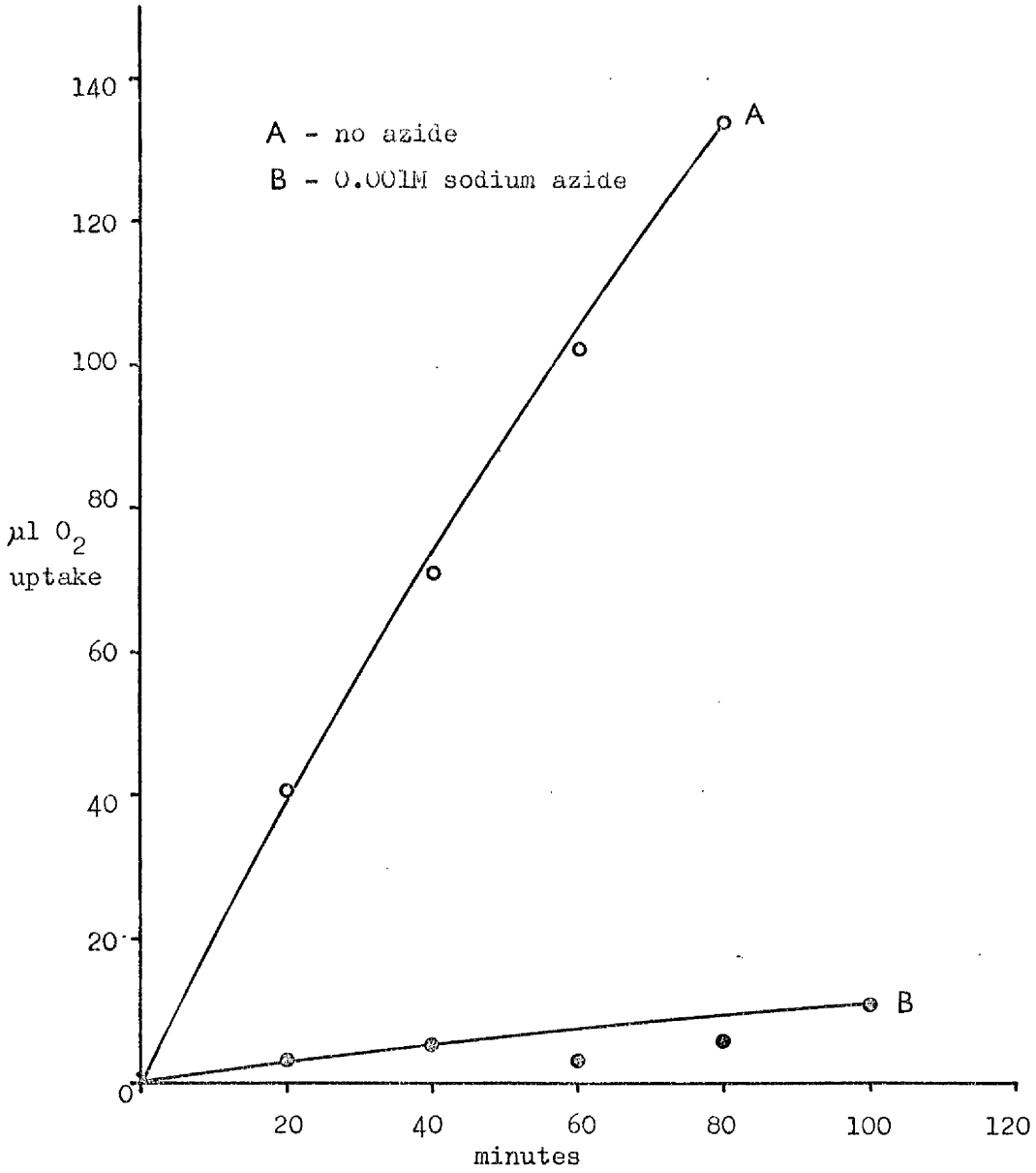


FIGURE 2

Effect of sodium azide on the endogenous respiration of Penicillium notatum mycelium.

Each flask contained 50 mg mycelial tissue in 0.01M phosphate buffer and 0.001M sodium azide was added to treatment B.

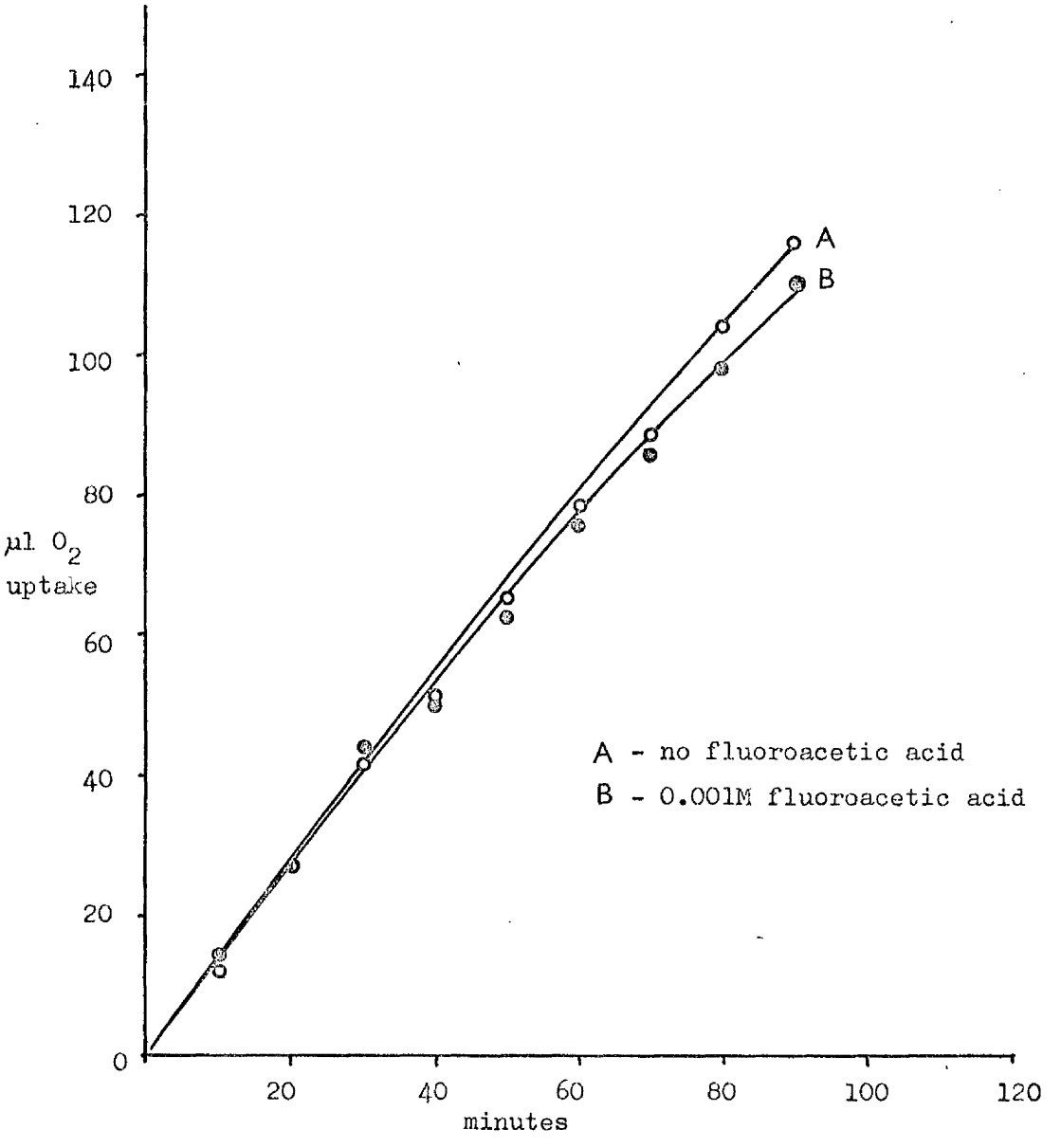


FIGURE 3

Effect of fluoroacetic acid on endogenous respiration of Penicillium notatum mycelium.

Each flask contained 50 mg mycelial tissue in 0.01M phosphate buffer and 0.001M fluoroacetic acid was added to treatment B.

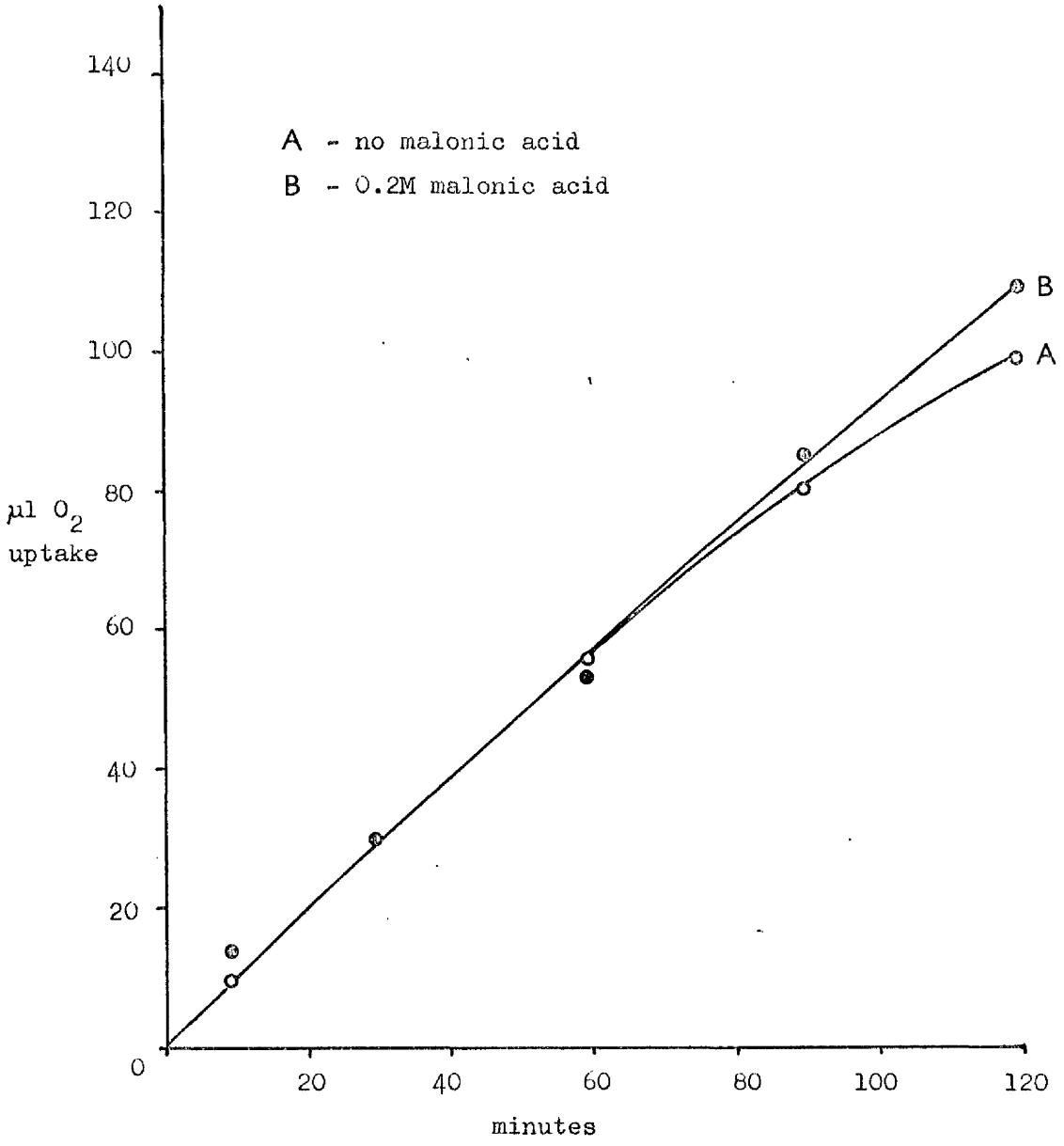


FIGURE 4

Effect of malonic acid on endogenous respiration of Penicillium notatum mycelium.

Each flask contained 50 mg mycelium in 0.01M phosphate buffer and 0.2M malonic acid was added to treatment B.

of mycelial tissue of P. notatum was then examined over a concentration range from 1 ppm to 5000 ppm. On incubation with these chemicals there was a progressive decrease in respiratory activity of mycelial discs with increase in concentration of the inhibitor as shown in Figures 5 and 6. The results, derived from two separate experiments, are summarised in Table 19.

The extent of inhibition was greater with γ-MCPB than with MCPA at concentrations between 50 ppm and 1500 ppm. Below 50 ppm neither herbicide had any effect on the oxygen uptake rate. Dose/response lines plotted from the results in Table 19 give ED 50 values of 5520 ppm and 300 ppm respectively for MCPA and γ-MCPB. Reference to Table 1 shows the corresponding ED 50 values for inhibition of P. notatum in petri dish culture to be 17030 ppm and 52 ppm respectively.

At the higher concentrations of γ-MCPB (1000 ppm and 1500 ppm) there was marked inhibition of oxygen uptake after only sixty minutes incubation. Further experiment, Table 20, showed that, at these concentrations, prolonged periods of incubation with the inhibitor, i.e. for more than 120 minutes prior to measurement of oxygen uptake, resulted in a complete inhibition of respiration.

2. The effect of MCPA and γ-MCPB on mitochondrial respiration of Penicillium notatum.

Further work was carried out on a particulate fraction isolated from homogenates of hand ground mycelium of P. notatum prepared as described in Part II, B.6 (b)

The succinic oxidase system, which has been used for the screening of many types of inhibitors, is the mitochondrial enzyme system catalysing the aerobic oxidation of succinate to molecular oxygen via the cytochromes in the tricarboxylic acid cycle interactions. It offers special advantages to the investigator as, unlike most

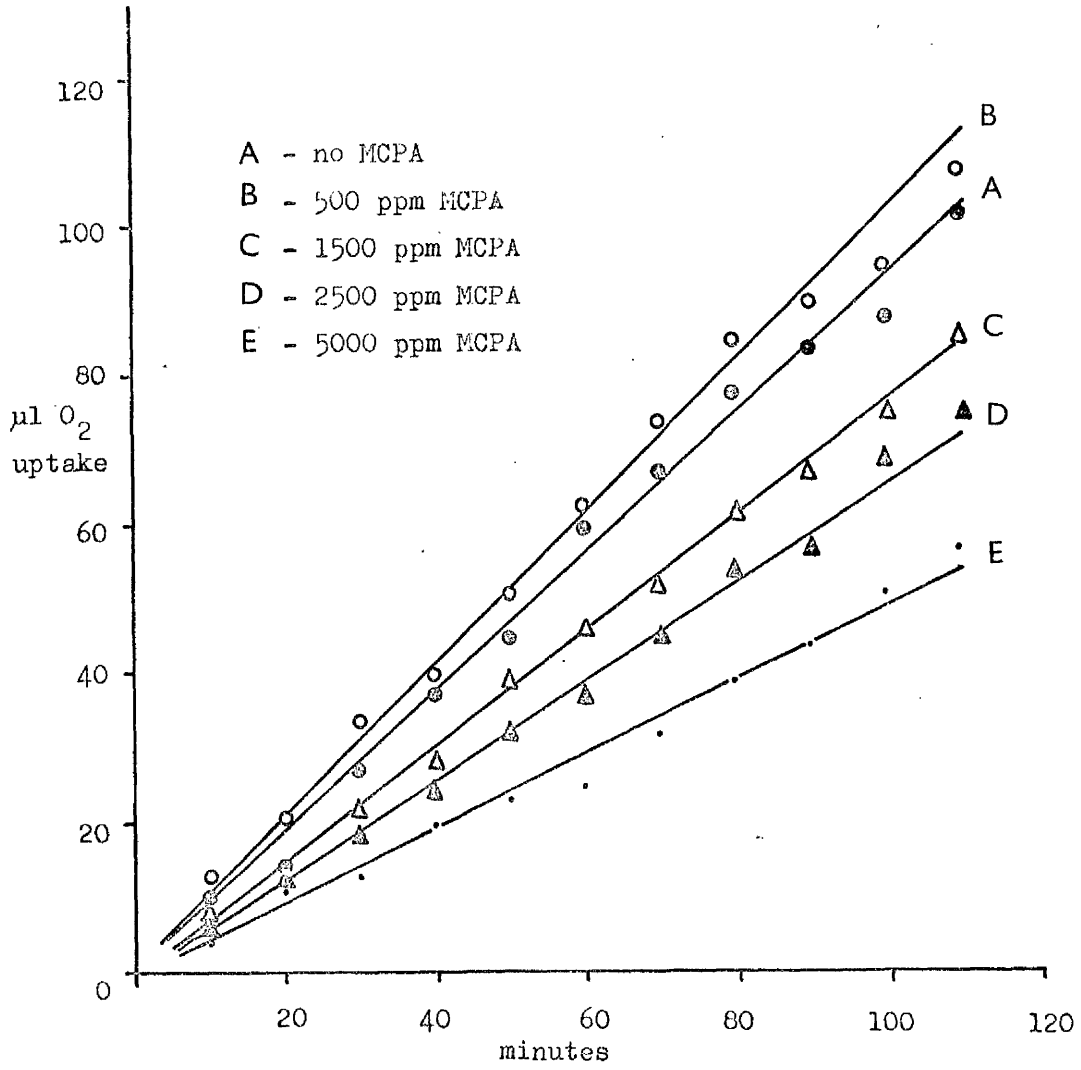


FIGURE 5

Effect of 4-chloro-2-methylphenoxyacetic acid (MCPA) on endogenous respiration of intact mycelium of Penicillium notatum.

Each flask contained 50 mg mycelial tissue in 0.01M phosphate buffer, and MCPA over a range 0 to 5000 ppm.

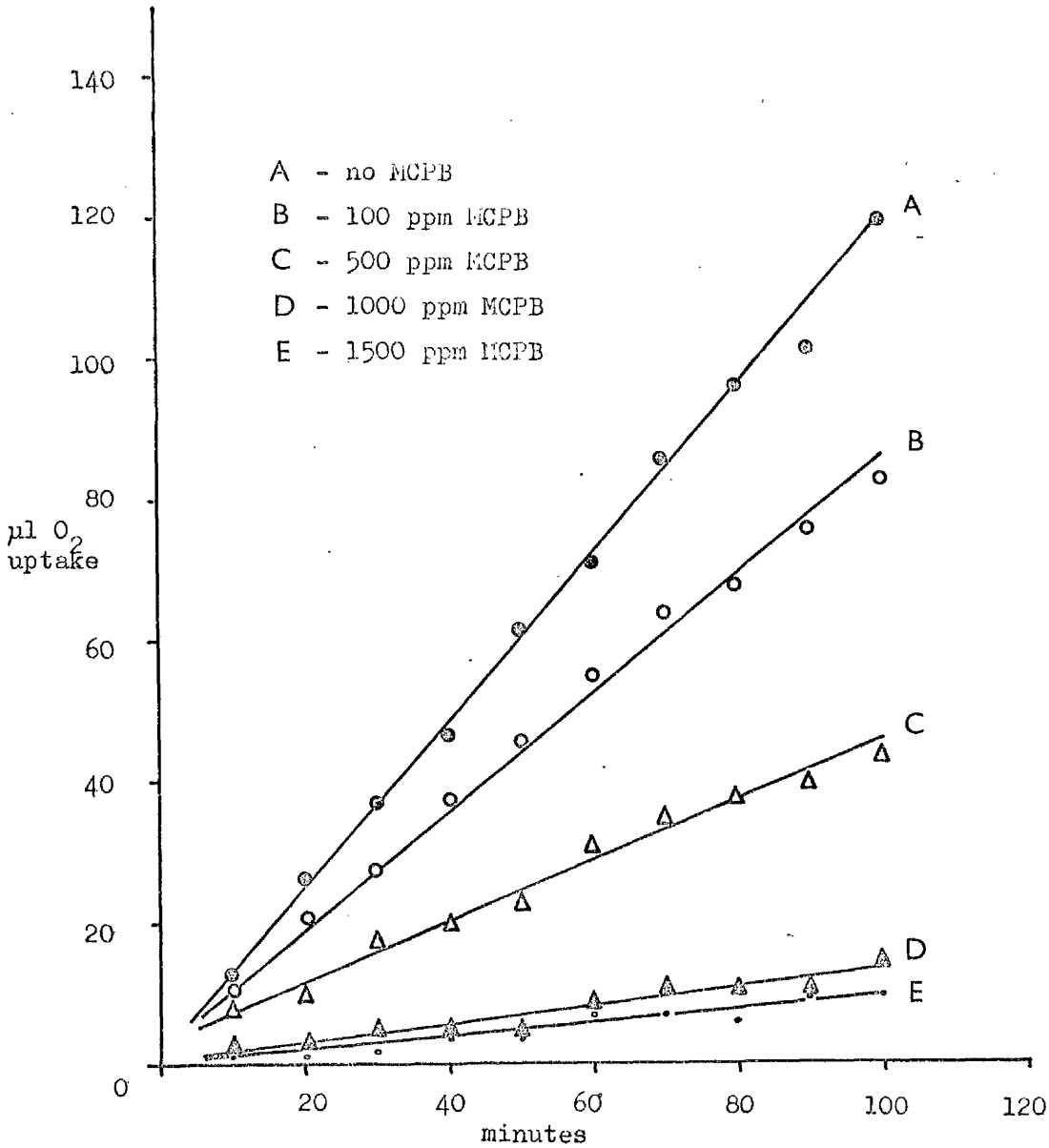


FIGURE 6

Effect of γ- 4-chloro-2-methylphenoxybutyric acid (MCPB) on endogenous respiration of intact mycelium of Penicillium notatum.

Each flask contained 50 mg mycelial tissue in 0.01M phosphate buffer, and MCPB over a range 0 to 1500 ppm.

TABLE 19

The effect of w-substituted phenoxycarboxylic acid herbicides (MCPA and Y-MCPB) on endogenous respiration of Penicillium notatum.

Results expressed as µl O_2/h/50 mg tissue

Concentration (ppm)	MCPA			Y-MCPB		
	Expt A	Expt B	% control	Expt A	Expt B	% control
5000	30		54			
2500	40		72			
1500	47		84	7		9
1000	44		79	9		12
500	61		109	26		37
100				50	73	71,70
50		79	103		87	84
10		73	95		104	100
5		72	94		105	100
1		75	98		105	100
Control no addition	56	77		71	104	
ED 50			5520			302

TABLE 20

The effect of incubation time on inhibition by Y-MCPB of mycelial respiration of Penicillium notatum.

Incubation period (minutes)	Percentage inhibition by Y-MCPB (1500 ppm)
0	46
30	14
60	10
120	0

intermediary metabolites, succinate, activated by its dehydrogenase, reacts directly with the cytochrome system without the necessity for intermediary carriers.

The activity of the complete succinic oxidase system was measured by determining the rate of oxygen uptake in the presence of excess succinate. Mitochondrial-type fractions from P. notatum were found to have considerable succinic oxidase activity which was sensitive to malonic acid as illustrated in Figure 7. Oxygen uptake rates by such cell-free extracts were from 150 to 250 μl O_2/hr using 0.02M potassium succinate as substrate. After examining the effects of various cofactors on the uptake of oxygen by these preparations using the references cited above for guidance, the final reaction mixture contained, per flask, 0.001M adenosine triphosphate (ATP), 0.1mM cytochrome c and 0.01M magnesium sulphate.

For inhibition studies with MCPA and γ-MCPB, cell-free extracts were incubated with the inhibitor under investigation at 30°C for thirty minutes prior to measurement of enzyme activity. The oxidation of succinate was inhibited by both compounds although inhibition by γ-MCPB was much greater than that of equivalent concentrations of MCPA. The results, given in Table 21, represent three experiments. Figure 8 illustrates the response of experiment A, Table 21.

The presence of other tricarboxylic acid cycle enzymes localised in the mitochondria were demonstrated in the cell-free extract when various intermediates, i.e. citrate, fumarate and malate, were supplied as substrates and oxidation rates compared with that of succinate. The results of three experiments are illustrated in Figure 9. Additional cofactors were required for maximum activity of citrate, fumarate and malate, namely coenzyme A (1.3μM)

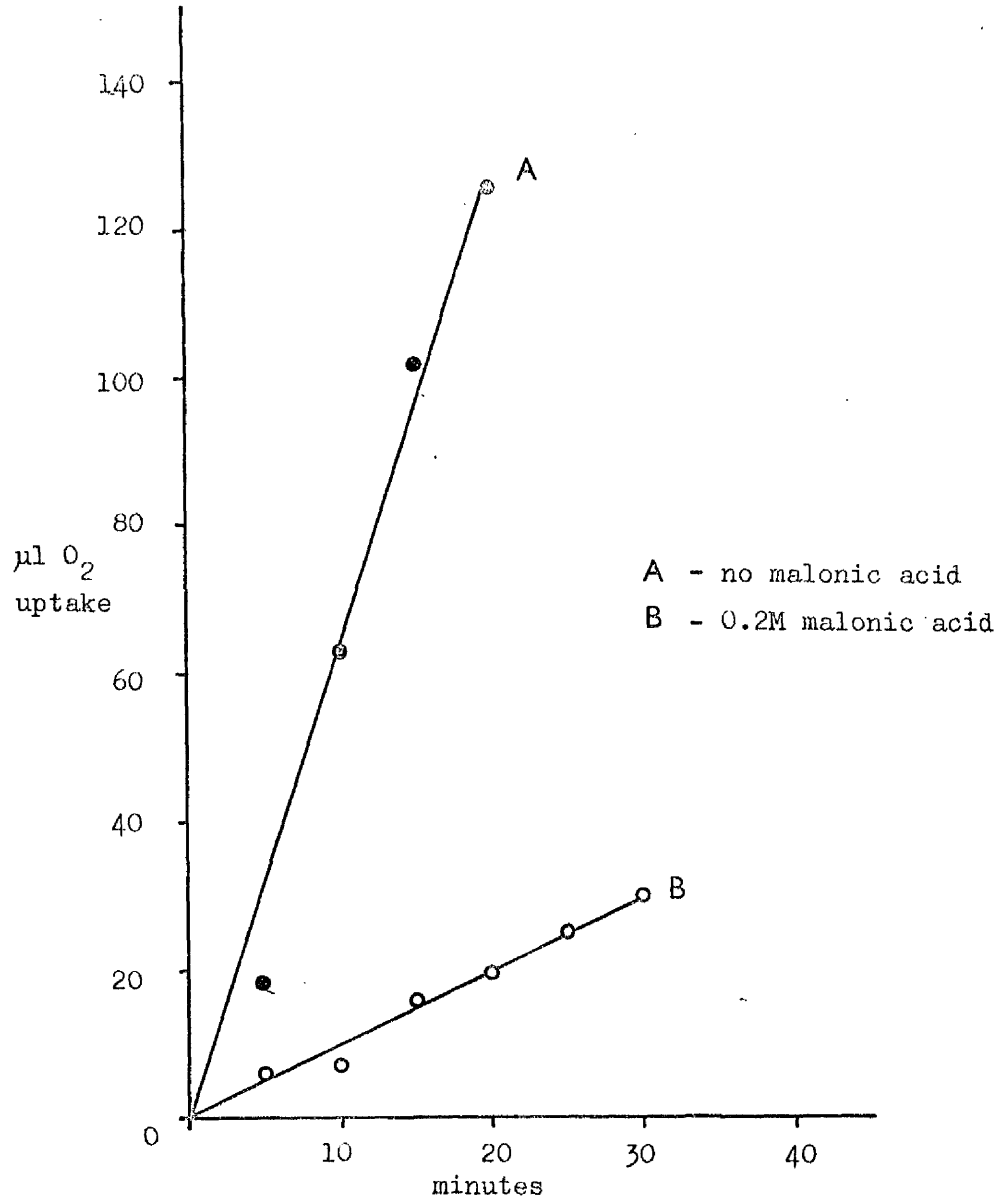


FIGURE 7

Effect of malonic acid on succinate oxidation by a cell-free extract of Penicillium notatum.

Each flask contained 0.4 ml cell-free extract in 0.08M phosphate/ 0.6M sucrose buffer at pH 7.3, 0.025M potassium succinate, 0.1mM cytochrome c, 0.001M ATP, 0.015M $MgSO_4$; 0.2M malonic acid was added to treatment B.

TABLE 21

The effect of the w-substituted phenoxycarboxylic acid herbicides (MCPA and Y-MCPB) on succinate oxidation by a cell free extract of Penicillium notatum.

Results expressed as µl O_2/h/0.4 ml extract*

Concentration (ppm)	MCPA			Y-MCPB			
	Expt A	Expt B	% control (mean)	Expt A	Expt B	Expt C	% control (mean)
5000	4		5				
1500	58	206	65	4	11	24	7
1000	65	248	76	46	29	170	44
100					97		84
10					111		96
1					111		96
Control no addition	90	312		90	116	312	
ED 50			2134				640

* as prepared, 8 g mycelium yielded 0.4 ml cell free extract.

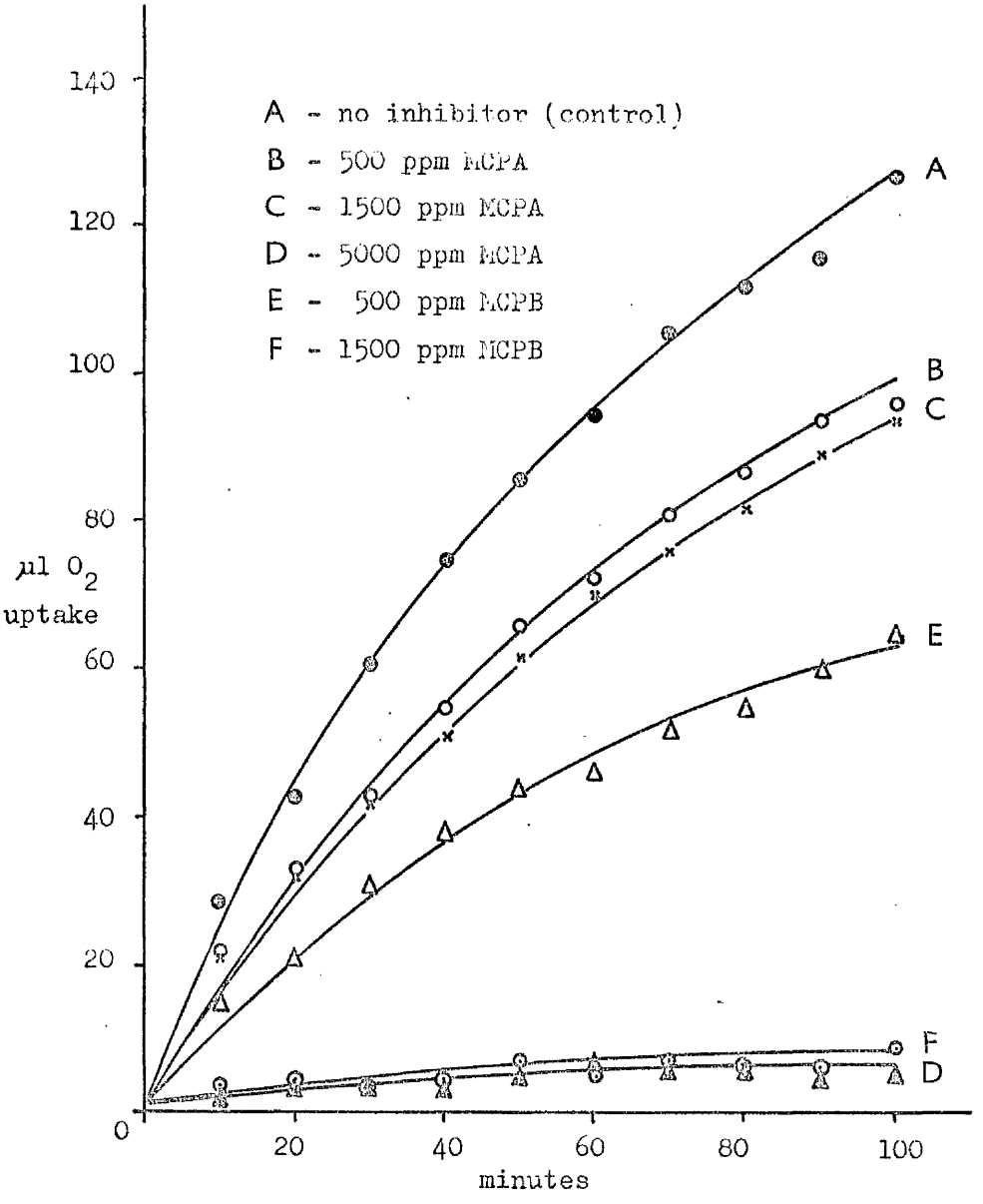


FIGURE 8

Effect of MCPA and MCPB on succinate oxidation by a cell-free extract of Penicillium notatum.

Each flask contained 0.4 ml cell-free extract in 0.08M phosphate/ 0.6M sucrose buffer at pH 7.3, 0.025M potassium succinate, 0.1mM cytochrome c, 0.001M ATP, 0.015M $MgSO_4$; MCPA and MCPB were added to treatments B to F at concentrations indicated above.

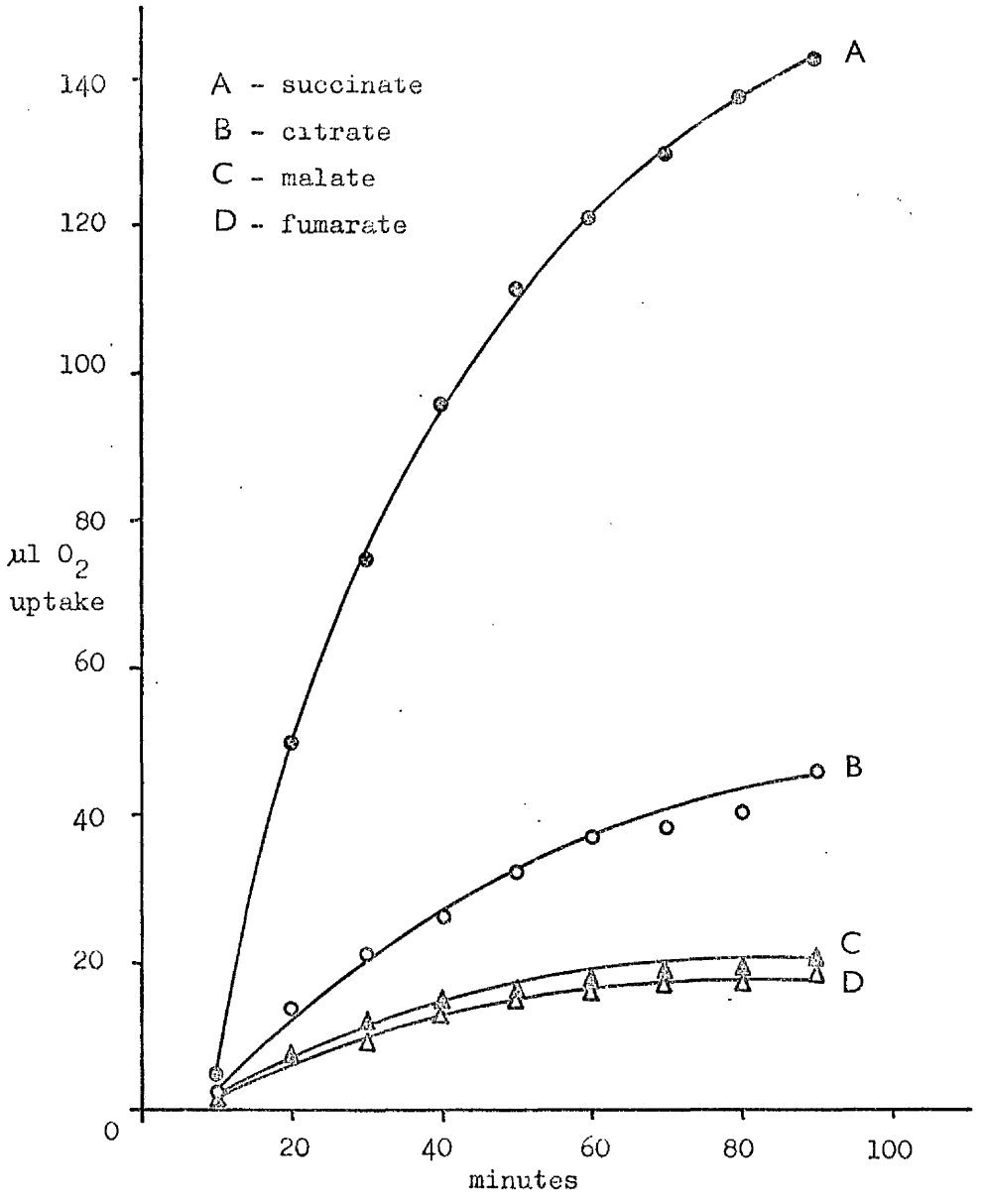


FIGURE 9

Oxidation of tricarboxylic acid cycle substrates compared with succinate by a cell-free extract of of Penicillium notatum.

Each flask contained 0.4 ml cell-free extract in 0.08M phosphate/ 0.6M sucrose buffer at pH 7.3, 0.025M substrate, 0.1mM cytochrome c, 0.001M ATP, 0.015M $MgSO_4$; treatments other than succinate required additional cofactors, i.e. 1.3µM coenzyme A, 13.0µM cocarboxylase, 13.0 µM NAD.

cocarboxylase (diphosphothiamine) (13.0 μM) and nicotinamide adenine nucleotide (NAD) (13.0 μM).

The oxidative capacities of these other tricarboxylic acid enzymes were, however, much lower than that of succinic oxidase which was used as a control in the experiments. α-ketoglutarate was also tested but no discernable oxidation was obtained. Inhibition by Y-MCPB at a level of 1500 ppm in the reaction mixture is summarised below:

Substrate	Percentage inhibition of oxygen uptake
succinate	94
citrate	95
fumarate	88
malate	83

The effect of Y-MCPB on the activity of the cytochrome a - cytochrome a_3 portion of the succinic oxidase system, i.e. cytochrome oxidase, was measured by determining the rate of oxidation in the presence of excess cytochrome c and a reducing agent, ascorbic acid, after the indirect method of Slater (1949a). It is assumed by this technique that cytochrome c is reduced by the ascorbic acid as soon as it is oxidised by the cytochrome oxidase oxygen and so the rate of oxygen uptake is a measure of the activity of the enzyme. It is likewise assumed that, in the study of the action of inhibitors, any decrease in the rate of oxygen uptake is due to the inhibition of the enzyme.

The results are illustrated in Figure 10. Y-MCPB at 1500 ppm reduced the control succinic oxidase activity by 78% but had no inhibitory effect on cytochrome oxidase activity. The presence of Y-MCPB in fact stimulated oxygen uptake to some extent.

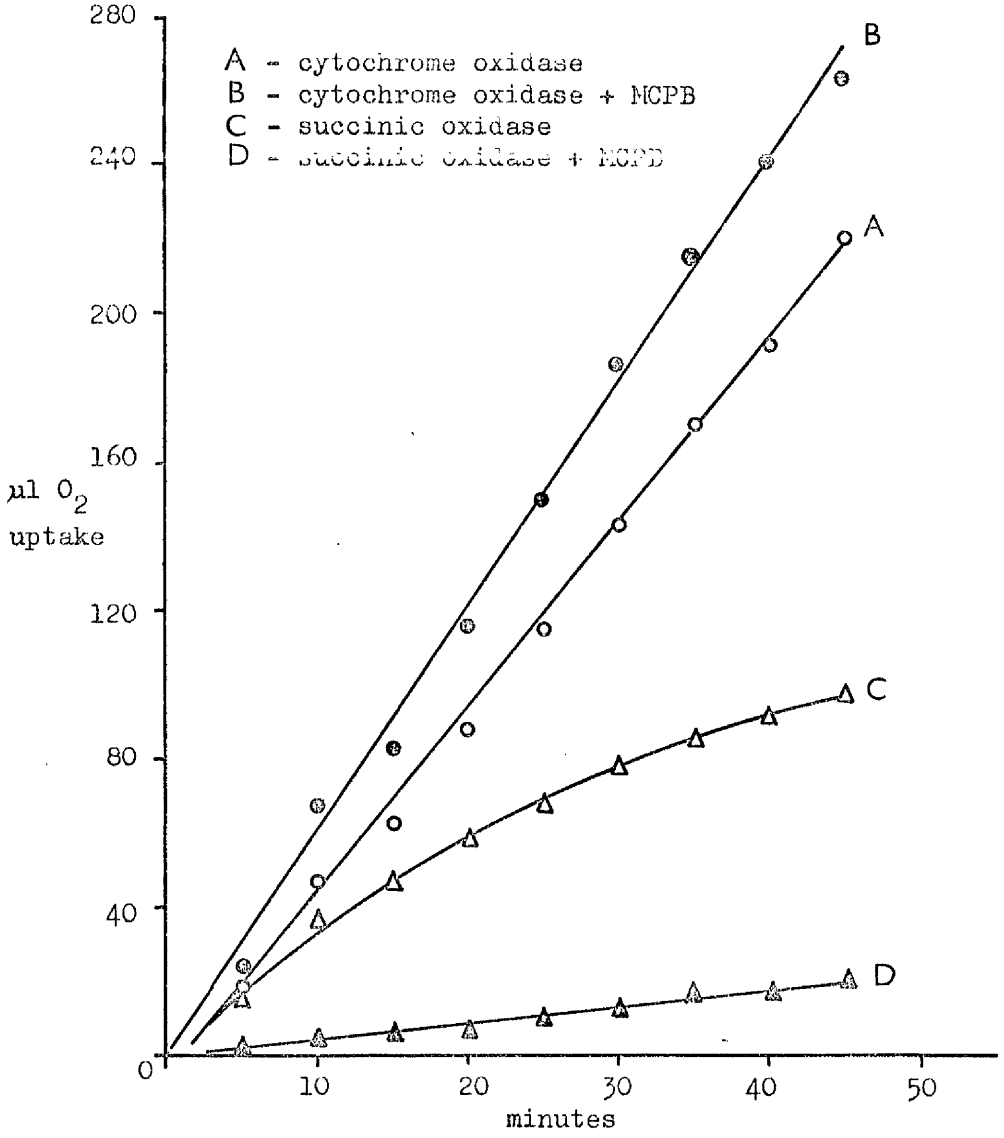


FIGURE 10

Effect of MCPB on succinic oxidase and cytochrome oxidase activity of a cell-free extract of Penicillium notatum.

Each flask contained -
(A,B) Cytochrome oxidase system - 0.2 ml cell-free extract in 0.08M phosphate/0.6M sucrose buffer at pH 7.3, 0.025M ascorbic acid, 0.2mM cytochrome c, 0.001M ATP, 0.015M $MgSO_4$.
(C,D) Succinic oxidase system - 0.4 ml cell-free extract in buffer as above, 0.025M potassium succinate, cofactors as above.
In treatments B and D MCPB was added to give 1500 ppm.

Therefore the inhibitory effect of high concentrations of γ-MCPB on the respiration of Penicillium notatum was assumed to occur at some point on the electron transport pathway between the site of succinate dehydrogenation and that of cytochrome oxidation.

IV. DISCUSSION

IV DISCUSSION

The investigation described in this report was originally conceived as a study of the side effects that might accompany the use in agricultural practice of the w-substituted phenoxycarboxylic acid group of herbicides and their consequent presence in the soil environment. Investigations reviewed in the literature (Fletcher, 1960; Bollen, 1961) have concluded that the application, either incidentally or deliberately, to the soil of w-substituted phenoxyacetic acid herbicides, i.e. MCPA, 2,4-D and 2,4,5-T, has no lasting effect detrimental to the micro-organisms in the soil. The present report submits further data on this subject, the α-propionic and γ-butyric acid forms of these herbicides being considered in addition to the more common acetic acids.

w-Substituted phenoxycarboxylic acid herbicides with three different forms of ring substitution, namely, 4-chloro-2-methyl-, 2,4-dichloro- and 2,4,5-trichloro-, were found to be non-toxic to micro-organisms in culture at low levels of concentration (5 ppm). These results therefore confirm those already published, concluding that the use of this group of herbicides at normal field rates is unlikely to have any adverse effect on the soil microfungi. Caution should be exercised, however, in extrapolating experimental laboratory results to the ecological situation in the field as it is difficult to relate agricultural rates of application of herbicides to dosage rates in vitro. The amount of herbicide reaching the soil, there to be diluted in the soil water is virtually impossible to determine with accuracy. However, calculations of concentrations of herbicide present in the soil suggest that less than 10 ppm are to be found in the top six inches, assuming uniform distribution (Fletcher, 1960; Bollen, 1961) following normal weedkilling treatments.

On several occasions during the experiments reported in this thesis, significant stimulation of fungal growth was observed. Although not followed up during the present project, these instances of growth at a rate exceeding 2 % of that of the control are brought together in Table 22 for the purposes of discussion.

In some of the experimental results given, e.g. Ascochyta pisi and Microsporum canis with MCPA, Aspergillus niger with 2,6-dichlorophenoxyacetic acid and 2,6-dimethylphenoxyacetic acid, and Penicillium notatum with phenoxyacetic acid and α-phenoxypropionic acid, significant increases in growth were observed throughout the concentration range tested, even at the 1000 ppm level. This may reflect utilisation of the chemicals in some way as a carbon source.

In most cases stimulation in growth rate appeared only at the lowest concentration tested, 5 ppm, or, if exceedingly pronounced, decreased as the concentration level rose. Most of these increases were significant by a t-test of percentage increase in colony diameter compared with the control. Later work (Smith & Shennan, 1966) showed that growth of Aspergillus niger in shake flasks, although inhibited by concentrations of MCPA and γ-MCPB above 100 ppm or 10 ppm respectively, was stimulated 10 % to 15 % by levels below 1 ppm of these same chemicals.

Such promotion of growth in fungi by certain of these w-substituted phenoxycarboxylic acids is assumed to be caused by a different biological mechanism than that producing inhibition, in many cases caused by higher concentrations of the same compound, and this stimulation may be allied, therefore, to the plant growth regulating activity of this group of chemicals.

As has been discussed in the introduction to this report, the

TABLE 22 . Stimulation of fungal growth by w-substituted phenoxycarboxylic acids

Results expressed as percentage increase in colony diameter compared with control (no addition)

Chemical	Organism	Concentration (ppm) 5	50	100	500	1000
4-chloro-2-methylphenoxyacetic acid	Aspergillus niger	7.2*	-	-	-	-
	Ascochyta pisi	7.6	13.1*	12.9*	14.0*	17.4**
	Microsporum canis	7.4*	7.9**	12.4***	8.7**	12.4***
	Pyrenophora avenae	41.8**	15.5**	-	-	-
2,6-dichlorophenoxyacetic acid	Aspergillus niger	no test	24.5***!	29.5***	-	-
2,6-dimethylphenoxyacetic acid	Aspergillus niger	no test	29.5***!	38.1***	no test	13.0**
α-2,4-dichlorophenoxypropionic acid	Aspergillus niger	no test	17.1***	-	-	-
	Penicillium notatum	36.7***	10.0*	11.0**	-	-
α-4-chloro-2-methylphenoxypropionic acid	Penicillium notatum	6.5*	4.2	-	-	-
β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid	Gibberella zeae	22.4***	14.2**	6.3	-	-
α-4-chloro-2-methylphenoxybutyric acid	Aspergillus niger	19.5***	-	-	-	-
α-methyl-γ-4-chloro-2-methylphenoxybutyric acid	Gibberella zeae	10.2**	-	-	-	-
β-methyl-γ-4-chloro-2-methylphenoxybutyric acid	Mucor hiemalis	11.2***	-	-	-	-
α-2,4-dichlorophenoxyisobutyric acid	Penicillium notatum	8.5**	2.0	-	-	-
phenoxyacetic acid	Penicillium notatum	-	2.0	-	3.7	12.5**
α-phenoxypropionic acid	Penicillium notatum	-	-	10.5**	-	12.3*
γ-phenoxybutyric acid	Penicillium notatum	8.1	-	-	-	-

- no increa

* t-test signific

* P =<0.05
** P =<0.01
*** P =<0.00

! 10 ppm

herbicidal effect of the w-substituted phenoxycarboxylic acid herbicides is thought to result from some form of auxin activity in the tissues of the plant and therefore these compounds are herbicidally effective at very low concentrations. In a review of auxins and fungi, Gruen (1959) concluded that, although auxin production is widespread among fungi belonging to different taxonomic groups, more than thirty years of work have furnished no evidence for a growth promoting role of auxins, particularly indoleacetic acid, in fungi. Assuming that fungi do require regulatory substances, biosynthesis must occur on any substrate permitting growth. While exogenous auxins can produce cell enlargement in fungi, these effects are not reported to be related to growth, and can be quite variable in some species depending on culture conditions.

Although low concentrations of the w-substituted phenoxycarboxylic acids were nontoxic to the organisms tested in this report, higher levels, above 100 ppm, were found to be inhibitory. The γ-butyric acid forms of these herbicides were among the most toxic of the compounds tested, but even MCPA and 2,4-D could be toxic at high concentrations.

Reports in the literature on in vitro effects of the w-substituted phenoxyacetic acid herbicides on micro-organisms are agreed that levels above 1000 ppm of 2,4-D are inhibitory to a wide selection of species (Stevenson & Mitchell, 1945; Culler, Weiser & Witman, 1948; Manil & Straszewska, 1950; Jensen & Petersen, 1952; Baldacci & Amici, 1954,1955; Johnson & Colmer, 1955, 1955a; Mitskovski, 1959; Virag, 1959; Mostafa & Gayed, 1960). 1000 ppm MCPA were reported as inhibitory to several soil fungi (Voderberg, 1961; Wilkinson & Lucas, 1969) and M.I.C. values for MCPA, 2,4-D and 2,4,5-T given in Table 8 agree generally with those found for a number of citrus fruit pathogens by Erickson, DeWolfe and Brannaman (1958).

That the fungitoxic effects of the w-substituted phenoxy-

carboxylic acids could be due to a different form of activity from their growth promoting role is supported by the conclusions of other authors on the mode of action of fungicides in general. In a review, Byrde(1965) considered it likely that fungicides in current use are characterised not by high toxicity on a weight/weight basis, but rather by their ability to be accummulated in fungal cells at concentrations greatly in excess of those in external solution. For instance, the LD 50s of various fungicides for mould spores range from 85 ppm to 10,000 ppm while the LD 50 for tomato of 2,4-D is 10 ppm (Millar & McCallan, 1956). This effect of accummulation in fungal cells may explain in part the present observation of the increased inhibition of γ-MCPB as compared with MCPA. Later work by colleagues (Kirkwood & Fletcher, 1970) with green algae, showed that the enhanced activity of γ-MCPB against growth of these organisms coincided with more efficient absorption of the chemical.

In the course of this report, emphasis has been laid on the influence of chemical structure on the fungitoxicity of the chemicals tested, exemplified by the difference in effect at higher concentrations (> 50 ppm) between the acetic and γ-butyric acid homologues of the w-substituted phenoxycarboxylic acid herbicides investigated. This initial observation led to the testing of other compounds, both with and without plant growth regulating activity, to explore this structure-activity relationship towards micro-organisms more fully. That mere increase in molecular weight does not necessarily increase toxicity is illustrated roughly in Figure 11 in which ED 50 values for all the compounds investigated with Pencillium notatum are plotted against their molecular weight. Although all chemicals with ED 50s below 200 ppm have molecular weights in excess of 225, the contrary does not hold true, as, for instance, 2,4,5-T with a molecular weight of 255.5 has an ED 50 of 24650 ppm.

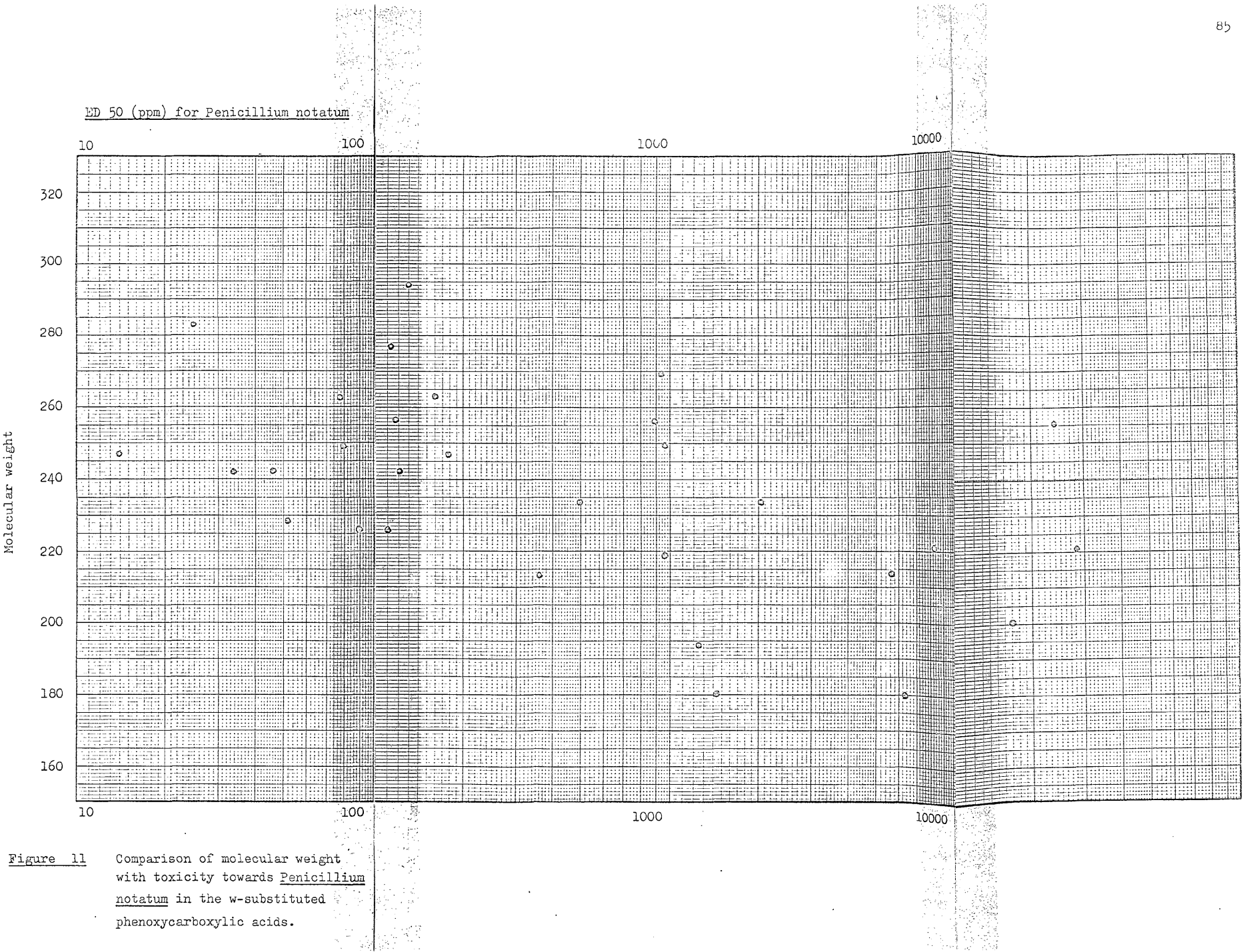


Figure 11 Comparison of molecular weight with toxicity towards Penicillium notatum in the w-substituted phenoxycarboxylic acids.

Three aspects of the structure-fungitoxicity relationship were explored, namely the influence of β-oxidative breakdown and the effects of changes in the side chain and ring of the phenoxycarboxylic acids.

The β-oxidative breakdown mechanism is the metabolic pathway by which most plants can metabolise long chain fatty acids and is likely to be responsible for the plant growth regulating activity of the higher members of the w-substituted phenoxycarboxylic acid herbicides as described in the introductory section.

That microbial β-oxidation of such compounds also occurs is in no doubt, as several authors have presented evidence for the operation of this process in micro-organisms. It is known that w-substituted phenoxycarboxylic acids such as γ-2,4-DB, γ-MCPB and higher homologues of these compounds are readily degraded by the soil microflora to forms toxic to higher plants (Brownbridge, 1956; Alexander & Aleem, 1961) and Webley, Duff and Farmer (1957) have shown that the actinomycete Nocardia opaca can bring about the β-oxidation of fatty acids.

One of the principal observations of the present report was the consistently wide discrepancy between fungitoxic activity of the acetic and γ-butyric members of the same homologous series and consequently an absence of alternation in effect as found in higher plants. The substituted phenoxyacetic acid, MCPA, was nontoxic to some fungi (Table 8) at levels as high as 10,000 ppm whereas γ-MCPB was highly toxic at 50 ppm or 100 ppm. It is suggested, therefore, that the inhibitory action of these compounds is due to the presence of the original compound and not to the final breakdown products resulting from a process of β-oxidation.

Considering the possibility of side chain degradation by β-oxidation the results obtained from Tables 10 and 11 indicate that toxicity towards fungi appears between the 2- and 4- carbon length

side chain forms of the w-substituted phenoxycarboxylic acids. The 2-carbon side chain (acetic acid) was least toxic, the 3-carbon (β-propionic) was intermediate, for Penicillium notatum, while 4-, 5- and 6- carbon chain acids were all highly toxic. Tests with Aspergillus niger (Table 10) showed the β-propionic acid to be as toxic as the higher homologues to this fungus. Figure 12 summarises these results for Penicillium notatum illustrating increase in fungitoxicity with increasing side chain carbon length in the w-substituted phenoxy-carboxylic acids tested.

Similar results have also been found by other authors. Byrde and Woodcock (1958) reported that, in a series of unsubstituted w-naphthyloxycarboxylic acids tested in vitro with Sclerotinia laxa, the acetic acid reduced growth by 10% while the β-propionic, γ-butyric, n-valeric and n-caproic acids gave inhibition ranging from 67% to 73%. As Sclerotinia laxa and Aspergillus niger were shown to be capable of β-oxidising these acids (Byrde, Harris & Woodcock, 1956; Byrde & Woodcock, 1957; Woodcock & Byrde, 1958) the higher homologues must have been fungitoxic per se. Otherwise the γ-butyric and n-caproic forms at least would have had an effect similar to the acetic acid to which they would have been degraded. Fawcett, Spencer and Wain (1957) testing a homologous series of w-phenoxyalkylcarboxylic acids (n = 1 to 10) on Botrytis fabae and Pythium ultimum reported that the acetic acid had no effect while growth was reduced 40% by the β-propionic acid and 95% by the γ-butyric, n-valeric, n-caproic and n-heptanoic acids. Therefore, although the side chains of these acids were capable of β-oxidation by higher plants, it was assumed that little or no degradation of the side chain had occured in the fungi tested.

In mono-substituted phenoxycarboxylic acids, Webley, Duff and

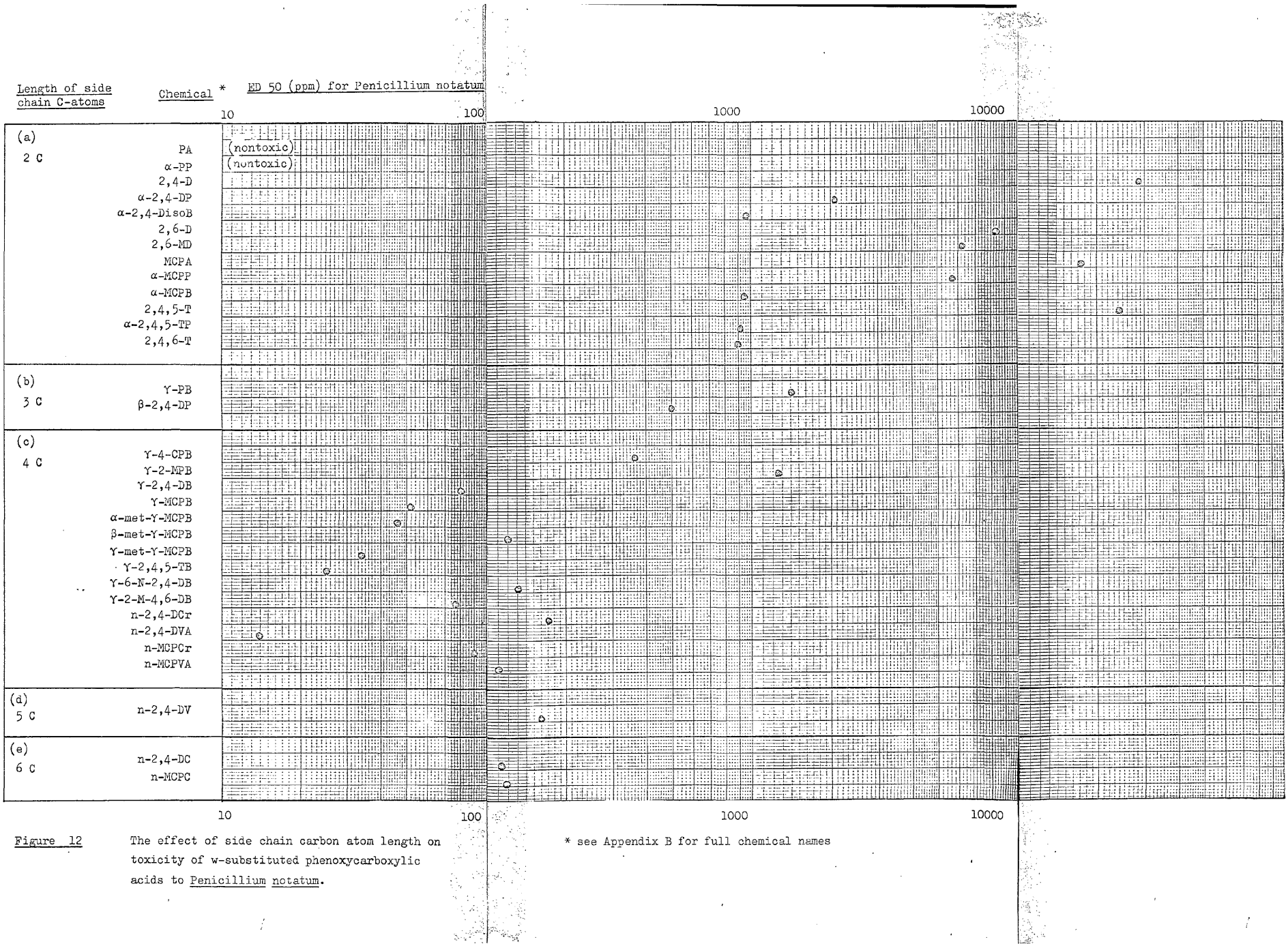


Figure 12 The effect of side chain carbon atom length on toxicity of w-substituted phenoxycarboxylic acids to Penicillium notatum.

* see Appendix B for full chemical names

Farmer (1957) established β-hydroxy acids as intermediates in the conversion by Nocardia opaca of 3- and 4-chlorophenoxybutyric acids to their corresponding acetic derivatives. The same authors (1958) showed that formation of the β-hydroxy intermediate was common to all w-aryloxybutyric acids including γ-naphthyl-, γ-naphthyloxy-, γ-phenyl- and γ-indolyl- series, However, a more detailed investigation of the di-substituted acids, γ-MCPB and γ-2,4-DB, showed that 50% and 20% respectively of the acids were partially converted, the β-hydroxy intermediate being detected in each case, but the corresponding acetates were not found. The fact that the β-oxidation process was blocked at the β-hydroxy intermediate in di-substituted acids was confirmed by the rapid conversion by N. opaca of n-4-chloro-2-methylphenoxy-caproic acid and n-2,4-dichlorophenoxycaproic acid to the corresponding γ-butyric acids, but no further. This agrees with the toxicity pattern for γ-MCPB and γ-2,4-DB found by the present author. As Webley et al (1957, 1958) also inferred that β-oxidation did take place if only to a limited extent, it was still possible that a breakdown product could be responsible for the toxicity. Table 14 however shows that β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid was nontoxic or equivalent in toxicity to MCPA towards the fungi tested.

Variation in the nature of the alkyl acid side chain in 2,4-D and MCPA by substitution to form the α-propionic, α-butyric and α-isobutyric analogues, can alter the growth regulating properties of the w-substituted phenoxycarboxylic acid herbicides. Apart from increasing molecular weight such changes render the molecule asymmetric and may hinder attachment to some free surface in the cell or impair penetration and movement of the intact molecule across cellular surfaces. Reports show that while the growth regulating properties of acetic, α-propionic and α-butyric acids are similar to one another, the

α-isobutyric homologues are much less effective, the only structural difference between the two butyric acids being the substitution of two methyl groups on either side of the α-carbon in the side chain, in contrast to the n-α-butyric acid form (Fawcett, Osborne, Wain & Walker, 1953; Vitou & Wain, 1959). Considering the fungitoxic properties of these same compounds it would appear from the results shown on Table 16 that, with Penicillium notatum, the n-α-substituted acid forms of MCPA, 2,4-D and 2,4,5-T became increasingly more toxic as molecular weight increased. The toxicities of α-4-chloro-2-methyl-phenoxybutyric acid and α-2,4-dichlorophenoxyisobutyric acid were similar to each other. While certain other fungi tested (Table 15) showed little difference in reaction to both the unsubstituted side chain of MCPA and its α-propionic derivative, α-MCPP, the α-butyric form, α-MCPB, was always more toxic, with the exception of Aspergillus niger. None of these α-substituted acids, however, had ED 50s as low as their γ-butyric analogues.

These conclusions are corroborated by reports on the fungi-toxicity of the α-substituted phenoxyacetic acids in the literature. Crowdy (1948) found little difference in toxicity towards Nectria galligena, the pathogen causing apple canker, between the acetic and α-propionic acids while β-propionic acid forms of the aryloxyaliphatic acids tested were far more toxic. Fawcett, Spencer and Wain (1955) reported that concentrations of n-α-substituted phenoxyacetic acids above 100 ppm were required to give 50% inhibition in six fungi tested in vitro.

The effect of ring substitution on the fungitoxicity of the few compounds available was surveyed and the results, for Penicillium notatum, are illustrated by way of summary in Figure 13.

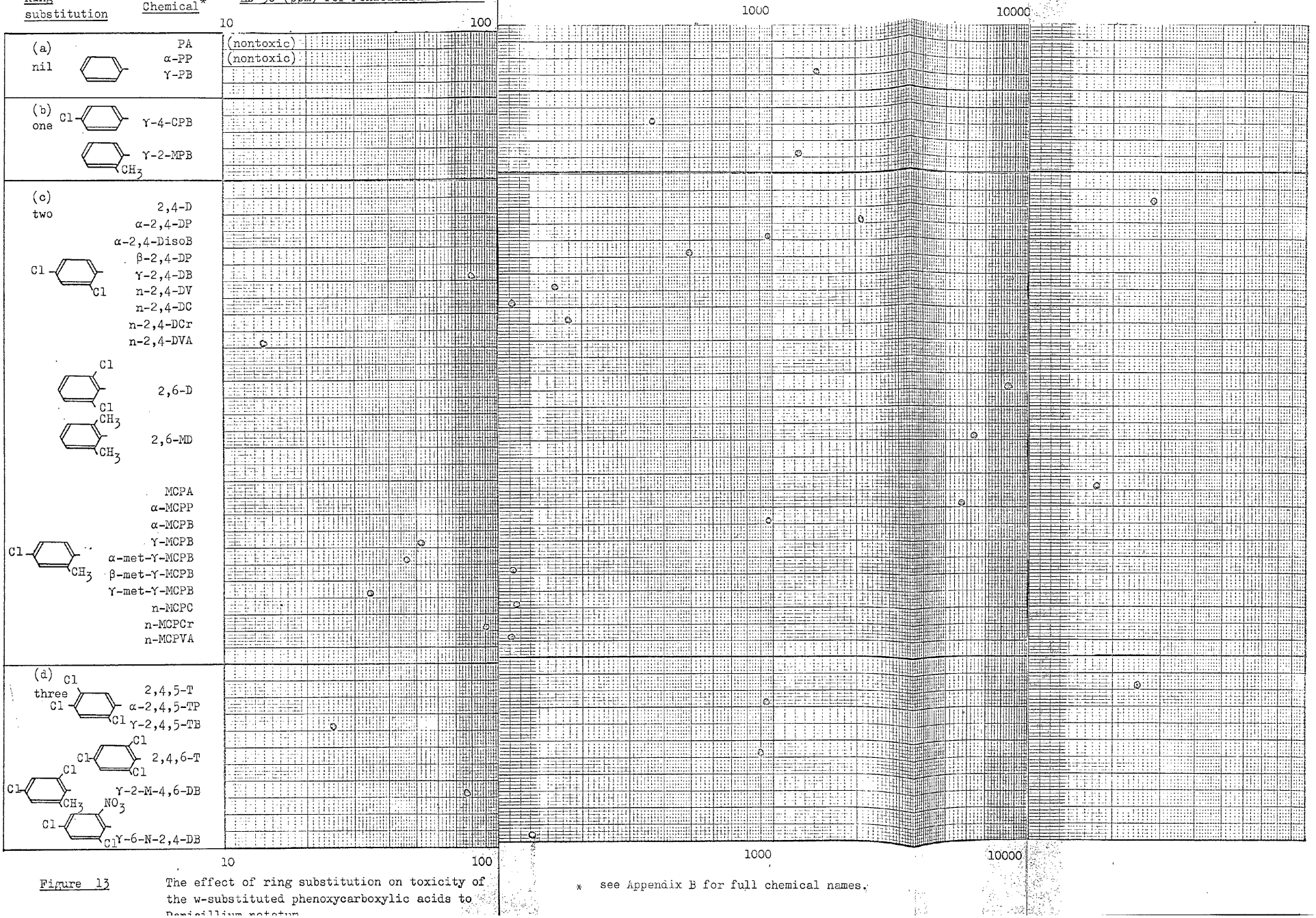


Figure 13 The effect of ring substitution on toxicity of the ω-substituted phenoxycarboxylic acids to Penicillium notatum

* see Appendix B for full chemical names.

In the positional isomers investigated in this report, i.e. 2,4- and 2,6-dichloro (or 2,6-dimethyl-) and 2,4,5- and 2,4,6-trichlorophenoxyacetic acids, an increase in toxicity towards Penicillium notatum associated with substitution in the other ortho (6-) position instead of the meta (5-) or para (4-) positions was found (Table 17) being particularly marked in 2,4,5-T (ED 50 of 24650 ppm) and 2,4,6-T (ED 50 of 900 ppm). This recalls the claim by some workers (Hansch, Muir & Metzenberg, 1951) of a "di-ortho" effect altering plant growth regulating activity. Similar tests on Aspergillus niger (Table 18) with 2,4-dichlorophenoxyacetic acid and 2,6-dichlorophenoxyacetic acid were inconclusive as the latter compound gave considerable stimulation of growth at 10 ppm and 100 ppm.

It would appear that increase in antifungal activity of the phenoxycarboxylic acids is also consistent with an increasing number of substituents in the ring. Phenoxyacetic acid was nontoxic, but some di- and tri- substituted acids were quite effective at high concentrations (Tables 17 and 18) while pentachlorophenoxyacetic acid, not tested here, is commonly used as a fungicide.

That differences in ring substitution can affect fungitoxicity is supported by reports on the influence of ring structure on the ease of microbial breakdown of such compounds in the soil environment. Biodegradability experiments have provided valuable evidence along these lines. For instance, the nucleus of halogenated phenoxycarboxylic acids has been reported to remain intact in soil for long periods in compounds containing a chlorine substituent in a position meta (3- or 5-) to the phenolic hydroxyl group (Alexander & Aleem, 1961; MacRae & Alexander, 1965). Thus, acids with substitutions of 3,4-dichloro- and 2,5-dichloro, and to a lesser extent, 2,4,5-trichloro- were resistant to attack irrespective of the form of the side chain.

The same authors report that when 2- and 4- positions were both substituted with chlorine, i.e. 2,4-D or γ-2,4-DB, breakdown was rapid. Methyl substitution, on the other hand, as in 4-chloro-2-methylphenoxy-acetic acid (MCPA) tended to result in resistance to attack. In soil experiments MCPA was also found to be more resistant to microbial attack than 2,4-D (Derose & Newman, 1948; Audus,1951). Webley, Duff and Farmer (1958) working with mono-substituted β-phenoxypropionic and γ-phenoxybutyric acids considered that ortho substitution in the 2- or 6- position by chlorine or methyl groups appeared to make the compound resistant to attack, the rate of β-oxidation by Nocardia opaca of meta, para and ortho isomers decreasing in that order. In the same paper it was further reported that the effect of di-substitution in the γ-butyric acid forms of these acids was to hinder conversion. A comparison by these authors between γ-2-methylphenoxybutyric acid and γ-4-chloro-2-methylphenoxybutyric acid (γ-MCPB) showed that there had been a greater conversion of the mono-methyl form, 30% and 15% of the β-hydroxy intermediate being formed from 2-methylphenoxybutyric acid and γ-MCPB respectively. Similarly when 3-chloro- and 4-chloro-phenoxybutyric acids were compared with γ-3,4-dichlorophenoxybutyric acid, the di-substituted compound was found to be converted much more slowly than the mono-substituted acids. The positional effect of the second substituent was also shown by Taylor and Wain (1962) reporting that a second chlorine group in the 3- or 4- position in the ring of 2-chlorophenoxy acids would reduce or increase respectively the β-oxidation rate of the compounds by several micro-organisms.

It has been shown, therefore, in the experimental work discussed above, that the w-substituted phenoxycarboxylic acids at low (herbicidal) concentrations had no harmful effect on fungi and other micro-organisms tested. However, at concentrations greater than 50 ppm there was a progressive increase in the toxicity of γ-MCPB to

most of the organisms, whereas at similar concentrations MCPA had considerably less effect. Postulating that γ-MCPB has an inherent protoplasmic toxicity in its own right at relatively high concentrations quite apart from its possible hormonal effect at low concentrations when perhaps converted in part to MCPA, further studies were carried out in an attempt to elucidate the site of this inhibition. Accordingly the inhibitory action of high doses of MCPA and γ-MCPB on the respiratory system of Penicillium notatum was investigated.

The results presented in Section III, 4, show that the inhibitory effect of high concentrations of the w-substituted phenoxycarboxylic acid herbicides tested on the growth of Penicillium notatum in petri dish culture were paralleled by a corresponding inhibition of the normal respiratory processes. Endogenous respiration of intact mycelial tissue was progressively inhibited with prolonged incubation with γ-MCPB and to a lesser extent, MCPA (Table 19). In a similar manner the succinic oxidase component of the respiratory electron transport chain was also inhibited (Table 21).

Surveying the literature, plant growth regulators have been shown to interfere with respiratory metabolism, uncoupling respiration from the formation of energy-rich phosphates in oxidative phosphorylation (Wort, 1961). 2,4-dichlorophenoxyacetic acid at concentrations between 10^{-4} and 10^{-3}M has been shown to have an uncoupling effect in rat liver tissue (Brody, 1952) and soya bean tissue (Switzer, 1971); 2,4-D, α-2,4-DP and 4-chlorophenoxyisobutyric acid all affected oxidative phosphorylation in cucumber mitochondria although this effect was not reported to be as great as that of the more pronounced uncoupling agents such as dinitrophenol (Stenlid & Saddick, 1962).

The concentrations of 2,4-D uncoupling oxidative phosphorylation (80 ppm to 400 ppm) in these reports above are higher than those

producing typical auxin effects (2 ppm to 10 ppm) and changes from auxin to anti-auxin properties caused by small changes in chemical structure e.g. the D- and L- forms respectively of α-2,4-dichlorophenoxypropionic acid, were reported not to be due to alteration in activity towards oxidative phosphorylation as both of these enantiomorphs had the same effect (Stenlid & Saddick, 1962). Concentrations of 2,4-D (10^{-3}M) which uncoupled phosphorylation in isolated plant mitochondria also completely inhibited growth of discs prepared from the same tissues (Wedding & Black, 1962). Uncoupling effects, therefore, do not seem to be associated with specific auxin activity.

There has been little work published on the effects of the w-substituted phenoxycarboxylic acid herbicides on the respiratory metabolism of micro-organisms, apart from a report of 2,4-D induced uncoupling of phosphorylation in Chlorella pyrenoidosa by Wedding and Black (1961) and studies on the effect of magnesium ion in protecting Azotobacter vinelandii from respiratory inhibition by 2,4-D and related chlorophenoxycarboxylic acids reported by Johnson and Colmer (1958).

The constitution and role of cytochrome mediated reactions in tissues of higher plants are well established, but relatively few studies have been made on the nature of the electron transport chain in fungi. Most fungi appear to respire via flavoprotein and a typical sequence of cytochromes as in higher plants and cytochrome oxidase has been implicated in the terminal respiration of a number of fungi, for instance, Myrothecium verrucaria (Darby & Goddard, 1950) and Penicillium notatum (Wolf, 1947).

Studies of the enzyme succinic dehydrogenase isolated from crude tissue extracts have been reported by several authors, for instance, Aspergillus niger (Martin, 1954), Neurospora crassa (Shepherd, 1951) and Myrothecium verrucaria (Hilton & Smith, 1959). Cell free extracts from yeast or other fungi which are capable of oxidative

phosphorylation are much more difficult to obtain and attempts to prepare such extracts from Penicillium notatum by the present author were unsuccessful.

The probable course of the electron transport chain is shown in Figure 14. Oxidised flavoproteins react with substrates such as succinate or with the reduced coenzyme NADH (Nicotinamide adenine dinucleotide). The resulting reduced flavine enzymes then release electrons through ubiquinone and the cytochrome sequence. It is not yet resolved whether ubiquinone and cytochromes b and c_1 are arranged in series or in parallel, and which are actually active in native mitochondria. After the electrons are passed through this region they then pass through cytochrome c to the cytochrome a + a_3 complex (cytochrome oxidase) where an oxygen molecule is bound.

As subsequent work by a colleague, Dr J.E.Smith (Smith & Shennan, 1966) showed that NADH oxidase as well as succinic oxidase was inhibited by Y-MCPB in cell free extracts of Aspergillus niger, and as the cytochrome oxidase portion of the system, i.e. the passage of electrons from the cytochrome a + a_3 complex to molecular oxygen. was not inhibited by Y-MCPB in either Penicillium notatum (Figure 10) or Aspergillus niger (Smith & Shennan, 1966) the probability is that the site of inhibition may be at some point on the electron transport chain common to both the succinate and NADH pathways and before the site of cytochrome oxidase. This would restrict the inhibitory activity of the Y-MCPB to the portion of the electron transport chain between the reduced flavoprotein enzymes produced by the oxidation of succinate and NADH, and the subsequent stages involving ubiquinone and cytochromes b and c. In the presence of Y-MCPB and MCPA the ubiquinone reductase complex in Aspergillus niger was inhibited 70% and 8% respectively (Smith & Shennan, 1966).

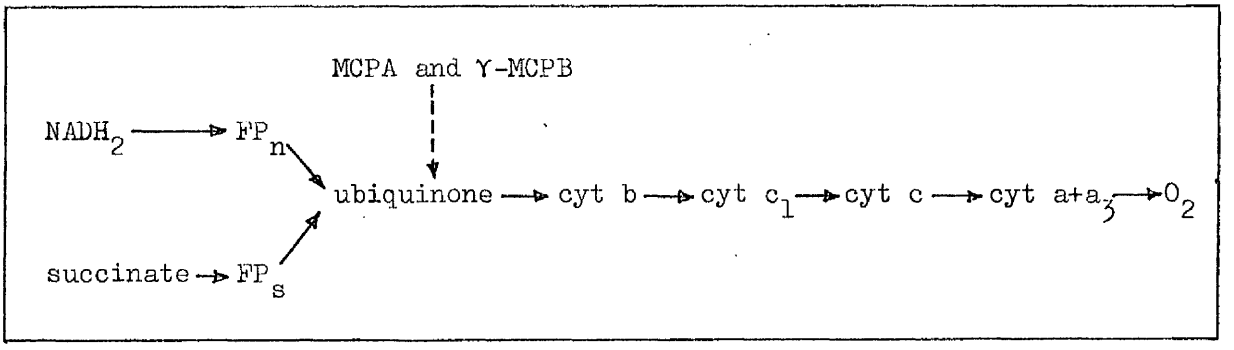


Figure 14

A generalised scheme of electron transport.

Abbreviations:

$NADH_2$	reduced nicotinamide adenine dinucleotide
FP_s	succinic dehydrogenase flavoprotein
FP_n	nucleotide dehydrogenase flavoprotein

V. CONCLUSIONS

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It is concluded from the results presented in this thesis on the effect of w-substituted phenoxycarboxylic acids on *in vitro* culture of commonly occuring microfungi that, as expected, the use of herbicides of this type at normal rates of application in the field is unlikely to affect adversely the microbial population of the soil. Indeed, stimulation of growth was observed in the course of the present report when certain combinations of fungi and w-substituted phenoxycarboxylic acids were tested. Further work, using more finely controllable experimental techniques to allow quantitative estimation of growth responses at concentration levels below, say, 1 ppm (approx. 5×10^{-6}M) would be required to elucidate whether or not this activity is akin to the growth regulating effects of these same chemicals in higher plants.

At higher concentrations many of the w-substituted phenoxycarboxylic acids were found to be toxic to the organisms tested. The acetic and α-propionic acid forms of the herbicides, MCPA, 2,4-D and 2,4,5-T were toxic to some fungi, having ED 50s ranging between 230 ppm and 10,000 ppm, or were nontoxic to other species showing no inhibitory effect throughout the concentration range tested. The γ-substituted phenoxybutyric acid forms of these herbicides, on the contrary, were consistently toxic at much lower levels than their acetic and α-propionic equivalents, a reversal of the relative activity of these homologues in higher plants.

Following investigations into the influence of chemical structure on the fungitoxicity of the w-substituted phenoxycarboxylic acids, it is concluded that β-oxidative breakdown of the longer side

chain acids is not necessary for activation as is the case for the growth promoting activity of these same compounds. No evidence for alternation in fungitoxicity was observed in homologous series of these acids and, therefore, it is suggested that the higher homologues of the series tested have an intrinsic toxicity towards micro-organisms irrespective of whether or not these same compounds are capable of being broken down by β-oxidation.

Surveying the variety of chemicals tested it would appear that increase in length of the carboxylic acid side chain in a homologous series from the least toxic acetic acid (2-carbon) to the γ-butyric acid (4-carbon) enhances toxicity. Longer side chain acids tested (5- and 6-carbon) had an effect equivalent to their γ-butyric acid homologues. Increase in toxicity is also apparently consistent with increase in number of substituents in the ring of the phenoxycarboxylic acids, and variation in activity depends on the type and position of these substitutions. Insufficient variety of compounds was available to confirm any such trends conclusively, but indications were present that substitution in both ortho (2,6-) positions led to greater toxicity than other patterns of di-substitution.

The toxicity of γ-MCPB to Penicillium notatum in petri dish and flask culture was paralleled by inhibition of the endogenous respiration of intact mycelial tissue. Further investigations using crude mitochondrial extracts of P. notatum showed that the inhibitory action of γ-MCPB was confined to that portion of the electron transport chain between the succinic oxidase system and the cytochrome mediated stages, as oxidation of succinate was shown to be adversely affected by the presence of γ-MCPB, whilst this compound had no effect on the cytochrome oxidase activity of Penicillium notatum.

γ-MCPB has therefore been shown to have an inherent non-specific toxicity towards micro-organisms at relatively high concentrations (> 100 ppm) in contrast to its growth promoting activity at lower concentrations (< 5 ppm) manifest in higher plants on breakdown by β-oxidation to MCPA.

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APPENDIX A

APPENDIX A

Index of organisms used and origin of cultures.

	Organism	Origin
1.	*Alternaria solani* (E.et M.) J.et Gr.	Botany Dept.W.S.A.C.
2.	*Armillaria mellea* (Vahl et Fr.) Quel.	C.M.I. no. 61755
3.	*Ascochyta pisi* Lib.	C.M.I. no. 54862
4.	*Aspergillus niger* van Tieghem	C.M.I. no. 17454
5.	*Botrytis cinerea* Pers.	C.M.I. no. 42089
6.	*Corticium solani* (Prill & Delacr.) Bourd & Galz.	C.M.I. no. 75144
7.	*Fusarium conglutinans* (Wollenw.) Snyder & Hansen	C.M.I. no. 98454
8.	*Fusarium nivale* (Fr.) Ces.	Botany Dept. W.S.A.C.
9.	*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen	C.M.I. no. 96339
10.	*Gibberella zeae* (Schw.) Petch.	C.M.I. no. 69695
11.	*Helminthosporium sativum* Pammel, King & Bakke	C.M.I. no. 75633
12.	*Microsporum canis* Bodin	Med. Mycol. Dept., Univ. Glasgow
13.	*Mucor hiemalis* Wehmer	C.M.I. no. 21216
14.	*Neurospora sitophila* Shear & Dodge	C.M.I. no. 63919
15.	*Penicillium chrysogenum* Thom	C.M.I. no. 40233
16.	*Penicillium notatum* Westling	C.M.I. no. 15378
17.	*Phytophthora parasitica* Dastur	C.M.I. no. 22176
18.	*Pyrenophora avenae* Ito & Kuribay	Botany Dept. W.S.A.C.
19.	*Pythium debaryanum* Hesse	C.M.I. no. 48558
20.	*Rhizopus stolonifer* (Ehrenb. et Fr.) Lind.	Botany Dept. W.S.A.C.
21.	*Trichoderma viride* Pers. et Fr.	C.M.I. no. 45553
22.	*Trichophyton sulphureum* C. Fox	Med. Mycol. Dept., Univ. Glasgow
23.	*Candida albicans* (Robin) Berkhout	Med. Mycol. Dept., Univ. Glasgow
24.	*Candida pulcherrima* (Lindner) Windisch	N.C.Y.C. no. 166
25.	*Saccharomyces acidifaciens* (Nickerson) n.c.	N.C.Y.C. no. 417
26.	*Saccharomyces cereviseae* Hansen	C.M.I. no. 61302

	Organism	Origin
27.	Saccharomyces fragilis Jorgensen	N.C.Y.C. no. 100
28.	Chlamydomonas subangulosa Fritsch & John	C.C.A.P. no. 11/28
29.	Chlorella vulgaris Beijerinck	C.C.A.P. no. 211/11a
30.	Dictyococcus terrestris (Chod. & Kol.) Vischer	C.C.A.P. no. 221/4

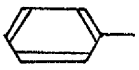
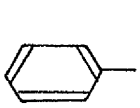
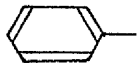
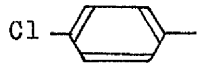
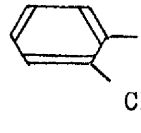
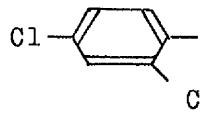
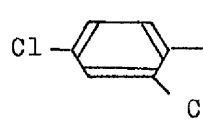
Abbreviations.

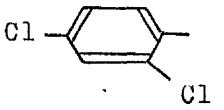
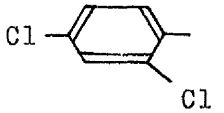
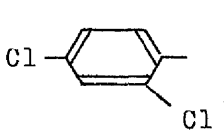
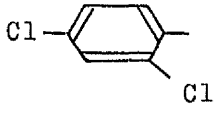
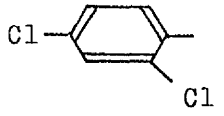
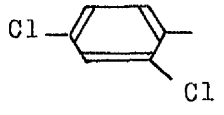
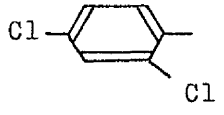
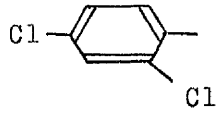
C.C.A.P.	Culture Collection of Algae and Protozoa, Botany School, Downing Street, Cambridge.
C.M.I.	Commonwealth Mycological Institute, Ferry Lane, Kew.
N.C.Y.C.	National Collection of Yeast Cultures, Brewing Research Institute, Nutfield, Surrey.
W.S.A.C.	West of Scotland Agricultural College.

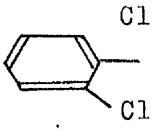
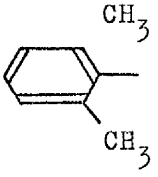
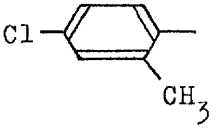
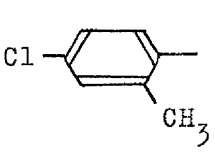
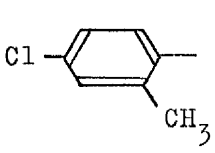
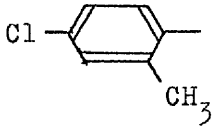
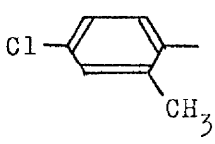
APPENDIX B

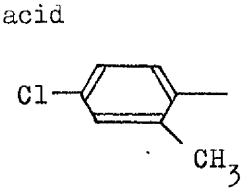
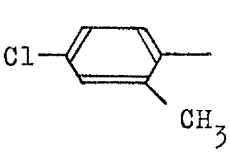
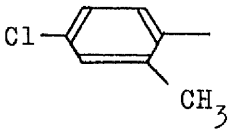
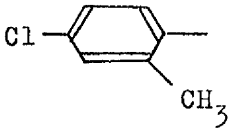
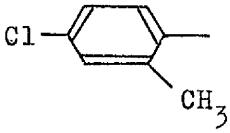
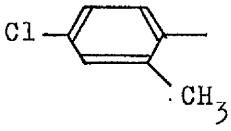
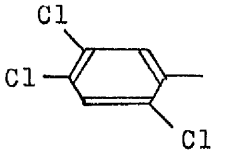
APPENDIX B

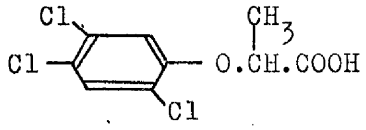
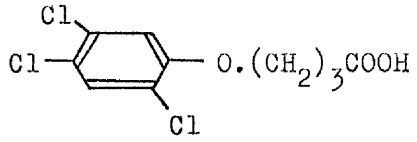
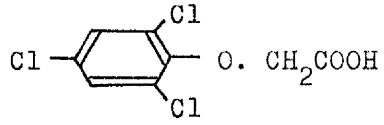
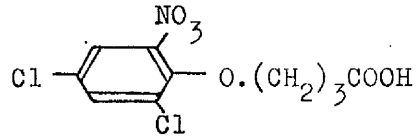
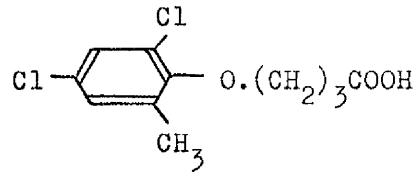
Index of phenoxycarboxylic acids tested.

	Chemical	Manufacturer's Ref. no.	Molecular weight	Formula
1.	Phenoxyacetic acid (PA) 	2608	152.0	$C_8H_8O_3$
2.	α-phenoxypropionic acid (α-PP) 	5152	166.1	$C_9H_{10}O_3$
3.	γ-phenoxybutyric acid (γ-PB) 	3130	180.0	$C_{10}H_{12}O_3$
4.	γ-4-chlorophenoxybutyric acid (γ-4-CPB) 	3045	213.5	$C_{10}H_{11}O_3Cl$
5.	γ-2-methylphenoxybutyric acid (γ-2-MPB) 	3249	194.0	$C_{11}H_{14}O_3$
6.	2,4-dichlorophenoxyacetic acid (2,4-D) 	-	221.0	$C_8H_8O_3Cl_2$
7.	α-2,4-dichlorophenoxypropionic acid (α-2,4-DP) 	5154	235.5	$C_9H_8O_3Cl_2$

Chemical	Manufacturer's Ref. no.	Molecular weight	Formula
8. β-2,4-dichlorophenoxypropionic acid (β-2,4-DP) 	7052	235.5	$C_9H_8O_3Cl_2$
9. γ-2,4-dichlorophenoxybutyric acid (γ-2,4-DB) 	-	249.0	$C_{10}H_{10}O_3Cl_2$
10. α-2,4-dichlorophenoxyisobutyric acid (α-2,4-DisoB) 	6952	249.0	$C_{10}H_{10}O_3Cl_2$
11. γ-2,4-dichlorophenoxycrotonic acid (γ-2,4-DCr) 	3008	247.0	$C_{10}H_8O_3Cl_2$
12. γ-2,4-dichlorophenoxyvinylacetic acid (γ-2,4-DVA) 	3597	247.0	$C_{10}H_8O_3Cl_2$
13. β-hydroxy-γ-2,4-dichlorophenoxybutyric acid 	3120	265.0	$C_{10}H_{10}O_4Cl_2$
14. δ-2,4-dichlorophenoxyvaleric acid (n-2,4-DV) 	-	263.0	$C_{11}H_{12}O_3Cl_2$
15. ε-2,4-dichlorophenoxycaproic acid (n-2,4-DC) 	4963	277.0	$C_{12}H_{14}O_3Cl_2$

	Chemical	Manufacturer's Ref. no.	Molecular weight	Formula
16.	2,6-dichlorophenoxyacetic acid (2,6-D) 	2899	221.0	$C_8H_8O_3Cl_2$
17.	2,6-dimethylphenoxyacetic acid (2,6-MD) 	6940	180.0	$C_{10}H_{12}O_3$
18.	4-chloro-2-methylphenoxy-acetic acid (MCPA) 	-	200.5	$C_9H_9O_3Cl$
19.	α-4-chloro-2-methylphenoxy-propionic acid (α-MCPP) 	3195	214.5	$C_{10}H_{11}O_3Cl$
20.	α-4-chloro-2-methylphenoxy-butyric acid (α-MCPB) 	5172	228.5	$C_{11}H_{13}O_3Cl$
21.	γ-4-chloro-2-methylphenoxy-butyric acid (γ-MCPB) 	3046	228.5	$C_{11}H_{13}O_3Cl$
22.	γ-(4-chloro-2-methyl)-α-methyl-phenoxybutyric acid (α-met-γ-MCPB) 	3279	242.5	$C_{12}H_{15}O_3Cl$

Chemical	Manufacturer's Ref. no.	Molecular weight	Formula
23. γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid (β-met-γ-MCPB) 	3453	242.5	$C_{12}H_{15}O_3Cl$
24. γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid (γ-met-γ-MCPB) 	3353	242.5	$C_{12}H_{15}O_3Cl$
25. γ-4-chloro-2-methylphenoxycrotonic acid (γ-MCPCr) 	3162	226.5	$C_{11}H_{11}O_3Cl$
26. γ-4-chloro-2-methylphenoxyvinylacetic acid (γ-MCPVA) 	3316	226.5	$C_{11}H_{11}O_3Cl$
27. β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid 	3274	244.5	$C_{11}H_{13}O_4Cl$
28. ε-4-chloro-2-methylphenoxycaproic acid (n-MCPC) 	4800	256.5	$C_{13}H_{17}O_3Cl$
29. 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) 	-	255.5	$C_8H_5O_3Cl_3$

Chemical	Manufacturer's Ref. no.	Molecular weight	Formula
30. α-2,4,5-trichlorophenoxypropionic acid (α-2,4,5-TP) 	4802	269.5	$C_9H_7O_3Cl_3$
31. γ-2,4,5-trichlorophenoxybutyric acid (γ-2,4,5-TB) 	2863	283.5	$C_{10}H_9O_3Cl_3$
32. 2,4,6-trichlorophenoxyacetic acid (2,4,6-T) 	2609	255.5	$C_8H_5O_3Cl_3$
33. γ-6-nitro-2,4-dichlorophenoxybutyric acid (γ-6-N-2,4-DB) 	4603	294.0	$C_{10}H_9O_5Cl_2N$
34. γ-2-methyl-4,6-dichlorophenoxybutyric acid (γ-2-M-4,6-DB) 	-	263.0	$C_{11}H_{13}O_3Cl_2$

APPENDIX C

APPENDIX C

Medium composition and preparation

1. Potato Dextrose Agar

Fresh diced potato	200g
Dextrose	20g
Agar	20g
Distilled water	1000ml

The potato was steamed in distilled water and an infusion prepared and clarified. This infusion was used to prepare the agar medium in the usual fashion.

2. Glucose Peptone Agar

Glucose	40g
Peptone	10g
Agar	20g
Distilled water	1000ml

3. Peptone Mineral Agar

Proteose peptone	1g
$K.NO_3$	0.2g
K_2HPO_4	0.02g
$Mg.SO_4$	0.02g
Agar	15g
Distilled water	1000ml

4. Glucose Peptone Broth

Peptone	10g
Dextrose	40g
Distilled water	1000ml

5. Bristol's Solution

Six stock solutions prepared each containing one of the following salts-

$Na\ NO_3$ 2.5 %

$Ca\ Cl_3$ 0.25 %

$Mg\ SO_4.7H_2O$ 0.75 %

$K_2\ HPO_4$ 0.75 %

$Na\ Cl$ 0.25 %

10 ml of each stock solution were added to 940 ml of distilled water together with one drop of 0.1 % $Fe\ Cl_3$ and 2 ml of a minor element solution. The medium was solidified with 1.5 % agar when required.

APPENDIX D

Statistical data

APPENDIX D 1. Data for computation of Analysis of Variance for effect of MCPA on Penicillium notatum on three occasions. Replicate diameters of colonies in mm.

Date	Concentration (ppm) 1000	500	100	50	5	Control no addn.
(a) May 1960	20.00	17.50	24.50	26.00	25.00	25.25
	24.00	21.50	25.00	26.00	25.50	24.00
	23.00	23.00	25.50	25.75	26.50	26.50
	21.00	24.75	24.50	26.50	25.00	24.50
	23.00	22.00	26.00	26.50	24.25	26.00
(b) Mar 1961	24.75	25.75	25.75	28.00	25.50	28.00
	24.00	25.25	25.50	25.00	26.00	31.00
	27.00	27.00	26.75	25.00	31.00	30.00
	25.75	24.00	25.50	26.00	29.00	28.00
	25.00	25.50	26.00	26.00	28.00	29.00
(c) Feb 1962	23.75	25.00	26.50	25.75	27.00	25.50
	24.75	23.50	25.25	26.75	26.50	27.00
	25.00	23.50	25.00	25.75	27.00	26.50
	23.00	25.00	23.50	23.50	27.00	26.00
	24.00	24.25	25.00	25.50	26.75	26.25
Σx	358.00	357.50	380.25	388.00	400.00	403.50
$\bar{x}$	23.87	23.83	25.35	25.87	26.67	26.90

APPENDIX D 2. Analysis of Variance with regression for data of effect of MCPA on Penicillium notatum on three occasions. Response vs. square root of dose.

(a) May 1960

Source of variation	df	SS	MS	F
Among groups	5	83.9003	16.7801	6.6087 ***
Linear regression	1	64.5015	64.5015	13.30 *
Deviations from regression	4	19.3988	4.8497	1.91 n.s.
Within groups (error)	24	60.9375	2.5391	
Total	29	144.7795		

Regression coefficient (b) -0.1303 ; s_b = 0.0356

95% con.lim. L_1 = -0.2291
L_2 = -0.0351

(b) March 1961

Source of variation	df	SS	MS	F
Among groups	5	60.9690	12.1938	6.67 ***
Linear regression	1	38.5015	38.5015	6.86 n.s.
Deviations from regression	4	22.4685	5.6171	3.0726 *
Within groups (error)	24	43.8750	1.8281	
Total	29	104.8683		

Regression coefficient (b) -0.1007 ; s_b = 0.0384

95% con.lim. L_1 = -0.2069
L_2 = -0.0055

(c) February 1962

Source of variation	df	SS	MS	F
Among groups	5	29.6435	5.9287	8.6236 ***
Linear regression	1	24.6785	24.6785	19.8812 *
Deviations from regression	4	4.9650	1.2413	1.8055 n.s.
Within groups (error)	24	16.5000	0.6875	
Total	29	46.1437		

Regression coefficient (b) -0.0806 ; s_b = 0.0181

95% con.lim. L_1 = -0.1306
L_2 = -0.0306

*** = P < 0.001
** = P < 0.01
* = P < 0.05
n.s.= not significant

APPENDIX D 3. Overall analysis of variance with regression for comparison of linear regression coefficients for MCPA.

Source of variation	df	SS	MS
Difference between means	2	70.9184	35.4592
Average linear regression	1	122.9925	122.9925
Difference between linear regression at three times	2	4.6890	2.3445
Total linear regression	3	127.6815	42.5605
Deviations from linear regression	4	10.3680	2.5920
Difference between deviations at three times	8	36.4643	4.5580
Total deviation from linear regression	12	46.8323	3.9027
Error	72	121.3125	1.6849
Total	89	366.7447	

$F_{[2][8]} = 0.5144$ n.s.

$F_{[8][72]} = 2.7052$ n.s.

APPENDIX D 4. Data for computation of Analysis of Variance for effect of γ-MCPB on Penicillium notatum on three occasions. Replicate diameters of colonies in mm.

Date	Concentration (ppm) 1000	500	100	50	5	Control no addn.
(a) May 1960	2.00	4.75	6.50	9.00	16.00	19.75
	1.75	4.00	8.00	9.00	16.25	22.50
	1.50	3.75	7.50	7.50	14.75	20.00
	1.75	4.00	8.75	7.75	15.00	20.50
	1.75	4.00	7.50	8.00	15.50	20.50
(b) Mar 1961	1.75	3.00	7.75	10.25	22.50	21.00
	1.75	3.00	7.00	8.75	22.00	23.00
	1.75	3.00	5.25	10.00	20.50	22.00
	2.00	4.25	7.75	10.00	21.25	22.50
	2.00	3.50	7.00	9.75	21.50	22.25
(c) Feb 1962	3.25	12.75	14.75	16.75	28.00	33.25
	7.00	11.75	14.50	16.75	27.75	32.00
	3.75	12.25	14.25	16.75	28.50	31.50
	3.90	13.25	15.00	17.00	27.00	30.25
	7.25	12.00	14.00	17.25	27.75	32.00
Σx	43.15	99.25	145.50	174.50	324.25	373.00
$\bar{x}$	2.88	6.62	9.70	11.63	21.62	24.87

APPENDIX D 5. Analysis of Variance with regression for data of effect of γ-MCPB on Penicillium notatum on three occasions. Response vs. logarithm of dose.

(a) May 1960

Source of variation	df	SS	MS	F
Among groups	5	1271.9750	254.5950	521.1771 ***
Linear regression	1	1260.4410	1260.4410	437.1219 ***
Deviations from regression	4	11.5340	2.8835	5.9028 **
Within groups (error)	24	11.7250	0.4885	
Total	29	1283.7000		

Regression coefficient (b) -6.1485

(b) March 1961

Source of variation	df	SS	MS	F
Among groups	5	1979.0000	395.8000	838.7370 ***
Linear regression	1	1894.9685	1894.9685	89.8886 ***
Deviations from regression	4	84.0730	21.0183	44.5397 ***
Within groups (error)	24	11.3250	0.4719	
Total	29	1990.4760		

Regression coefficient (b) -7.5389

(c) February 1962

Source of variation	df	SS	MS	F
Among groups	5	2497.4504	499.4901	982.0863 ***
Linear regression	1	2416.0050	2416.0050	29.6641 **
Deviations from regression	4	81.4455	20.36.6	40.0346 ***
Within groups (error)	24	12.2056	0.5086	
Total	29	2509.6560		

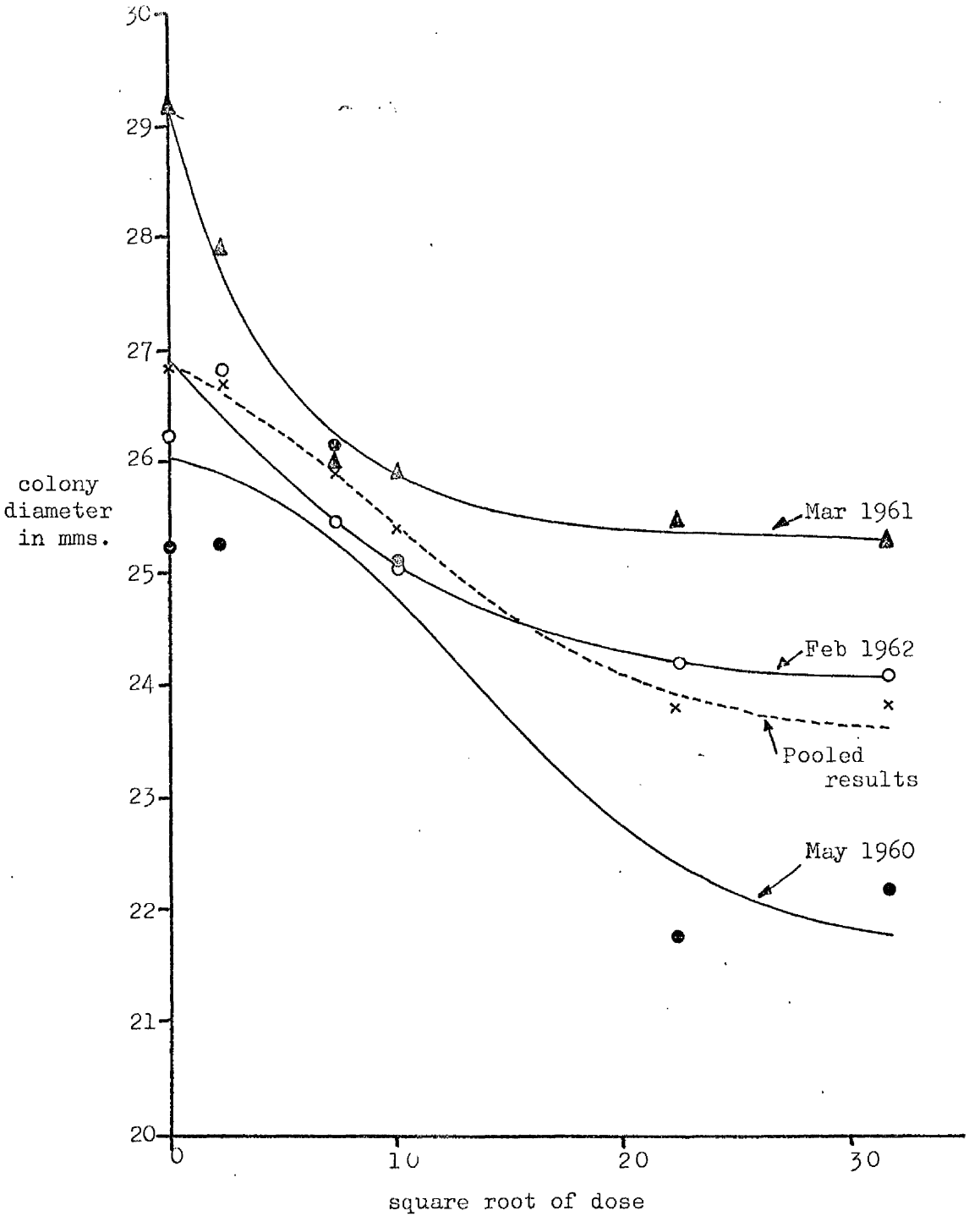
Regression coefficient (b) -8.5125

APPENDIX D 6. Overall analysis of variance with regression for comparison of linear regression coefficients for Y-MCPB.

Source of variation	df	SS	MS
Difference between means	2	1245.5848	622.7924
Average linear regression	1	5475.0735	5475.0735
Difference between linear regression at three times	2	96.3410	48.1705
Total linear regression	3	5571.4145	1857.1382
Deviations from linear regression	4	89.5125	22.3781
Difference between deviations at three times	8	87.5400	10.9425
Total deviation from linear regression	12	177.0525	14.7544
Error	72	35.2556	0.4897
Total	89	7029.3074	

$F_{[2][8]} = 4.4021$ n.s.

$F_{[8][72]} = 10.4528$ *** ($P < 0.001$)



APPENDIX D 7.

Response vs. square root of dose curves plotted for three separate experiments on the effect of 4-chloro-2-methylphenoxyacetic acid (MCPA) on Penicillium notatum in petri dish culture.

APPENDIX D 8. Analysis of variance with regression for data of effect of various w-substituted phenoxycarboxylic acids on fungi quoted in the text.

Response vs. square root of dose.

(a) Penicillium notatum. 4-chloro-2-methylphenoxyacetic acid, static flask culture (Table 3)

Source of variation	df	SS	MS	F
Among groups	5	0.0396	0.0079	3.95 **
Linear regression	1	0.0175	0.0175	3.182 n.s.
Deviations from regression	4	0.0220	0.0055	2.75 n.s.
Within groups (error)	24	0.0469	0.0020	
Total	29	0.0865		

Regression coefficient b = -0.0021

(b) Penicillium notatum. 4-chloro-2-methylphenoxyacetic acid, shake flask culture (Table 3)

Source of variation	df	SS	MS	F
Among groups	5	0.1888	0.0378	1.9688 n.s.
Linear regression	1	0.0524	0.0524	1.6324 n.s.
Deviations from regression	4	0.1285	0.0321	1.6719 n.s.
Within groups (error)	24	0.4607	0.0192	
Total	29	0.6495		

Regression coefficient b = -0.0042

(c) Penicillium notatum. 2,4-dichlorophenoxyacetic acid (Table 1 etc.)

Source of variation	df	SS	MS	F
Among groups	5	16.5625	3.3125	6.657 ***
Linear regression	1	4.5665	4.5665	1.6615 n.s.
Deviations from regression	4	10.9940	2.7485	5.5235 **
Within groups (error)	24	11.9500	0.4976	
Total	29	28.5125		

Regression coefficient b = -0.0347

APPENDIX D 8. (cont'd)

(d) Penicillium notatum. β-hydroxy-γ-2,4-dichlorophenoxybutyric acid (Table 13)

Source of variation	df	SS	MS	F	
Among groups	5	57.4245	11.4849	4.5316	**
Linear regression	1	15.2500	15.2500	1.4356	n.s.
Deviations from regression	4	42.4900	10.6225	4.1913	*
Within groups (error)	24	60.8255	2.5344		
Total	29	118.2500			

Regression coefficient b = -0.063

(e) Penicillium notatum. α-4-chloro-2-methylphenoxypropionic acid (Table 1 etc.)

Source of variation	df	SS	MS	F	
Among groups	5	228.2500	45.6500	38.15	***
Linear regression	1	148.5365	148.5365	74.50	**
Deviations from regression	4	79.7135	19.9284	16.74	***
Within groups (error)	24	28.5750	1.1906		
Total	29	256.8250			

Regression coefficient b = -0.1977

(f) Penicillium notatum. β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid (Table 13)

Source of variation	df	SS	MS	F	
Among groups	5	18.6417	3.7283	1.2467	n.s.
Linear regression	1	16.8550	16.8550	37.5557	**
Deviations from regression	4	1.7950	0.4488	0.1501	n.s.
Within groups (error)	24	71.7950	2.9906		
Total	29	90.4167			

Regression coefficient b = -0.0666

APPENDIX D 8. (cont'd)

(g) Penicillium notatum. 2,4,5-trichlorophenoxyacetic acid (Table 1)

Source of variation	df	SS	MS	F
Among groups	5	24.5874	4.9175	17.2665 ***
Linear regression	1	13.9440	13.9440	6.4371 n.s.
Deviations from regression	4	8.6649	2.1662	7.6060 **
Within groups (error)	12	3.4176	0.2848	
Total	17	28.0050		

Regression coefficient b = -0.0782

(h) Ascochyta pisi. 4-chloro-2-methylphenoxyacetic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	149.7604	29.9521	3.1622 *
Linear regression	1	99.1505	99.1505	7.7386 *
Deviations from regression	4	51.2495	12.8124	1.3527 n.s.
Within groups (error)	24	227.3250	9.4719	
Total	29	377.0854		

Regression coefficient b = +0.1616

(i) Ascochyta pisi. α-4-chloro-2-methylphenoxypropionic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	67.4750	13.4950	1.7162 n.s.
Linear regression	1	0.3800	0.3800	0.0217 n.s.
Deviations from regression	4	69.9600	17.4900	2.2242 n.s.
Within groups (error)	24	188.7250	7.8635	
Total	29	256.2000		

Regression coefficient b =-0.01

APPENDIX D 8. (cont'd)

(j) Corticium solani 4-chloro-2-methylphenoxyacetic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	576.9750	115.3950	44.7771 ***
Linear regression	1	409.2805	409.2805	9.7625 *
Deviations from regression	4	167.6945	41.9236	16.2677 ***
Within groups (error)	24	61.8500	2.5771	
Total	29	638.8250		

Regression coefficient b =-0.3260

(k) Corticium solani. α-4-chloro-2-methylphenoxypropionic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	73.3417	14.6683	5.6326 **
Linear regression	1	45.3600	45.3600	6.4846 n.s.
Deviations from regression	4	27.9800	6.9950	2.6860 n.s.
Within groups (error)	24	62.5000	2.6042	
Total	29	135.8417		

Regression coefficient b = -0.1090

(l) Microsporum canis. 4-chloro-2-methylphenoxyacetic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	74.3417	14.8683	9.94 ***
Linear regression	1	33.0900	33.0900	3.2087 n.s.
Deviations from regression	4	41.2500	0.3125	6.8943 ***
Within groups (error)	24	35.9000	1.4958	
Total	29	110.2418		

Regression coefficient b = +0.093

APPENDIX D 8. (cont'd)

(m) Microsporum canis. α-4-chloro-2-methylphenoxypropionic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	19.3000	3.8600	1.2389 n.s.
Linear regression	1	0.4300	0.4300	0.0911 n.s.
Deviations from regression	4	18.8700	4.7175	1.5142 n.s.
Within groups (error)	24	74.7750	3.1156	
Total	29	94.0750		

Regression coefficient b = -0.011

(n) Microsporum canis. α-4-chloro-2-methylphenoxybutyric acid (Table 15)

Source of variation	df	SS	MS	F
Among groups	5	120.9312	24.1862	3.1292 *
Linear regression	1	114.3500	114.3500	69.4588 **
Deviations from regression	4	6.5850	1.6463	0.2130 n.s.
Within groups (error)	24	185.5000	7.7292	
Total	29	306.4312		

Regression coefficient b = -0.174

(o) Microsporum canis. β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid (Table 14)

Source of variation	df	SS	MS	F
Among groups	5	91.1500	38.2300	5.5912 **
Linear regression	1	55.8650	55.8650	1.6802 n.s.
Deviations from regression	4	133.0000	33.2500	4.8629 **
Within groups (error)	24	164.1000	6.8375	
Total	29	355.2500		

Regression coefficient b = -0.121

APPENDIX D 8. (cont'd)

(p) Mucor hiemalis. 4-chloro-2-methylphenoxyacetic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	21.7750	4.3550	5.8716 **
Linear regression	1	4.6450	4.6450	1.6846 n.s.
Deviations from regression	4	17.1300	4.2825	5.7739 **
Within groups (error)	24	17.8000	0.7417	
Total	29	39.5750		

Regression coefficient b = -0.035

(q) Mucor hiemalis. α-4-chloro-2-methylphenoxypropionic acid (Table 5)

Source of variation	df	SS	MS	F
Among groups	5	309.5705	61.9141	27.1517 ***
Linear regression	1	155.8750	155.8750	4.0586 n.s.
Deviations from regression	4	153.6250	38.4063	16.8427 ***
Within groups (error)	24	54.7260	2.2803	
Total	29	364.2965		

Regression coefficient b = -0.203

APPENDIX E

Experimental data

ASPERGILLUS NIGER

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration(ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
γ-4-chlorophenoxybutyric acid	1.5 ± 0.25	(no test)	20.2 ± 0.15	30.7 ± 0.29*	(no test)	33.9 ± 0.86	237
γ-2-methylphenoxy butyric acid	5.7 ± 0.37	12.0 ± 0.52	30.4 ± 0.38	33.7 ± 0.46	39.5 ± 0.42	39.8 ± 0.76	290
2, 4-dichlorophenoxyacetic acid	24.0 ± 0.72	26.9 ± 0.23	38.7 ± 0.72	(no test)	(no test)	41.6 ± 0.49	1210
α-2, 4-dichlorophenoxypropionic acid	9.8 ± 0.49	12.8 ± 0.93	35.5 ± 0.43	47.7 ± 0.56	(no test)	40.7 ± 0.62	324
β-2, 4-dichlorophenoxypropionic acid	0.0	4.7 ± 0.35	22.0 ± 0.13	37.5 ± 0.22	(no test)	41.6 ± 0.49	140
γ-2, 4-dichlorophenoxybutyric acid	1.3 ± 0.09	2.0 ± 0.31	26.2 ± 0.75	35.5 ± 0.67	(no test)	41.6 ± 0.49	190
δ-2, 4-dichlorophenoxyvaleric acid	0.0	1.7 ± 0.11	32.2 ± 0.40	39.4 ± 0.43	42.6 ± 0.25	51.0 ± 0.27	114
2, 6-dichlorophenoxyacetic acid	27.4 ± 0.49	(no test)	43.9 ± 0.40	42.2 ± 0.66*	(no test)	33.9 ± 0.86	6560
2, 6-dimethylphenoxyacetic acid	38.3 ± 0.98	(no test)	46.8 ± 0.26	43.8 ± 0.34*	(no test)	33.9 ± 0.86	non-toxic
4-chloro-2-methylphenoxyacetic acid	11.2 ± 0.89	17.8 ± 0.58	22.9 ± 0.50	38.5 ± 0.73	45.2 ± 0.85	42.2 ± 0.56	230
α-4-chloro-2-methylphenoxypropionic acid	30.7 ± 1.04	28.7 ± 0.71	34.0 ± 0.54	39.4 ± 0.54	46.9 ± 1.55	48.8 ± 0.60	713
α-4-chloro-2-methylphenoxybutyric acid	9.7 ± 0.56	14.2 ± 0.46	24.8 ± 0.97	33.8 ± 0.37	46.9 ± 0.67	39.2 ± 0.75	270
γ- 4-chloro-2-methylphenoxybutyric acid	0.0	4.9 ± 0.19	13.7 ± 0.28	22.8 ± 0.59	37.0 ± 0.64	40.4 ± 0.17	64
γ-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	0.0	4.3 ± 0.08	7.9 ± 0.46	16.8 ± 0.18	35.6 ± 0.64	36.6 ± 1.06	46
γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	(no test)	1.0 ± 0.22	7.1 ± 0.10	15.8 ± 0.32	32.7 ± 0.66	36.3 ± 0.73	49
γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid	2.1 ± 0.25	8.9 ± 0.32	9.9 ± 0.40	16.3 ± 0.26	32.4 ± 0.95	34.5 ± 0.59	55
ε-4-chloro-2-methylphenoxycaproic acid	0.0	1.5 ± 0.26	26.8 ± 0.14	37.4 ± 0.34	49.4 ± 0.27	53.0 ± 0.16	112
2, 4, 6-trichlorophenoxyacetic acid	16.1 ± 0.62	(no test)	26.3 ± 0.49	34.0 ± 0.63	(no test)	33.9 ± 0.86	818
γ-2-methyl-4, 6-dichlorophenoxybutyric acid	0.0	(no test)	16.7 ± 0.19	25.3 ± 0.12*	(no test)	33.9 ± 0.86	94

* 10 ppm

ASCOCHYTA PISI

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	46.5 ± 1.43	45.2 ± 1.48	44.7 ± 1.30	44.8 ± 0.85	42.6 ± 1.63	39.6 ± 1.35	non-toxic (R)
∝-4-chloro-2-methylphenoxypropionic acid	38.5 ± 1.45	38.5 ± 1.50	41.6 ± 1.35	37.8 ± 1.48	37.1 ± 0.82	40.0 ± 0.65	non-toxic (R)
ɣ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	7.3 ± 0.26	20.1 ± 1.21	29.8 ± 0.81	36.5 ± 1.15	46

CORTICIUM SOLANI

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	68.5 ± 0.98	70.1 ± 0.39	80.9 ± 0.93	76.8 ± 0.49	76.8 ± 0.78	77.6 ± 0.51	16900 (R)
∝-4-chloro-2-methylphenoxypropionic acid	63.6 ± 0.83	63.7 ± 0.74	64.5 ± 0.57	64.6 ± 0.51	68.0 ± 0.96	66.3 ± 0.62	non-toxic (R)
ɣ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	28.5 ± 0.50	36.8 ± 1.23	58.5 ± 0.81	58.1 ± 0.64	94

GIBBERELLA ZEAE

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	51.7 ± 0.77	64.3 ± 0.58	66.8 ± 0.86	66.9 ± 0.78	76.7 ± 0.49	76.4 ± 0.40	3250
α-4-chloro-2-methylphenoxypropionic acid	55.7 ± 1.12	62.9 ± 1.00	74.7 ± 0.80	76.0 ± 1.23	79.6 ± 1.33	81.0 ± 0.84	2600
α-4-chloro-2-methylphenoxybutyric acid	40.5 ± 0.35	53.5 ± 0.42	62.6 ± 0.81	64.8 ± 0.90	60.2 ± 0.60	62.5 ± 0.50	1730
γ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	28.1 ± 0.39	37.7 ± 0.44	57.8 ± 0.47	64.5 ± 0.78	77
γ-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	1.1 ± 0.32	6.9 ± 0.20	28.9 ± 0.32	43.0 ± 1.44	61.8 ± 1.15	55.9 ± 0.98	150
γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	5.3 ± 0.41	21.4 ± 0.62	52.9 ± 0.56	59.9 ± 0.46	77.5 ± 1.37	85.0 ± 0.32	190
γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid	0.0	2.4 ± 0.22	42.0 ± 0.92	51.5 ± 1.77	69.7 ± 2.03	72.9 ± 2.27	137
γ-4-chloro-2-methylphenoxycrotonic acid	(no test)	11.4 ± 0.64	30.7 ± 0.34	35.1 ± 0.53	47.3 ± 1.02	55.1 ± 0.60	144
β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid	39.9 ± 1.62	50.2 ± 2.92	57.0 ± 1.19	61.2 ± 0.51	65.6 ± 0.99	53.6 ± 2.01	2430
ε-4-chloro-2-methylphenoxycaproic acid	0.0	0.0	19.4 ± 1.09	22.9 ± 0.41	48.0 ± 0.72	53.2 ± 0.64	55

MICROSPORUM CANIS

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	42.6 ± 0.53	41.2 ± 0.68	42.6 ± 0.24	40.9 ± 0.51	40.7 ± 0.73	37.9 ± 0.43	non-toxic (R)
α-4-chloro2-methylphenoxypropionic acid	30.1 ± 0.50	30.0 ± 0.69	28.5 ± 0.45	29.4 ± 0.56	30.3 ± 0.66	31.1 ± 1.27	non-toxic (R)
α-4-chloro-2-methylphenoxybutyric acid	32.0 ± 0.63	34.2 ± 0.75	35.6 ± 2.44	35.5 ± 0.71	37.5 ± 1.20	38.0 ± 0.65	11450 (R)
γ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	24.8 ± 0.43	32.8 ± 0.20	33.8 ± 0.51	44.5 ± 1.16	121
γ-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	0.0	7.3 ± 0.51	37.2 ± 0.56	40.0 ± 0.76	46.9 ± 1.33	46.9 ± 0.58	231
γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	0.0	0.0	22.9 ± 0.23	29.2 ± 0.77	36.1 ± 1.17	43.4 ± 1.47	123
γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid	0.0	4.8 ± 0.25	28.1 ± 0.43	31.2 ± 0.77	36.2 ± 1.33	35.9 ± 0.98	177
δ-4-chloro-2-methylphenoxycrotonic acid	0.0	11.1 ± 0.43	37.4 ± 0.24	38.7 ± 1.24	39.7 ± 1.02	38.9 ± 1.33	335
β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid	35.3 ± 1.31	35.1 ± 1.04	32.9 ± 1.06	35.4 ± 1.06	38.2 ± 0.85	40.7 ± 0.46	non-toxic (R)
ε 4-chloro-2-methylphenoxycaproic acid	0.0	0.0	0.0	25.7 ± 0.56	36.8 ± 1.07	39.9 ± 1.08	110

MUCOR HIEMALIS

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	51.4 ± 0.25	51.3 ± 0.25	52.7 ± 0.46	50.0 ± 0.35	52.3 ± 0.26	52.9 ± 0.60	non-toxic (R)
α-4-chloro-2-methylphenoxypropionic acid	38.9 ± 0.43	35.0 ± 1.18	37.9 ± 0.41	39.7 ± 0.37	43.8 ± 0.68	44.1 ± 0.55	12276 (R)
α-4-chloro-2-methylphenoxybutyric acid	23.0 ± 1.58	29.9 ± 0.93	40.3 ± 0.89	43.4 ± 0.62	42.5 ± 0.27	42.9 ± 0.51	1156
γ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	10.5 ± 0.42	22.6 ± 2.19	40.1 ± 0.29	41.6 ± 0.40	55
γ-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	0.0	0.0	20.0 ± 0.27	30.1 ± 0.33	39.3 ± 0.80	40.3 ± 0.90	98
γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	0.0	0.0	5.3 ± 0.56	15.4 ± 1.28	40.7 ± 0.34	36.6 ± 0.53	38
γ-(4-chloro-2-methyl)-δ-methylphenoxybutyric acid	0.0	0.0	3.8 ± 0.37	23.9 ± 0.45	35.8 ± 1.36	53.8 ± 1.45	21
ε-4-chloro-2-methylphenoxycaproic acid	0.0	0.0	7.5 ± 0.55	17.8 ± 0.60	43.1 ± 1.18	50.6 ± 2.03	35

PENICILLIUM NOTATUM

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
phenoxyacetic acid	23.6 ± 0.53	21.8 ± 0.53	20.5 ± 0.39	21.4 ± 0.56	20.3 ± 0.51	21.0 ± 0.26	non-toxic
α-phenoxypropionic acid	16.0 ± 0.93	13.4 ± 0.28	15.8 ± 0.52	14.5 ± 0.45	13.4 ± 0.29	14.3 ± 0.25	non-toxic
γ-phenoxybutyric acid	10.3 ± 0.16	12.1 ± 0.23	14.7 ± 0.15	17.3 ± 0.29	19.3 ± 0.42	17.8 ± 0.98	1436
δ-4-chlorophenoxybutyric acid	8.3 ± 0.56	(no test)	17.5 ± 0.20	24.3 ± 0.35*	(no test)	27.1 ± 0.16	365
γ-2-methylphenoxybutyric acid	10.7 ± 0.10	11.1 ± 0.34	13.4 ± 0.38	11.7 ± 0.27	14.4 ± 0.67	17.7 ± 0.15	1253
2, 4-dichlorophenoxyacetic acid	19.5 ± 0.76	19.8 ± 0.30	19.6 ± 0.22	20.8 ± 0.34	19.5 ± 0.45	21.3 ± 0.33	28560 (R)
α-2, 4-dichlorophenoxypropionic acid	12.7 ± 0.43	16.7 ± 0.35	19.3 ± 0.26	19.2 ± 0.38	23.8 ± 0.26	17.4 ± 0.38	2088
β-2, 4-dichlorophenoxypropionic acid	3.5 ± 0.24	12.1 ± 0.49	14.9 ± 0.38	16.4 ± 0.52	(no test)	23.6 ± 0.46	500
γ-2, 4-dichlorophenoxybutyric acid	1.9 ± 0.19	3.3 ± 0.30	12.0 ± 0.36	14.0 ± 0.73	22.8 ± 0.14	25.5 ± 0.64	80
β-2, 4-dichlorophenoxyisobutyric acid	(no test)	12.1 ± 0.36**	12.2 ± 0.17	14.2 ± 0.84	15.1 ± 0.33	13.9 ± 0.06	986
γ-2, 4-dichlorophenoxycrotonic acid	3.5 ± 0.53	10.5 ± 0.35	11.8 ± 0.53	12.6 ± 0.54	22.6 ± 0.61	22.9 ± 0.74	177
γ-2, 4-dichlorophenoxyvinylacetic acid	1.3 ± 0.40	3.0 ± 0.33	10.2 ± 0.30	14.7 ± 0.46	26.5 ± 0.49	28.2 ± 0.38	14
β-hydroxy-γ-2, 4-dichlorophenoxybutyric acid	18.5 ± 1.19	20.1 ± 0.19	21.1 ± 0.94	18.2 ± 0.34	20.0 ± 0.65	22.2 ± 0.40	non-toxic (R)
δ-2, 4-dichlorophenoxyvaleric acid	0.0	0.0	22.1 ± 0.37	30.0 ± 0.32	34.8 ± 0.90	37.3 ± 0.72	160
ε-2, 4-dichlorophenoxycaproic acid	0.0	2.4 ± 0.38	10.5 ± 0.17	11.1 ± 0.37	11.9 ± 0.38	19.1 ± 0.33	112
2, 6-dichlorophenoxyacetic acid	22.1 ± 0.67	(no test)	25.0 ± 0.12	25.7 ± 0.15*	(no test)	27.1 ± 0.16	8280
2, 6-dimethylphenoxyacetic acid	21.6 ± 0.05	(no test)	24.2 ± 0.22	25.6 ± 0.17*	(no test)	27.1 ± 0.16	6400
4-chloro-2-methylphenoxyacetic acid	25.3 ± 0.51	25.5 ± 0.48	25.9 ± 0.23	26.0 ± 0.55	27.9 ± 1.01	29.3 ± 0.58	17030 (R)
α-4-chloro-2-methylphenoxypropionic acid	18.9 ± 0.52	24.1 ± 0.73	22.0 ± 0.50	26.4 ± 0.33	26.9 ± 0.26	25.3 ± 0.46	5700 (R)
α-4-chloro-2-methylphenoxybutyric acid	8.7 ± 0.26	10.6 ± 0.53	12.3 ± 0.58	13.8 ± 0.84	14.9 ± 0.39	17.8 ± 0.73	960
γ-4-chloro-2-methylphenoxybutyric acid	1.8 ± 0.06	3.4 ± 0.23	7.0 ± 0.46	9.8 ± 0.26	21.6 ± 0.33	22.2 ± 0.33	52
ε-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	0.9 ± 0.06	2.0 ± 0.21	6.0 ± 0.41	7.2 ± 0.10	15.3 ± 0.35	17.8 ± 0.73	46
δ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	1.4 ± 0.17	5.6 ± 0.48	9.1 ± 0.22	10.2 ± 0.34	15.2 ± 0.46	16.8 ± 0.20	121
γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid	1.1 ± 0.09	1.2 ± 0.15	6.0 ± 0.21	9.0 ± 0.36	17.8 ± 0.48	23.9 ± 0.19	34
δ-4-chloro-2-methylphenoxycrotonic acid	(no test)	9.1 ± 0.36	10.9 ± 1.00	13.4 ± 0.20	19.3 ± 0.26	22.7 ± 0.12	90
δ-4-chloro-2-methylphenoxyvinylacetic acid	6.5 ± 0.41	9.7 ± 0.52	10.5 ± 0.40	15.1 ± 0.43	20.0 ± 0.53	21.0 ± 0.69	110
β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid	18.8 ± 0.22	19.1 ± 0.94	20.1 ± 0.46	20.2 ± 0.68	20.3 ± 1.34	21.2 ± 0.29	non-toxic (R)
ε-4-chloro-2-methylphenoxycaproic acid	0.0	3.7 ± 0.47	14.3 ± 0.37	15.2 ± 0.40	20.6 ± 0.17	26.3 ± 0.21	117
2, 4, 5-trichlorophenoxyacetic acid	23.3 ± 0.25	23.1 ± 0.08	23.4 ± 0.22	24.5 ± 0.63	24.6 ± 0.22	26.3 ± 0.08	24650 (R)
α-2, 4, 5-trichlorophenoxypropionic acid	(no test)	20.0 ± 0.10	27.9 ± 0.45	29.5 ± 0.65	31.0 ± 1.02	32.4 ± 0.48	936
γ-2, 4, 5-trichlorophenoxybutyric acid	(no test)	2.2 ± 0.10	8.2 ± 0.09	10.5 ± 0.32	19.4 ± 0.22	29.7 ± 0.17	25
2, 4, 5-trichlorophenoxyacetic acid	16.7 ± 0.37	(no test)	21.8 ± 0.18	24.3 ± 0.71*	(no test)	27.1 ± 0.16	900
δ-6-nitro-2, 4-dichlorophenoxybutyric acid	0.0	0.0	8.3 ± 0.97	12.6 ± 0.20	13.4 ± 0.64	16.2 ± 0.14	130
γ-2-methyl-4, 6-dichlorophenoxybutyric acid	1.7 ± 0.51	(no test)	10.7 ± 0.19	17.6 ± 0.39*	(no test)	27.1 ± 0.16	77

* 10 ppm ** 200 ppm

PYRENOPHORA AVENAE

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	9.6 ± 0.62	24.1 ± 0.89	28.9 ± 0.24	34.3 ± 1.26	42.1 ± 3.42	29.7 ± 0.62	767
α-4-chloro-2-methylphenoxypropionic acid	39.6 ± 1.30	38.4 ± 1.50	50.9 ± 2.08	51.5 ± 1.27	69.8 ± 2.03	66.9 ± 1.04	1037
α-4-chloro-2-methylphenoxybutyric acid	5.7 ± 0.81	12.6 ± 2.12	19.7 ± 3.20	39.0 ± 3.90	46.8 ± 1.89	45.7 ± 1.04	121
γ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	22.2 ± 0.49	36.8 ± 1.31	79.7 ± 0.51	79.5 ± 0.47	55
γ-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	0.0	0.0	15.1 ± 0.51	24.3 ± 1.29	54.7 ± 1.77	53.5 ± 0.92	55
γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	0.0	0.0	6.0 ± 0.97	7.8 ± 1.25	23.4 ± 1.11	22.0 ± 0.52	52
γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid	0.0	0.0	13.2 ± 2.15	19.4 ± 1.53	45.0 ± 2.85	51.7 ± 1.99	40
δ-4-chloro-2-methylphenoxycrotonic acid	(no test)	0.0	8.0 ± 0.27	11.8 ± 0.96	24.8 ± 2.01	26.1 ± 1.40	55
β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid	20.3 ± 1.45	35.1 ± 1.83	39.9 ± 1.49	27.7 ± 1.69	42.4 ± 2.16	47.8 ± 1.66	888
ε-4-chloro-2-methylphenoxycaproic acid	0.0	0.0	12.3 ± 0.27	15.6 ± 0.93	35.7 ± 1.48	38.0 ± 2.49	53

PYTHIUM DEBARYANUM

EXPERIMENTAL RESULTS, EXPRESSED AS MEAN COLONY DIAMETER IN mm.

Chemical	Concentration (ppm) 1000	500	100	50	5	Control No addition	ED 50 (ppm)
4-chloro-2-methylphenoxyacetic acid	0.0	15.9 ± 0.54	44.5 ± 1.43	49.0 ± 0.74	53.9 ± 0.87	54.9 ± 0.62	300
α-4-chloro-2-methylphenoxypropionic acid	4.7 ± 0.20	19.6 ± 0.48	44.5 ± 0.65	47.7 ± 0.49	48.6 ± 0.75	48.5 ± 0.35	424
α-4-chloro-2-methylphenoxybutyric acid	(no test)	25.2 ± 1.02	38.3 ± 0.70	48.8 ± 1.93	64.5 ± 1.99	63.9 ± 1.24	144
γ-4-chloro-2-methylphenoxybutyric acid	0.0	0.0	15.3 ± 0.56	25.3 ± 0.82	42.5 ± 0.42	45.5 ± 1.52	61
β-(4-chloro-2-methyl)-α-methylphenoxybutyric acid	0.0	3.8 ± 0.20	27.4 ± 0.76	37.1 ± 1.04	54.5 ± 1.05	57.1 ± 0.48	92
γ-(4-chloro-2-methyl)-β-methylphenoxybutyric acid	0.0	0.0	13.4 ± 0.93	28.4 ± 0.32	44.9 ± 1.65	48.4 ± 2.59	58
γ-(4-chloro-2-methyl)-γ-methylphenoxybutyric acid	0.0	0.0	17.3 ± 1.08	28.0 ± 1.38	41.7 ± 0.68	43.7 ± 0.41	74
γ-4-chloro-2-methylphenoxycrotonic acid	(no test)	1.6 ± 0.51	28.4 ± 0.58	32.4 ± 1.44	59.1 ± 3.04	55.9 ± 1.58	93
β-hydroxy-γ-4-chloro-2-methylphenoxybutyric acid	2.1 ± 0.52	16.7 ± 2.21	53.2 ± 2.21	60.3 ± 1.33	60.8 ± 1.37	66.7 ± 1.63	275
ε-4-chloro-2-methylphenoxycaproic acid	0.0	0.0	10.7 ± 0.60	33.9 ± 0.89	66.4 ± 1.31	68.5 ± 1.58	49