STUDIES IN THE PRODUCTION AND EFFECTS OF VOLATILE FUNGAL METABOLITES.

A thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Science.

by

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GENERAL INTRODUCTION

Previous work in this department has shown that many fungi may produce volatile metabolites, other than carbon dioxide, which can affect the growth of living things. These effects may result from the action of a single metabolite, or from the action of a mixture of several metabolites including common respiratory ones. The work reported in this thesis was designed to extend these observations; it has comprised:-

I. The continuation of a survey of the genus Fomes for the occurrence of volatile metabolites and the identification and examination of effects of biologically active substances seen during this survey.

Section IA describes a survey of a wide range of species of the genus Fomes. This work was designed primarily to obtain information relating to the distribution of volatile metabolites with biological activity within a smaller taxonomic group than had been examined in detail in previous surveys. It was approached along two lines: survey by biological assay of culture gases and survey by gas chromatographic analysis of culture gases.

During this survey several points of interest arose. Some of these were examined further and are described in subsequent sections:-

Section IB describes the investigation of the biological activity of culture gases from Fomes scutellatus, a species shown to produce culture gases with biological activity during the survey of the genus Fomes. This investigation was brought to a successful conclusion by the identification of hydrogen cyanide in the culture gases and the

demonstration that the concentrations of this metabolite detected in the gases of F. scutellatus could, under certain conditions, account for the biological activity of these gases.

Section IC describes an investigation of the biological activity of the volatile metabolites of Fomes noxius, another species shown to produce culture gases with biological activity in the survey of the genus Fomes. This investigation has only been partially completed. The metabolites so far identified in the culture gases of this species cannot account for the biological activity observed under the conditions examined. The possibility of the presence of additional components in the culture gases is partially investigated and discussed.

II. The commencement of a survey of the genus Clitocybe for the occurrence of volatile metabolites and the identification and examination of the effects of biologically active substances seen during this survey.

Section IIA describes a survey of a range of species of the genus Clitocybe. This work was done as a logical extension to the Fomes survey and, except in the inclusion of an analysis for hydrogen cyanide (HCN) production, was carried out in a similar way: survey by biological assay, survey by gas chromatographic analysis of culture gases and survey by examination for HCN production.

Section IIB describes a brief analysis of the effects of individual culture gases produced by four of the species which were found to be biologically active during the survey.

III. This section describes a reinvestigation of the effects of fungal volatiles on zygospore production by Rhizopus sexualis (Smith) Callen.

This developed from previous survey work carried out by Martin (1963) by invitation from Professor Hawker.

IV. This section describes the extension of laboratory observations to the possible part such metabolites may play in ecological interactions in nature.

This consists of a brief study of the effects of the culture gases of F. scutellatus and of F. noxius acting through a thin layer of soil. This section is intended only as a preliminary study. The implications of this work in relation to possible extension of work along this line are discussed.

REVIEW OF THE LITERATURE ON VOLATILE METABOLITE PRODUCTION BY FUNGI

Up until about 1962 there were very few reports of volatile fungal metabolites affecting living things. Apart from carbon dioxide, only a small number of these biologically active metabolites had been identified.

Ethylene was one of the most commonly reported compounds. It was identified from Blastomyces dermatidis and Histoplasma capsulatum (Nickerson, 1948) using the 'triple response of pea seedlings' described by Knight & Crocker (1913) as a means of identifying this compound. Ethylene was also suspected as a gaseous product of Penicillium digitatum by Miller, Winston & Fisher (1940) and by Biale (1940) and its identity subsequently confirmed by chemical tests by Young, Pratt & Biale (1951) and by Fergus (1954), using the 'triple response'. In recent studies of ethylene, Stahmann, Clare & Woodbury (1966) have shown that it is also produced by sweet potato tissue infected with black rot where it can increase resistance to disease and induce enzyme activity in infected tissue. In addition, there is an increasing interest in the interaction of this compound with plant growth hormones, e.g. with gibberellin (Scott & Leopold, 1967), with indoleacetic acid (Burg & Burg, 1967; Chadwick & Burg, 1970), stressing the likely significance of the rôle this compound may play in living systems.

An observation on the inhibition of germination of uredospores of Puccinia graminis var. tritici by gases produced by the same species (Allen, 1955) lead to the identification of trimethylethylene as the volatile

metabolite responsible for this effect (Forsyth, 1955). In addition, Allen (1957) and French, Massey & Weintraub (1957) detected a volatile substance also produced which could overcome the self-inhibition of germination of uredospores and stimulate the formation of infection structures. Pelargonaldehyde (n-nonanal) was identified as the substance responsible for this stimulation (French & Weintraub, 1957). Subsequently French (1961) showed that many related unsaturated terpene aldehydes, several straight chain and terpene alcohols and many terpenoid hydrocarbons were also active. He suggests that active compounds may function as the substrate required to remove a block in metabolism produced by an endogenous inhibitor.

Trimethylethylene has also been identified as a self-inhibitor from uredospores of Uromyces phaseoli (Yarwood, 1956) and from Tilletia levis (Hanna, Vickery & Pucher, 1932). Pelargonaldehyde has been identified as a stimulant, promoting growth of several wood rotting fungi (Fries, 1960). In this case, however, it was detected as a volatile product of decaying wood and not as a fungal product.

Ammonia was identified as the substance produced by Mucor plumbens which could account for the stimulation of sporangia production in Pilobolus kleinii by gases from M. plumbens (Page, 1959).

The production of gaseous hydrogen cyanide by fungi and its biological effects have been reported by several authors. These are reviewed in Sections IB and IIA of this thesis.

Finally, Challenger, Higgenbottom & Ellis (1933) identified trimethylarsine and dimethylarsine as the toxic volatiles produced by

Penicillium brevicaule growing in rotting wallpaper.

The small number of investigations in which the active gaseous metabolites were identified is probably accounted for by the lack of analytical techniques suitable for the detection of gaseous products. This is especially likely in view of the low concentrations in which some of these volatiles have since been shown to be active. Another factor contributing to this is the difficulty in obtaining samples in a suitable form for analysis by traditional chemical techniques from the conditions in which the biological effects can be demonstrated.

By 1959, more refined means of chemical analysis were becoming available. Mass spectrometry was used by McTeague, Hutchinson & Reed (1959) to identify a previously unidentified volatile substance produced by the mycelium of *Agaricus campestris*. This compound is responsible for stimulating the germination of spores of the same species, a phenomenon also reported by Stănek (1959). In this publication, McTeague *et al.* suggested that 2,3-dimethyl-1-pentene was the substance involved, but this was not verified since no synthetic material was available. Subsequent work has caused them to modify their suggestion (Lösel, 1964) in view of the recognized activity of phosphorous pentoxide, the drying agent used by them in the preparation for spectrometry. The suggestion put forward is that the drying treatment had converted iso-amyl alcohol or iso-valeric acid, both of which were shown in *A. campestris* (Lösel, 1964 and 1967) to stimulate spore germination, into 2,3-dimethyl-1-pentene. No confirmation of the presence of this compound

was obtained from gas chromatographic analysis of the culture gases of A. campestris by Lockard & Kneebone (1962). However, stimulation of spore germination with authentic samples of ethyl alcohol, ethyl acetate, acetaldehyde, ethylene and acetylene, the compounds they, and recently Tschierpe & Sinden (1965), identified could not be demonstrated (Lösel, 1964).

There were several records in the literature at this time which described instances where the biological activity of volatile fungal metabolites had been observed, but no identification successfully undertaken.

The volatile antibiotics reported in the genus Trichoderma (Bilai, 1956; Dymovych, 1960; Martin, 1963) remain unidentified as do the volatile metabolites produced by colonies of wild yeast and Torulopsis sanguinea reported to stimulate germination of various Gasteromycetes and Hymenomycetes (Fries, 1943). Similarly, the compound produced by Mucor spinosus which stimulates the growth of Phytophthora citrophthora has not been identified (Bitancourt & Rosetti, 1951).

There are several reports of volatile metabolites affecting sexual reproduction in fungi. Moreau & Moruzi (1931) claimed that the production of fertile perithecia in Neurospora crassa is not dependent upon physical contact between opposite strains, but they may form after gaseous contact between '+' and '-' strains. Banbury (1954 and 1955) reported that zygophores of the two mating types in Mucor mucedo produced different volatile metabolites, each of which stimulated growth of zygophores of its own mating type and retarded growth in zygophores of the opposite type. Plempel (1962) confirmed the presence of a volatile attractant, but could

not demonstrate the mutual repulsion of zygophores from like strains.

A volatile stimulant evolved by zygospore producing cultures of Rhizopus sexualis has been reported by Hepden & Hawker (1961). This metabolite, which is discussed more fully in Section III of this thesis, stimulates zygospore production at temperatures normally too low to allow zygospores to be produced. Again, no identification of this compound has been made although there were indications that a basic compound, which was not ammonia, but might be methylamine, was involved.

The most commonly detected metabolite at this time was, of course, carbon dioxide and its universal occurrence as a product of respiration of fungi and other organisms well known.

The effects of carbon dioxide on higher plants are very variable depending to a great extent on other environmental factors as well as the tolerance of the particular plant. Kidd (1914) reached the conclusion that many plants may require the presence of a minimal amount of carbon dioxide for germination to occur. On the other hand, higher concentrations may inhibit germination. The roots of plants growing in soil tend to be subjected to concentrations of carbon dioxide higher than atmospheric and are thought to be commonly tolerant of concentrations of up to about 9-10% (e.g. Russell, 1961). However, it is not clear to what extent these effects may be due to other factors such as oxygen deficiency in environments where carbon dioxide concentrations are high. It is accepted that small increases in atmospheric carbon dioxide concentration enhance photosynthesis, although high levels may be toxic.

Bacteria are generally considered to be tolerant of high carbon dioxide concentrations.

The effects of carbon dioxide on fungi are also variable. Early observations on the activity of carbon dioxide on fungi were made by Brown (1922). He reported that the growth of several fruit-rotting fungi, including species of Botrytis, Fusarium and Alternaria, was reduced in the presence of atmospheres containing 10% and 20% carbon dioxide whereas concentrations of 2% had little effect. He does, however, stress that the generally inhibitory effects of high concentrations of carbon dioxide could be modified by other environmental factors such as temperature and pH. An apparent contradiction of Brown's report by Rippel & Heilmann (1930), who found that Botrytis cinerea was stimulated by concentrations of up to 2% carbon dioxide, can be explained by the fact that Rippell et al. based their comparisons on observations of growth in air free of carbon dioxide whilst Brown used ordinary air as a control environment. Barinova (1961), also using air free from carbon dioxide as a control, reported stimulation of growth of Aspergillus niger and Rhizopus sexualis in the presence of carbon dioxide.

Other reports of carbon dioxide producing morphological effects include those by Barnett & Lilly (1958) who showed that accumulation of carbon dioxide in poorly ventilated vessels containing Choanephora cucurbitarum could lead to inhibition of sporulation in this species, and by Denny (1933) who found that concentrations of carbon dioxide of up to 32% had no inhibitory effect on

Neurospora sitophila. Bright, Dixon & Whymper (1949) demonstrated the stimulation of sporulation in Saccharomyces cerevisiae as a result of increased carbon dioxide production. In this case, an increase in ethanol production was shown to contribute to the effect.

Stimulatory effects have been reported by several other authors. Platz, Durrell & Howe (1927) showed that concentrations of carbon dioxide up to 15% stimulated germination of chlamydospores of Ustilago zeae. Stimulatory effects on growth have been reported by Golding (1940) with Penicillium roqueforti, by Gundersen (1961) with Fomes annosus and by Thacker & Good (1952) who reported that several species which inhabit the trunks of maple trees, where the carbon dioxide concentrations are usually between 5% and 10%, can tolerate this and higher concentrations of carbon dioxide.

Timonin (1935) examined the effects of carbon dioxide building up in soil and found that the soil fungi he examined could grow well in concentration of the order of that found in soil. Hollis (1948) also studying carbon dioxide in the soil found that Fusarium oxysporum and F. camartii grew in concentrations of carbon dioxide up to 48%. On the other hand, Burges & Fenton (1953) showed that concentrations of over 5% carbon dioxide inhibited the growth of Penicillium nigricans, Gliomastix convoluta, Zygorrhynchus vuillemini and several other fungi which had been growing in the top 5 cm. of soil, whereas Zygorrhynchus taken from a depth of below 10 cm could withstand concentrations of up to 20%. These observations caused them to suggest that carbon dioxide concentration was a critical factor in the distribution of soil fungi and not oxygen level as had been suggested by Bisby, Timonin & James, (1935). The observations of Abeygunawardena & Wood (1957) add

support to this hypothesis. They found that the effect of different carbon dioxide concentrations on the germination of sclerotia of Sclerotium rolfsii depended upon the depth at which the sclerotia had been buried. Durbin (1959), who showed that strains of Rhizoctonia solani isolated from different soil depths varied in carbon dioxide tolerance, also suggested that carbon dioxide played a critical role in determining the habitat of the species he examined.

There have been many other reports of differing carbon dioxide tolerance of soil fungi, including the work of Stover (1958) and Stover & Froiburg (1958) on the survival of Fusarium oxysporum in flooded soils. They showed that this species could tolerate up to 23.5% carbon dioxide in the soil, although concentrations of 2.25% were optimal for multiplication and concentrations of 4.25% optimal for growth.

Many further reports are reviewed by McCauley & Griffin (1969(a)). Their observations confirm those of earlier workers in that concentrations of greater than 0.2% oxygen had no inhibitory effect on the activity of a range of soil fungi, while carbon dioxide concentrations were shown to influence the survival and competitive value of the species examined. Subsequent investigations (McCauley & Griffin 1969(b)) indicated that in soils the bicarbonate ion, rather than carbon dioxide directly, was affecting the growth of the soil fungi examined.

Obviously, the effects of carbon dioxide depend not only upon the tolerance of the fungus to pure carbon dioxide, or the interaction with the physical environment indicated above, but also upon the interaction with

other gases or non-volatile metabolites of that fungus and of other organisms. This has already been mentioned in connection with ethanol (Bright et al., 1949) and will be discussed in more detail later in this review.

The introduction of gas chromatography as a suitable tool for use in biological research has facilitated the identification of volatile products and permitted a more quantitative approach to investigating particular problems. However, despite this, and despite an appreciation of the possible biological significance of volatile metabolite production in biological systems indicated by the earlier isolated reports, the number of investigations of volatile metabolite production in fungi is still small.

Investigation of the occurrence and properties of volatile fungal metabolites has been developed in this Department for several years. Following the report of the stimulation of growth of fern prothalli by the volatile metabolites of several fungi (Hutchinson & Fahim, 1958) a survey was made to examine the occurrence of biologically active volatile metabolites in a large number of fungi selected to represent a wide range of taxonomic groups (Martin, 1963; Dick & Hutchinson, 1966). The results of this survey indicated that the production of biologically active metabolites by fungi was of frequent occurrence, 42 out of 61 species examined producing activity against at least one of the 5 species of fungi used in the biological assay. In addition, the production of these

metabolites was found to show no particular correlation with the taxonomic relationships of the species examined. No successful gas analysis was made at this time. Another survey by Hutchinson (1967), extending previous observations on the effects of volatile fungal metabolites on the growth of fern prothalli, showed that 13 of the 16 species examined stimulated and 2 inhibited the growth of fern prothalli, again indicating the frequency of occurrence of biological effects of volatile fungal metabolites.

As gas chromatographic techniques became suitable for sampling the emanations from cultures, the metabolites produced by the two species shown to be most active in Martin's survey were identified. In the first of these, Fomes annosus, Glen, Hutchinson & McCorkindale (1966) identified hexa-1,3,5-triyne in the culture gases and showed that concentrations of this single unusual metabolite, equal to those produced by F. annosus, could account for the inhibition of growth and sporulation of Aspergillus niger, inhibition of Chaetomium globosum and inhibition of germination of cress seeds observed in the presence of culture gases of this species. In the second, Saccharomyces cerevisiae, a mixture of respiratory products was identified in the culture gases, i.e. carbon dioxide, ethanol, ethyl acetate, n-propanol, isobutanol, and two isopentanols, (Glen & Hutchinson, 1969). They showed that the inhibition of sporulation in A. niger and inhibition of cress seed germination observed in the presence of culture gases of this species could be accounted for by the presence of metabolites at

concentrations equivalent to those detected in yeast gases. Of these, ethanol was shown to be the single most inhibitory substance to growth of A. niger, while carbon dioxide was the most inhibitory to sporulation of this species. Ethanol and 3-methylbutanol were most inhibitory to germination of cress.

The reports of the above compounds as gaseous products of yeast growing under aerobic conditions cannot be compared directly with the extensive studies on the fermentation products of the same species (e.g. Stevens, 1960; Nordström, 1964, 1967), a larger number of compounds possibly reaching detectable concentrations in the fermentation products of this fungus than in the culture gases alone.

Norrman & Fries (1968) and Norrman (1969) carried out an investigation on another species of yeast, Dipodascus aggregatus, similar to that on Saccharomyces cerevisiae described above except that they used the concentrated distillation residues from culture medium and mycelium for gas analysis on which to base biological tests with identified components. A wider range of compounds was detected in this analysis (Norrman, 1969). The major components were ethyl acetate, ethyl propionate and ethanol, although smaller amounts of 26 other compounds, mainly acids and esters were found. Amongst these were two compounds, n-butyl formate and n-propyl n-butyrate, which had not previously been reported as fungal metabolites. This identification on the whole agrees with the previous reports on the metabolites produced by D. aggregatus (Drawert & Rapp, 1966;

Nyman, 1966; Nordström, 1967) although there are some quantitative differences reported by Norrman. Previous to this publication, Norrman & Fries (1968) had reported that various concentrations of many of the compounds subsequently identified in D. aggregatus could stimulate the production of conidia and inhibit the linear growth of Pestalotia rhododendri, although the effects produced by the culture gases of D. aggregatus were different to those produced by the authentic compounds tested.

Respiratory products have also been reported from cultures of Ceratocystis spp. and Thielaviopsis spp. (Collins & Morgan, 1960, 1962; Collins & Kalnins, 1965 (a) and (b), 1966). The following compounds were identified from steam distillates of cultures extracts, methanol, ethanol, acetone, 2-methylpropanal, furfural, 3-methylbutanol, 2-heptanone, acetaldehyde, isobutanol, ethylacetate, isobutyric acid, acetic acid, propionic acid, 2-hexanol, ~~furfuraldehyde~~. Collins & Kalnins (1965 (a)) suggest that some of these products may be active in nature, attracting insects especially Drosophila spp. to mycelium of Ceratocystis fimbriata wilt of oak. Investigations by Dorsey & Leach (1954) report similar attraction of beetles to trees infected with Endoconidiophora fagacearum. In connection with the attraction of insects by volatile emanations of fungi, Muto & Sugwara (1965), and Locquin (1967) examined the attraction of house flies to gases produced by sporophores of Amanita muscaria. The compounds responsible have not been identified

in this case, although Muta et al. (1965) report that an esterified residue of a long chain fatty acid has attractant properties similar to that of A. muscaria.

Ethanol, and to a lesser extent 1-propanol, isopropanol and 1-butanol, have been shown to stimulate rhizomorph production in Armillaria mellea (Weinhold, 1963; Weinhold & Garraway, 1966; Garraway & Weinhold, 1965 1968; Raabe & Hurlimann, 1967). Carbonyl compounds are thought to be responsible for the increased sexual affinity of germinating smut spores, leading to fusion of the promycelial cells (Nielson, 1966). The identity of these compounds is not yet known, nor of the volatile factors released by the smut fungi, their hosts and facultative parasites which cause germination to change from sporadial to mycelial.

Investigations by Smith & Robinson (1969) have shown that the respiratory gases, ethanol and acetaldehyde, are produced by Fusarium oxysporum, Aspergillus niger and Geotrichium candidum and could account for the stimulation of growth of fern prothalli produced by culture gases of these species. These observations could be of relevance in connection with the stimulation reported by Hutchinson (1967).

In the extensive studies of the volatile factors responsible for soil 'sporostasis', (Robinson & Park, 1965; Park & Robinson, 1966; Park, Robinson & Garrett, 1968; Robinson & Garrett, 1969) the gases acetaldehyde, ethanol, acetone, n-propanol, isobutanol, ethyl acetate, isobutylacetate, propionaldehyde and n-butyraldehyde have been detected.

Each substance was shown to be active in concentrations comparable with those in aspirated culture gases.

Hora & Baker (1970) have also reported the induction of soil fungistastis by volatile factors in the soil, but they have made no identification of compounds involved. A similar type of factor may be responsible for the failure of conidia of Glomerella cingulata to germinate under crowded conditions, although, again, no identification has been made (Lingappa & Lingappa, 1966).

The ecological implications of much of this work have recently been receiving more attention, and a publication by Owens, Gilbert, Griebel & Menzies (1969), where the rate of respiration of soil fungi and bacteria was shown to rise in response to the presence of volatile emanations produced by plant residues, stresses the importance of this aspect. Also work on volatile residues of fungicides (Latham & Linn, 1968) and on the stimulatory effect of the volatile sulphur compounds produced by Allium spp. on germination of Sclerotium cepivorum (King & Coley-Smith, 1969(a), (b) and (c)) indicate this trend in present work on volatile metabolites, not only of fungi, but in higher plants.

Most of the work reviewed here has been initiated by biological observations or has had immediate biological implications suggested by it. In addition to this information, however, there are numerous reports of the identification of volatile or potentially volatile metabolites in fungal extracts which have been found in the course of purely analytical

work on fungal metabolites. Miller (1961) reviewed the metabolites reported from fungi and other microbes. A wide range of categories of organic compounds appear in this review, and although many of these are fairly involatile, it is possible that under certain conditions molecules may be released into the atmosphere. Although most of this work has no immediate relevance to reported biological interactions, obviously this chemical information may be shown to be useful in future investigations.

GENERAL METHODS.

GENERAL METHODS

This section describes the methods used generally in many parts of the work. Those used specifically for particular investigations are described separately in the relevant sections.

Throughout this work the term 'test' organism or 'test' solution refers to the organism or solution whose gaseous products were being examined. The term 'assay' organism refers to the organism which was used to measure the biological effect of gases produced by fungal cultures or by standard solutions.

1. Culture Media.

The species used in this work were inoculated on to the surface of one of the following media:-

a) 5% malt agar (50g Oxoid malt extract, 20g Oxoid agar in 1 l deionized water)

b) 2% malt agar (20g Oxoid malt extract, 20g Oxoid agar in 1 l deionized water)

c) 1% malt agar (10g Oxoid malt extract, 20g Oxoid agar in 1 l deionized water)

d) ½% water agar (5g Oxoid agar in 1 l deionized water)

e) Potato dextrose agar (200g potatoes, 20g Oxoid dextrose, 20g Oxoid agar made up to 1 l with deionized water)

f) Bouillon agar (10g Lab. Lemco beef extract, 10g Difco peptone, 5g Na Cl, 20g Oxoid agar in 1 l deionized water, adjusted to pH 7 with Na OH)

Media were autoclaved at 120°C for 20 minutes.

2. Biological Assay Assemblies.

The following types of assay assemblies were used in tests which examined the biological activity of gases from fungal cultures, and of gases from authentic samples of constituents identified in culture gases.

a) Paired petri dish assemblies.

These consist of the bases of two petri dishes (diameter, 9 cm) held together with two crossed rubber bands and separated by a sterile cellophane disc.

In all tests, one dish contained either a culture of test fungus grown on the appropriate culture medium, uninoculated medium of the same composition, a known volume of test solution or a similar volume of sterile deionized water. The other dish of each assembly contained an assay organism inoculated on the appropriate culture medium.

b) Paired bottle assemblies.

These consist of two bottles connected by a glass 'T' piece (bore diameter, 10 mm; 'T' bar length, 90 mm; single arm length, 25 mm) fitted into the necks of the bottles with rubber bungs at either end of the long arm. The short arm of the 'T' piece was fitted with a rubber

serum cap to allow gas samples to be withdrawn for analysis.

In all tests, one bottle contained fungus or other material being tested, the other bottle contained a culture of the assay organism.

c) Soil tube assay assemblies.

The special apparatus used in this work is described in the appropriate section below.

d) Single bottle assay assemblies.

These were used only for tests with authentic mixtures of carbon dioxide and air.

They consist of a single bottle containing assay organism inoculated on the appropriate medium, and sealed during the experiment with a rubber serum cap.

To attain the appropriate concentration of carbon dioxide in tests, the pressure of the air in the bottles was reduced by calculated amounts and then returned to atmospheric by allowing authentic carbon dioxide to enter the bottles.

3. Test Species.

The name and source of each culture examined is given in the section relating to that particular investigation.

a) Fomes and Clitocybe.

These species were inoculated on to the surface of 20 ml aliquots of 5% malt agar in petri dishes or on to the surface of 120 ml aliquots

of the same medium in 1 l Roux bottles. This medium is known to be suitable for the growth of these species although it is appreciated that this particular medium is not necessarily optimal for the growth of each.

Cultures were incubated in low-intensity diffuse light at 24°C until mycelial growth covered more than 90% of the agar surface. The cultures were then paired with the appropriate assay organism in the appropriate test assemblies.

Test species grew at different rates, thus cultures of different species were of different ages when tested. However, all tests with any one species were carried out using cultures of similar age.

b) Rhizopus sexualis.

The methods relating to this species are described in the section dealing with this investigation.

4. Assay Species.

a) Lettuce (Lactuca sativa L. var. sativa, cultivar Grand Rapids).

Tests with lettuce were carried out as follows:-

Seeds were surface sterilized by shaking for one minute in an aqueous solution of mercuric chloride (1g $HgCl_2$, 1 l deionized water) followed by four washes of about one minute each in sterile deionized water. The trivial reduction in germination caused by this process was distributed at random in controls and all other test assemblies,

and it was thought that its possible effects did not justify analysis at present. Ten seeds were place approximately equidistant from each other on the surface of 20 ml aliquots of ½% water agar in petri dishes or on the surface of 70 ml aliquots of ½% water agar in large glass bottles (volume, 770 ml). The inoculated vessels were paired in the appropriate assembly type.

Assay assemblies containing lettuce were incubated at 24°C under warm white fluorescent tubes. In tests using paired petri dish assemblies, the lettuce seeds were in the upper dish. After seven days, the number of germinated* seeds and the length of the shoot and of the root of the germinated seedlings was measured. Any other morphological effects were assessed visually.

(* Germination was defined here as the growth of a radicle until its external length was more than the diameter of the seed on its short axis.)

b) Aspergillus niger van Teigham (Glasgow University Collection No. 1).

Tests with A. niger were carried out as follows:-

An aqueous suspension of conidia of A. niger was made by pouring 1 ml sterile deionized water into a test tube culture of Aspergillus and shaking to dislodge the spores. This suspension was mixed with 2% malt agar at approximately 45°C and the mixture immediately poured into petri dishes. Dishes were incubated for 18 hours at 24°C in diffuse light. Discs (diameter, 4.0 mm) were cut from the incubated

suspension and each placed centrally on the surface of 20 ml aliquots of 2% malt agar in petri dishes. By this procedure, colonies of regular shape were formed and no spores were dropped on the agar surface, thus preventing the formation of additional colonies. Inoculated dishes were used in paired petri dish assemblies, using plates inoculated from the same suspension in any one experiment.

Paired assemblies containing A. niger were incubated at 24°C in diffuse light for seven days. The A. niger colonies were in the upper dish of assay assemblies during incubation. After seven days, the linear growth was assessed by taking the mean of values for two colony diameters at right angles to one another from each colony. At the same time sporulation was assessed visually with the aid of a Watson binocular microscope (x100), using the following scale:-

\+ = conidial heads occupying 0 - 25% of the field *

2+ = " " " 25 - 50% " " "

3+ = " " " 50 - 75% " " "

4+ = " " " 75 -100% " " "

(* A field was a standard area of approximately 16 mm^2.)

Five fields along a transect of each colony were examined, two at the periphery, two at positions half-way between the inoculum disc and the outside edge, and one in the centre, adjacent to the inoculum disc. Average numerical values for each colony were calculated on this scale.

It was appreciated that the above assessments were subjective.

However, no analysis of the consistency of results obtained was carried out since the survey was limited to scanning for gross effects which would be obviously significant by such measurements.

c) Bacteria.

The species used as assay organisms are listed in the sections dealing with the investigations in which they were used.

Aqueous suspensions from young cultures (1 to 7 days old) were streaked on to the surface of 20 ml aliquots of Bouillon agar in petri dishes. Inoculated dishes were used in paired petri dish assemblies.

Assemblies with bacteria were incubated at 24°C in diffuse light for seven days. The bacteria were in the upper dish in assemblies during incubation. After seven days the amount of growth in each streak was assessed by eye and recorded on the following scale:-

- = no growth

$\pm$ = poor growth, less than the control

+ = growth equal to control

$\ddagger$ = growth more than the control

d) Rhizopus sexualis.

The methods for inoculation and assessment of morphogenetic changes in this species are given in the section dealing with this investigation.

Experiments with all test and assay species were replicated suitably throughout. The numbers of replicates and the numbers of individuals measured in each case are stated in the relevant places.

5. Gas Chromatographic Analysis.

a) Gas chromatograph conditions.

Apparatus.

All gas analysis was carried out using an Aerograph Model 204 gas chromatograph.

Flame ionization detectors were used in most analyses of organic volatiles. The advantages of this type detector include its insensitivity to air and water vapour, products invariably present in the culture gases of living organisms. Examination via this detector thus avoids the use of drying agents which several authors, including Lösel (1964), have suggested may lead to confusing results. Also, the response of this type of detector is linearly related to concentration over a wide range, making it suitable for quantitative analysis.

Micro cross-section detectors were used in the analysis of carbon dioxide and oxygen for which this detector system is particularly suitable.

Operating conditions.

Throughout this work carbon dioxide and oxygen were examined under the conditions given in text Table 1.

Table 1. Operating conditions used for chromatographic analysis of carbon dioxide and oxygen.

Gas being analysed	Column				Carrier gas and Flow rate (ml/min)
	Dimensions	Material	Packing	Temp.	
Oxygen	10ft. x 1/4in.	Stainless Steel	Molecular Sieve 5A 30/60 mesh	65°C	Helium 22
Carbon dioxide	6ft. x 1/4in.	Stainless Steel	Porapak R	65°C	Helium 30

The operating conditions used in the analysis of volatiles other than carbon dioxide and oxygen are given in the appropriate sections dealing with each particular analysis.

b) Source of samples.

For most of the work, appropriate volumes of gas were removed from Roux bottle cultures of test species or from test assemblies using a gas tight syringe, and injected directly into the chromatograph. In some cases, culture gases were condensed by passing them through a trap or a precolumn immersed in solid carbon dioxide. Samples were taken similarly from these traps after they had been allowed to return to room temperature.

c) Identification of culture gases.

By retention time comparisons.

The retention times of peaks produced by identified compounds were compared with the retention times of authentic material.

By syringe reactions for functional group analysis.

This is based on the technique described by Hoff & Feit (1964) in which suitable reagents are introduced into syringes containing gas samples before the samples are injected into the gas chromatograph. Many functional groups can be analysed by selection of suitable reagents and comparison of peak pattern before and after treatment.

d) Quantitative measurements of components of culture gases.

Carbon dioxide and oxygen concentrations were estimated by comparison with peak heights produced by samples of air from a well-ventilated corridor. Concentrations of other components were estimated by comparison of peak heights given by culture gases with those given by samples from the headspace above authentic solutions of known concentration.

SECTION IA

A SURVEY OF THE GENUS *FOMES* FOR THE OCCURRENCE OF VOLATILE METABOLITES.

SECTION 1A. A SURVEY OF THE GENUS FOMES FOR THE OCCURRENCE OF VOLATILE METABOLITES

Introduction.

The first departmental survey (Dick & Hutchinson, 1967) was done by testing the effects of gases from fungal cultures on a range of other fungi. This method was found to have the following disadvantages:-

a) It took a long time to examine each species.

b) It involved a risk of putting substantial effort into examining biological interactions before finding out that the particular active species either did not produce sufficient gaseous metabolites to be identified by gas chromatography, or that the active metabolites were not recognisable by the available gas chromatographic techniques.

An alternative of starting the survey by the relatively quick gas chromatographic analysis of culture gases was therefore examined. This has the advantage of eliminating immediately the interactions which are not likely to respond easily to gas chromatographic analysis. A number of these eliminated interactions were found to be biologically interesting, however. The survey was therefore continued using both biological and gas chromatographic screening.

In the previous survey, fungi from a wide range of taxonomic groups were examined. The time therefore seemed ripe for a more concentrated

investigation of a particular group. The survey was first extended by examining the genus Fomes because of the particularly interesting and unusual metabolites produced by F. annosus (Glen et al., 1966). The only additional information on volatile metabolite production in this genus was the report of the production of acetaldehyde, ethyl acetate and ethanol, also by F. annosus (Nord & Vitucci, 1946).

The range of assay organisms was also extended in this survey by including lettuce as convenient representative Angiosperm and by including a range of common saprophytic bacteria. To enable this to be done in the time available the range of fungi was reduced to one species, Aspergillus niger. This species was chosen because it was the one with least sensitivity, and most convenient constant growth habit of the five examined in the previous survey. It seemed likely that a metabolite affecting this relatively insensitive species would be likely to be highly active, and/or be produced in sufficiently large quantity for further examination to be practicable.

Methods.

Appendix Table I lists the 33 species which were examined.

1. Biological assay.

This was carried using paired petri dish assemblies.

All Fomes cultures were grown on 5% malt agar which had been

found to be a convenient medium by Glen et al., (1966). The species generally grow slowly, and the diameter of colonies on agar tends to be limited by staling or other factors in the growth medium. To overcome this, each culture was inoculated with five pieces of mycelium placed approximately equidistant from each other. However, mycelium of F. scutellatus grew very little when inoculated in this way, the inocula apparently inhibiting each other. For this species, therefore, a single central inoculum was used, and the incubation period lengthened accordingly.

The activity of cultures was assayed in the following tests:

a) Lettuce.

In tests with lettuce, each experiment consisted of three to six dishes of lettuce (inoculated as described in the General Methods, paragraph 4(a), paired with cultures of a species of Fomes, and three to six dishes of lettuce paired with uninoculated 5% malt agar. The percentage germination of lettuce seeds and root and shoot growth of germinated seedlings were measured as described in the above mentioned paragraph of the General Methods.

b) Aspergillus niger.

In tests with A. niger, each experiment consisted of four to six dishes with A. niger (inoculated as described in the General Methods), paragraph 4(b) paired with cultures of a species of Fomes and four to six dishes of A. niger paired with uninoculated 5% malt agar. The

diameters and sporulation of A. niger colonies were measured as described in the General Methods.

c) Bacteria.

The following were chosen as a convenient range of common species representing several morphological types:-

(1) Mycobacterium phlei NCTC 8151

(2) Micrococcus roseus NCTC 7523

(3) Corynebacterium xerose University of Glasgow Microbiology Department AC/X/65

(4) Staphylococcus aureus Oxford H.

(5) Bacillus subtilis NCTC 3160

(6) Klebsiella aerogenes NCTC 418

(7) Pseudomonas aeruginosa NCTC 7244

(8) Rhizobium sp. Rothamstead Experimental Station catologue number 2001

In these tests, species (1) to (4) were streaked on one plate and species (5) to (8) streaked on another. The preparation and inoculation of these plates is described in the General Methods, paragraph 4(c). Each experiment consisted of three dishes with species (1) to (4) and three with species (5) to (8) paired with cultures of a species of Fomes and three dishes of each type paired with uninoculated 5% malt agar. The growth of bacterial streaks was estimated by the method described in the General Methods.

The Rhizobium culture was obtained when tests with Fomes species were already underway. Subsequent tests did not indicate any exceptional

sensitivity of this species, thus no repetition of tests where this species had been omitted were made.

Initially all tests were carried out once for each test fungus, but where biological effects were observed, activity was examined in two further replicate experiments. Tests with bacteria and F. noxius and F. scutellatus were repeated twice before the investigations on the activity of these species were extended.

2. Gas chromatographic assay.

This was carried out on cultures in Roux bottles, using the media and inoculation methods described for the biological assay. Gas samples were taken from bottles containing cultures incubated as described in the General Methods, paragraph 5(a) and gas liquid chromatograph (G.L.C.) traces from these were compared with traces produced from similar samples from bottles containing uninoculated 5% malt agar.

The conditions of analysis are given in text Table 2.

Table 2. Operating conditions used for chromatographic analysis of *Fomes* culture gases.

Dimensions	Material	Packing	Temperature (°C)	Carrier gas and flow rate (ml/min)	Hydrogen flow rate (ml/min)
5ft x 1/8in	Stainless steel	10% Carbowax 1500 (polyethylene Glycol)	70 programmed to 100 (4°/min)	Helium 36	30
6ft x 1/8in	Stainless steel	15% Dinonyl phthalate (DNP)	70 programmed to 100 (4°/min)	Helium 36	30
6ft x 1/8in	Stainless steel	20% Carbowax 20M	70 programmed to 100 (4°/min)	Helium 36	30
6ft x 1/8in	Stainless steel	Porapak R	70	Helium 36	30

60/80 mesh Chromosorb W was used as a support in all these columns.

The Carbowax 20M column is recommended for the detection and separation of a wide range of alcohols, aldehydes, esters, ketones, phenols, aromatic hydrocarbons and aliphatic chlorine compounds. DNP is recommended for the detection and separation of ethers and hydrocarbons. Under the above conditions, Carbowax 20M does not retain very low molecular weight compounds

sufficiently long for peaks to be determined clearly from the injection shock peaks. Carbowax 1500 and Porapak R retain such compounds for a longer time, and they were used therefore when such peaks were seen using Carbowax 20M and DNP.

Results.

1. Biological Survey.

a) Interactions with lettuce.

The results are recorded in appendix Table II: summarized and analysed in appendix Table V, and illustrated in text Figures (i), (a), (b), (c) and (d).

In all cases when inhibition occurred, other than with F. noxius, the effects were of general stunting of growth. No other characteristic morphological differences were observed between seedlings paired with test fungus and those in control treatments. With F. noxius characteristic morphological differences appeared. The roots of affected seedlings were stunted and shrivelled and were browner in colour than roots of seedlings in control treatments. Typical examples are shown in Plate I.

b) Interactions with Aspergillus niger.

The results are recorded in appendix Table III; they are summarized and analysed in appendix Table V, and illustrated in text Figures (ii) (a) and (b).

Figure (i)(a). Differences in % germination of lettuce seeds in assemblies containing lettuce paired with Fomes cultures compared with controls, paired with uninoculated 5% malt agar.

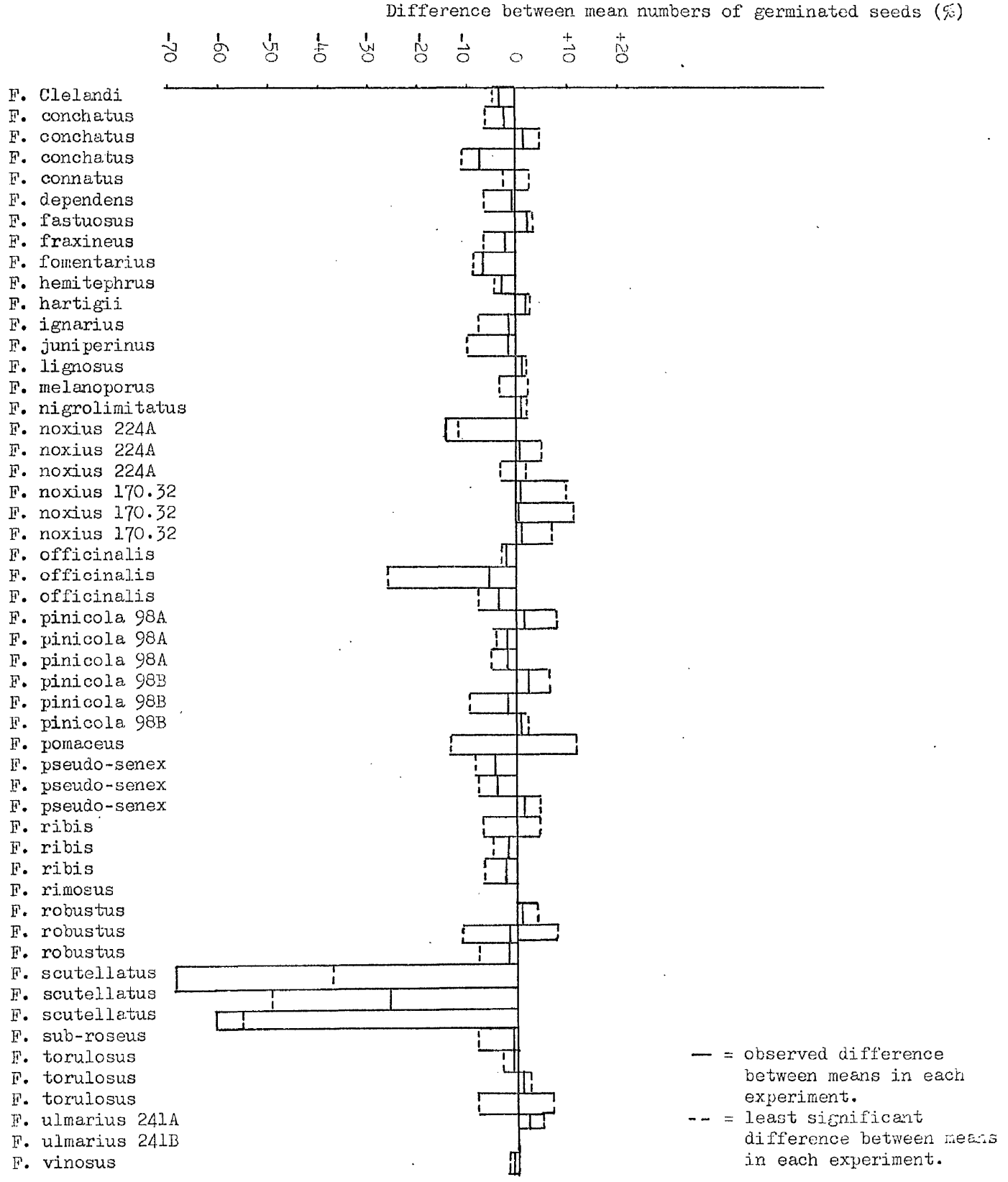


Figure (i)(b). Differences in mean lengths of shoots of germinated seedlings in assemblies containing lettuce paired with Fomes cultures compared with controls, paired with uninoculated 5% malt agar.

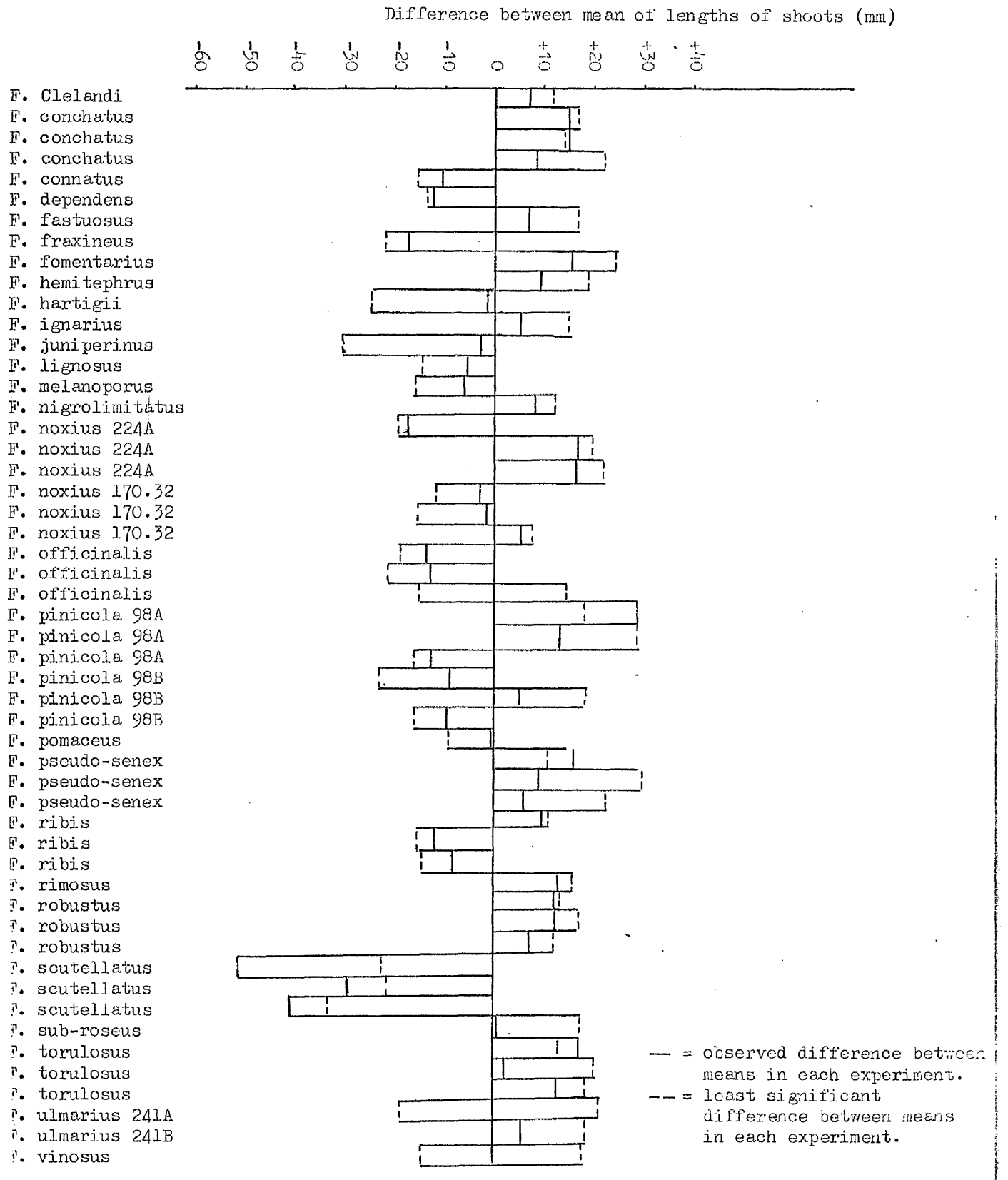


Figure (i)(c). Differences between mean lengths of roots of germinated seedlings in assemblies containing lettuce paired with Fomes cultures compared with controls, paired with uninoculated 5% malt agar.

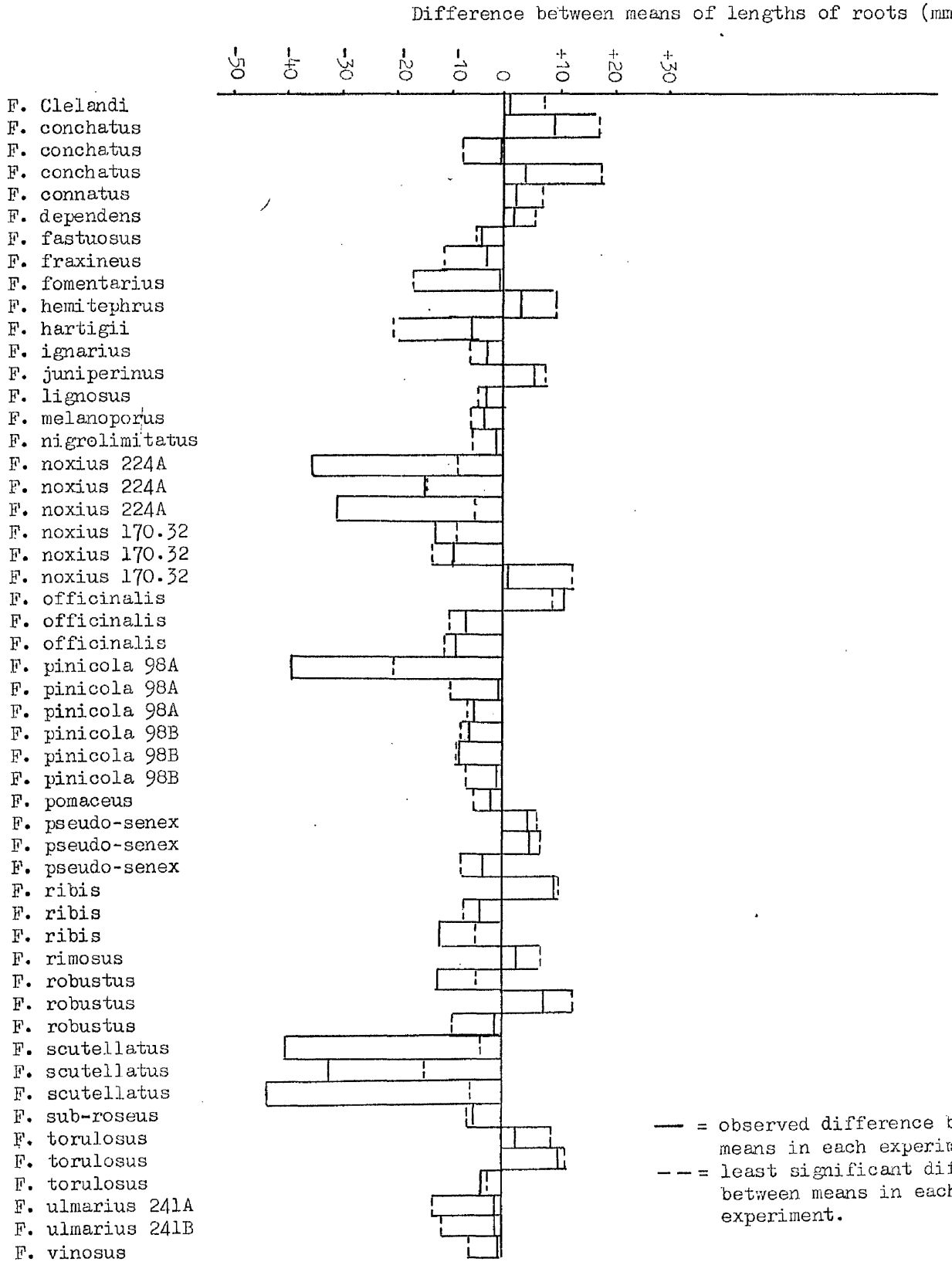


Figure (i)(d). Differences in mean lengths of shoots + roots of germinated seedlings in assemblies containing lettuce paired with Fomes cultures compared with controls, paired with uninoculated 5% malt agar.

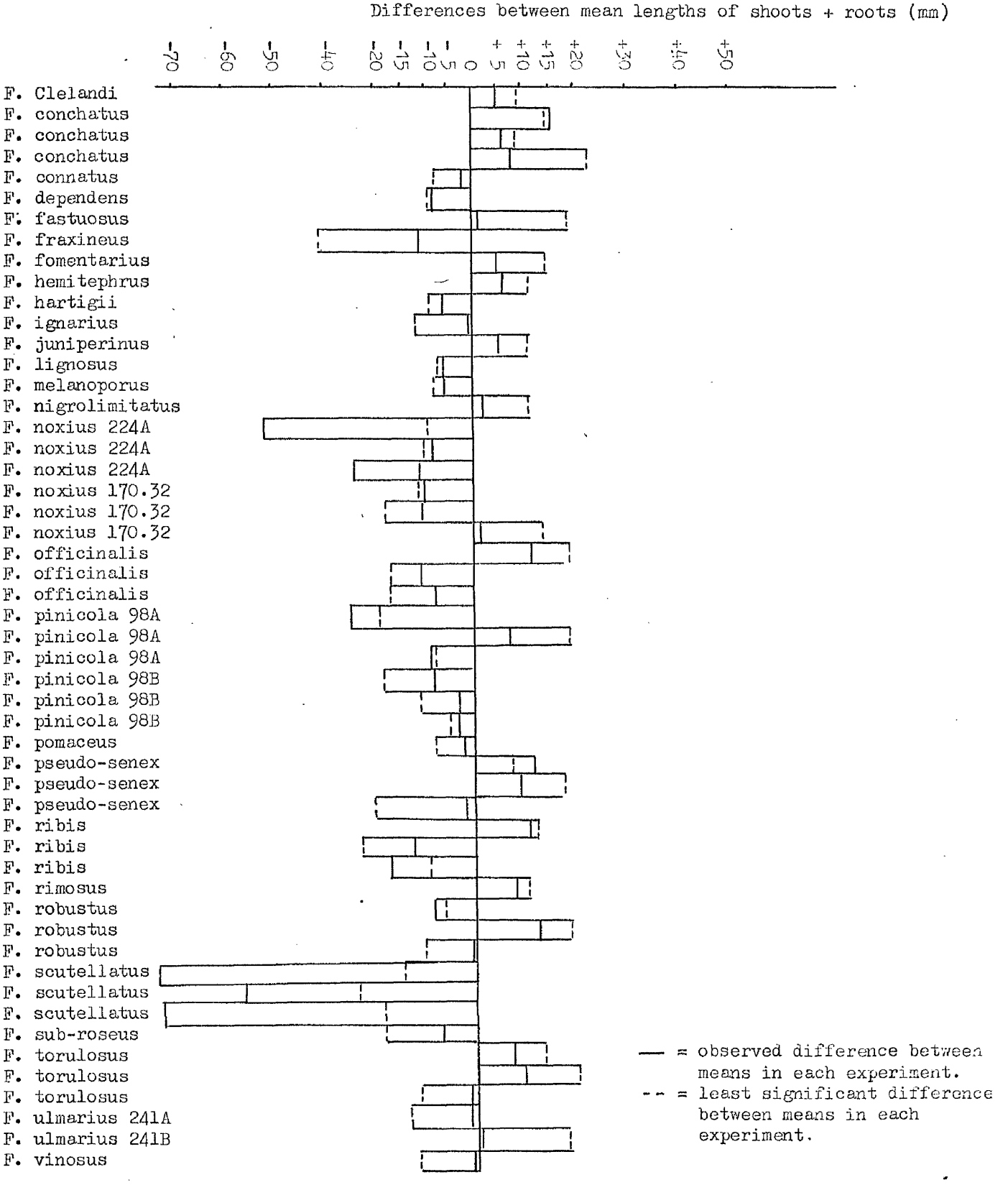


Figure (ii)(a). Differences between means of diameters of A. niger colonies in assemblies containing A. niger paired with Fomes cultures compared with controls, paired with uninoculated 5% malt agar.

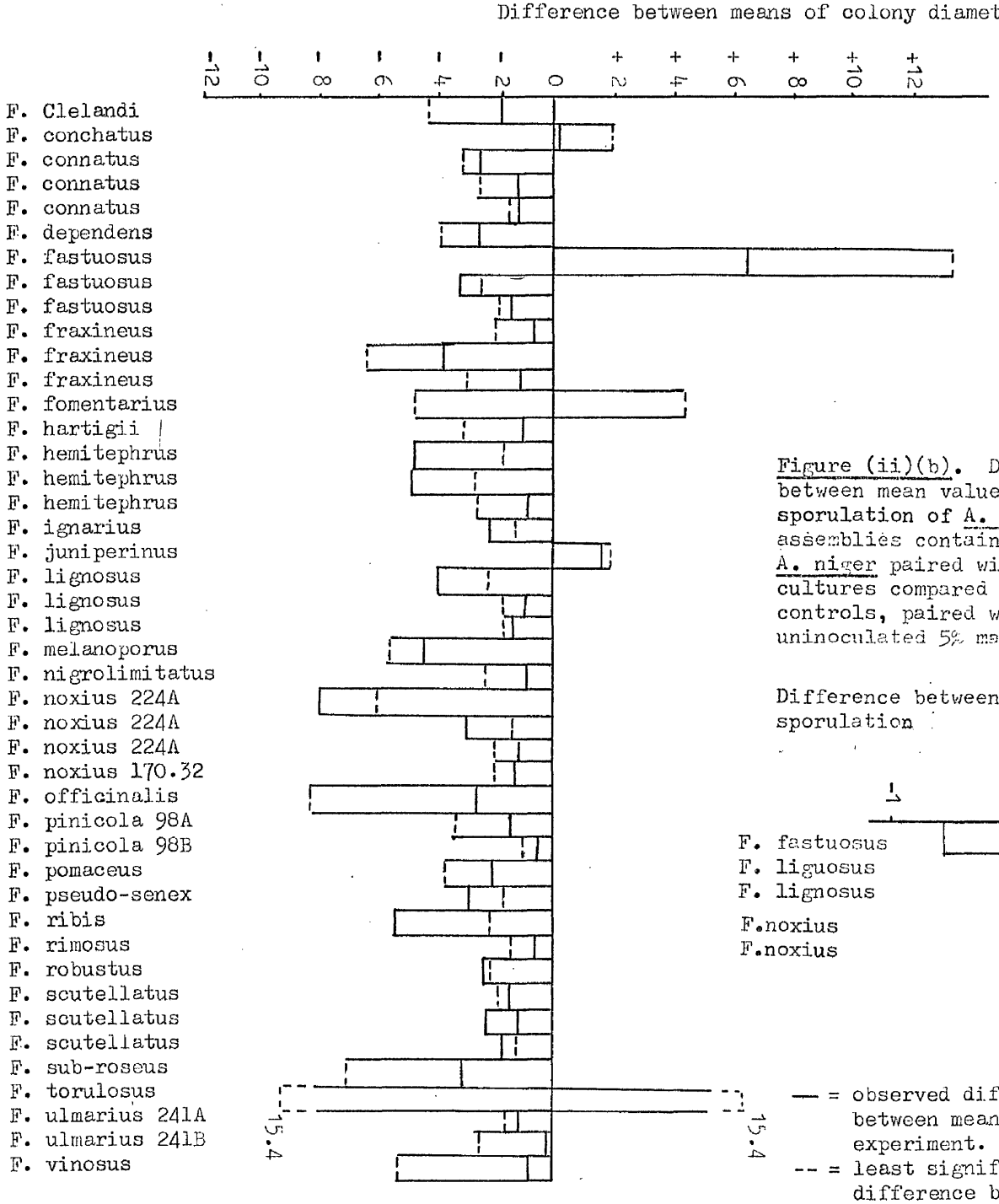


Figure (ii)(b). Differences between mean values for sporulation of A. niger in assemblies containing A. niger paired with Fomes cultures compared with controls, paired with uninoculated 5% malt agar.

— = observed difference between means in each experiment.

-- = least significant difference between means in each experiment.

Plate 1.

Lettuce seedlings grown in the presence of a culture of *F. noxius*.

Lettuce seedlings grown in the presence of uninoculated 5% malt agar.

Table 3. Species producing biological activity in survey.

Assay species / Facet of growth affected / Test species	Lettuce				A. niger		Bacteria
	% germination	Shoot length	Root length	Shoot + Root length	Colony diameter	Sporulation	Streak growth
F. conchatus		+		+			
F. connatus					-		
F. fastuosus					-		
F. fraxineus						-	
F. hemitephrus					--		++
F. ignarius					-		
F. lignosus					-		
F. noxius 224A	-		---	--	--	++	
F. noxius 170.32			-				
F. officinalis			+		-		
F. pinicola 98A		+	-	--			
F. pinicola 98B			-				
F. pseudo-senex		+		+			
F. ribis			-	-	-		
F. robustus			-	-			
F. scutellatus	--	---	---	---	--		
F. torulosus		+	-				

- = inhibitory effect, recorded in one experiment.
+ = stimulatory effect, recorded in one experiment.

(c) Interactions with bacteria.

The results are recorded in appendix Table IV.

Text Table 3 summarizes the results of all tests in which a significant biological effect was recorded.

2. Gas chromatographic survey.

The patterns of traces obtained show consistences which permit the arrangement of some of the species into groups:-

Group I.

Illustration : Text Figure (iii).

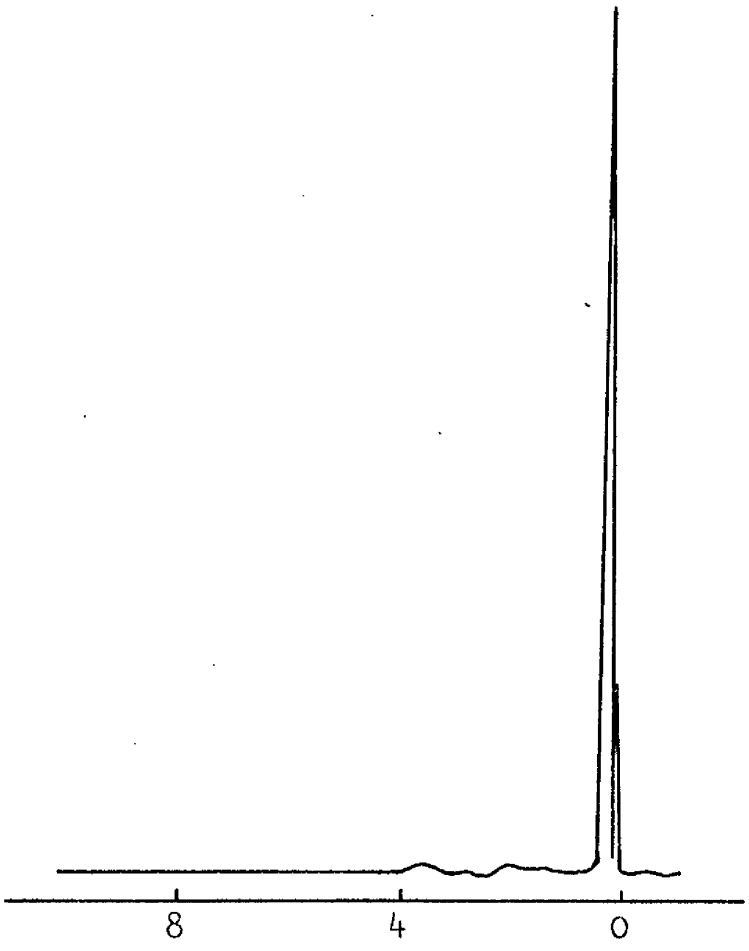
Description : An injection peak followed by an irregular number of small peaks, some barely distinguishable from background noise.

Cultures giving this pattern: Uninoculated 5% malt agar, F. connatus, F. dependens, F. fastuosus, F. ferruginosus, F. fraxineus, F. hartigii, F. ignarius, F. juniperinus, F. lignosus, F. nigrolimitatus, F. officinalis, F. pinicola 98A, F. pinicola 98B, F. pseudo-senex, F. scutellatus and F. torulosus.

Figure (iii). Group I.

Typical G.L.C. trace produced by an 8 week old culture of F. scutellatus.

Column: Carbowax 20M



Group II.

Illustration : Text Figure (iv) (a), (b) and (c).

Description : An injection peak followed by a single prominent peak. Carbowax 20M and DNP do not separate this peak clearly from the injection peak, but Porapak R retains this compound for a longer time, giving a clearly separate single peak.

Cultures following this pattern: F. conchatus, F. occidentalis, F. pomaceus, F. ribis, F. rimosus and F. vinosus.

Group III.

Illustration : Text Figure (v) (a) and (b).

Description : An injection peak followed by two prominent peaks. Carbowax 20M and DNP do not separate the first of these peaks clearly from the injection peak. The retention time of this peak is slightly longer (of the order of 1.6 min on Carbowax 20M and 1.6 min on DNP, compared with 0.8 min on Carbowax 20M and 1.0 min on DNP for Group II).

Cultures producing this pattern: F. Clelandi, F. fomentarius, F. hemitephrus and F. sub-roseus.

No general pattern was seen which applied to more than one species of the remainder. These patterns are described below as follows:-

Figure (iv). Group II.

Typical G.L.C. traces produced by 5 week old cultures of F. conchatus.

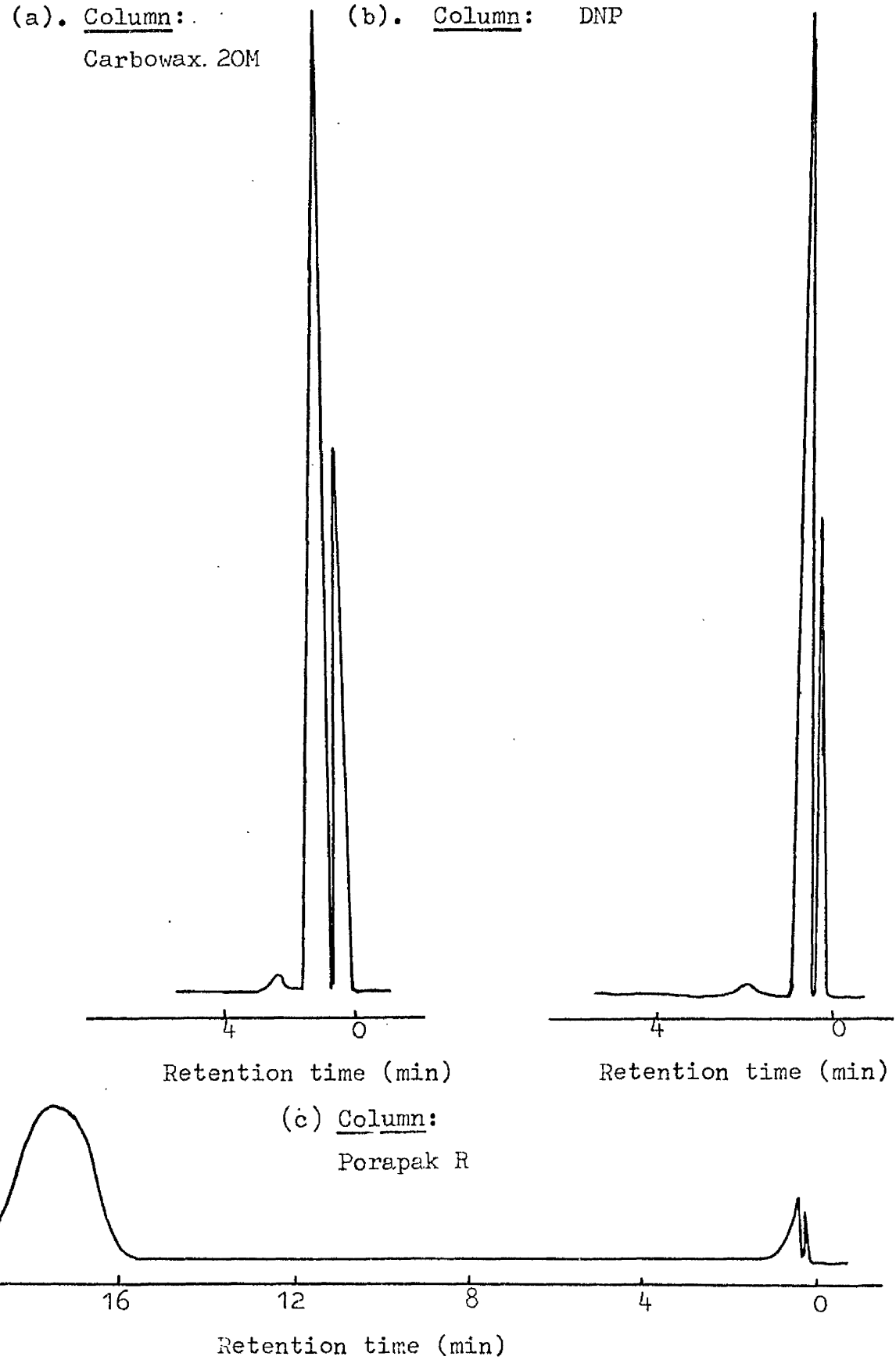
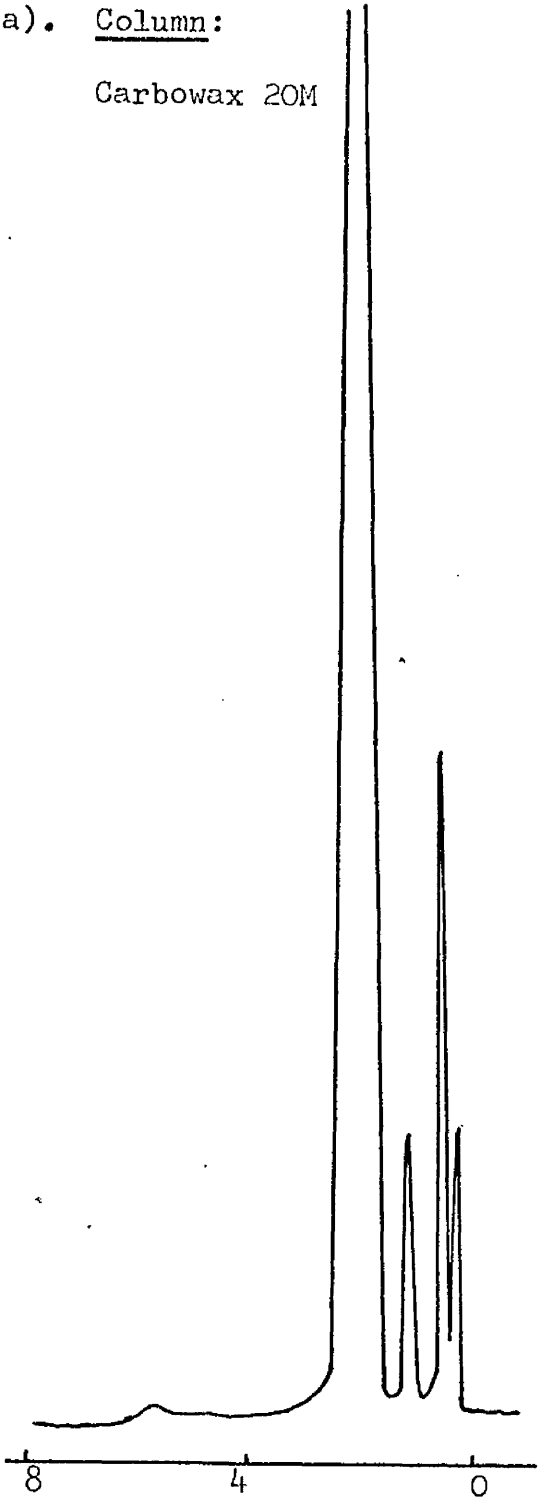


Figure (v). Group III.

Typical G.L.C. traces produced by 5 week old cultures of F. Clelandi.

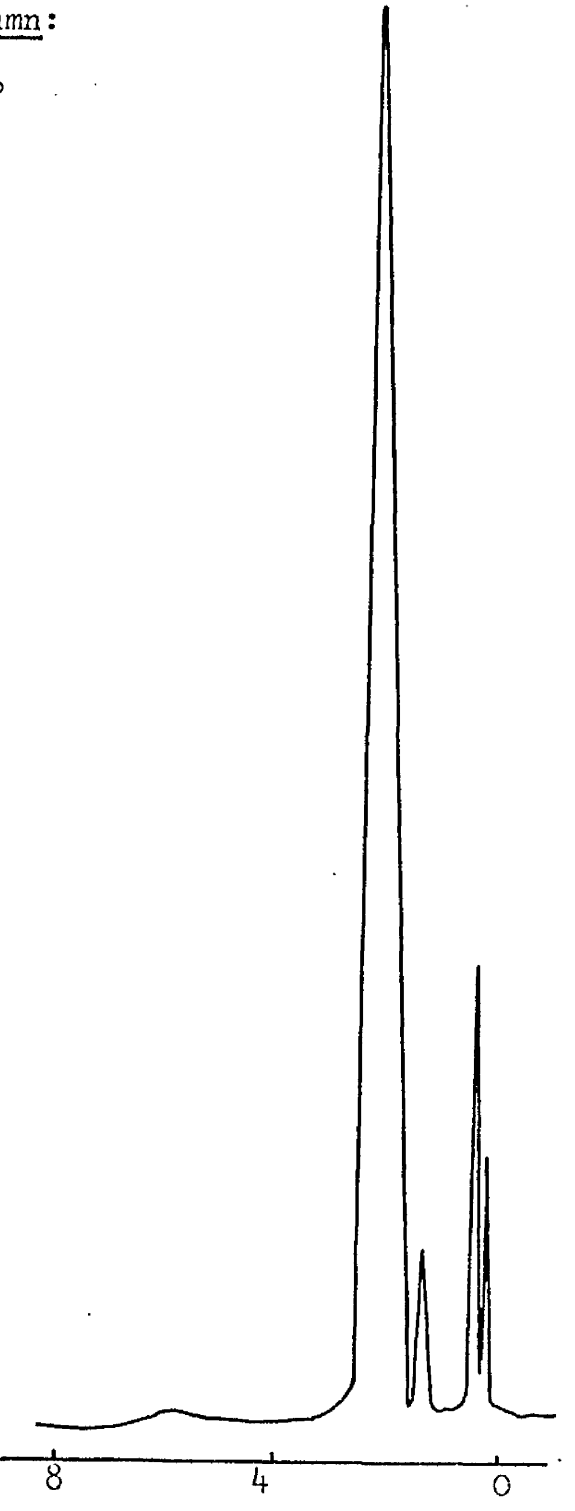
(a). Column:

Carbowax 20M



(b). Column:

DNP



The pattern (Pattern IV) produced by F. melanoporus (text Figure (vi)[1](a) and (b) shows an injection peak followed by a single prominent peak. This peak has a slightly longer retention time than that produced by Groups II and III, (of the order of 2.0 min on Carbowax 20M and 2.0 min on DNP).

The pattern (Pattern V) produced by F. ulmarius 241A and 241B (text Figure (vi)[2](a), (b) and (c)) shows an injection peak followed by three prominent peaks on Carbowax 20M and DNP. These columns do not separate the first of these peaks clearly from the injection peak. An additional peak is detectable on Carbowax 1500.

The pattern (Pattern VI) produced by F. robustus (text Figure (vii) (a) and (b)) shows an injection peak followed by four prominent peaks. The retention times of these peaks are of the same order as those detected in Pattern V. No accurate comparison has yet been made. The relative peak heights differ in the two Patterns. In Pattern VI the height of the third unknown peak is proportionately larger than the equivalent peak in Group V.

The pattern (Pattern VII) produced by F. noxius 224A (text Figure (viii) (a), (b) and (c)) also shows an injection peak followed by four prominent peaks. Carbowax 20M and DNP do not separate the first of these peaks clearly from the injection peak under these conditions. Carbowax 1500 retains this peak for a longer time giving a clearly

Figure (vi)[1]. Pattern IV.

Typical G.L.C. traces from 5 week old cultures of F. melanoporus.

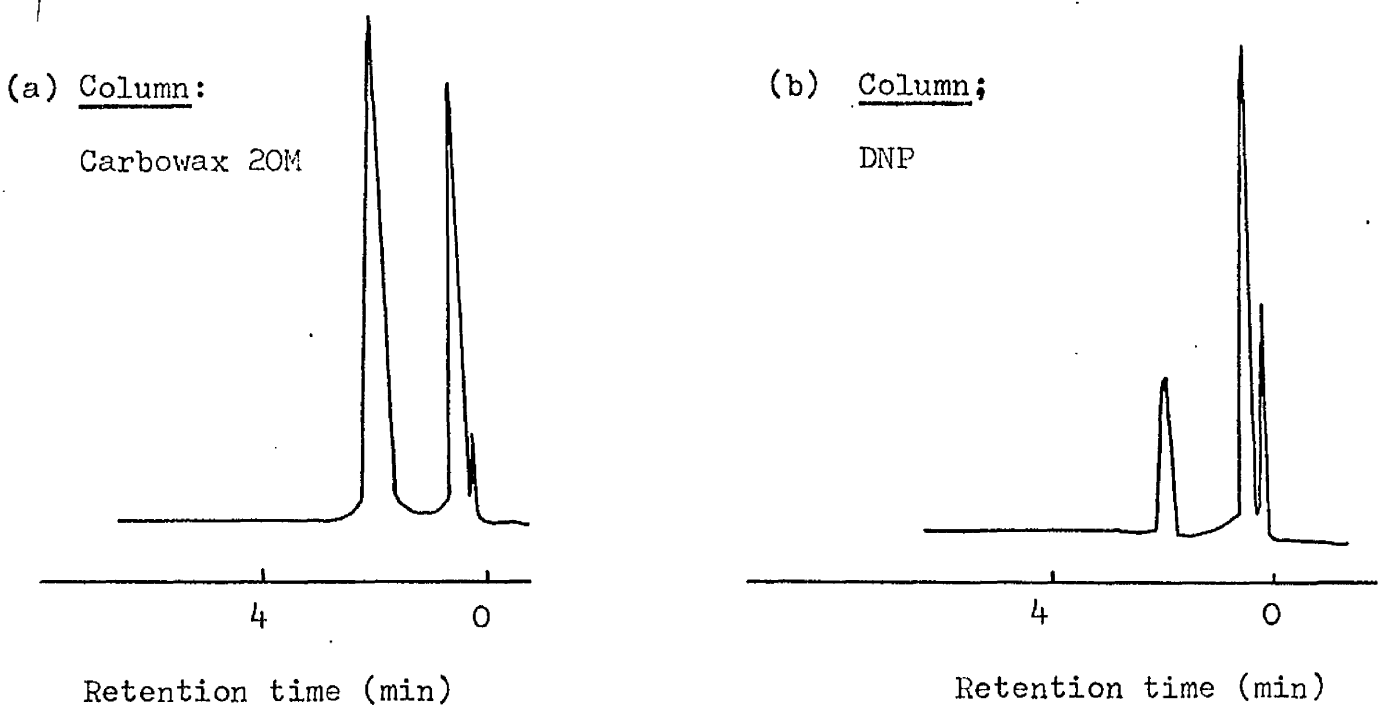
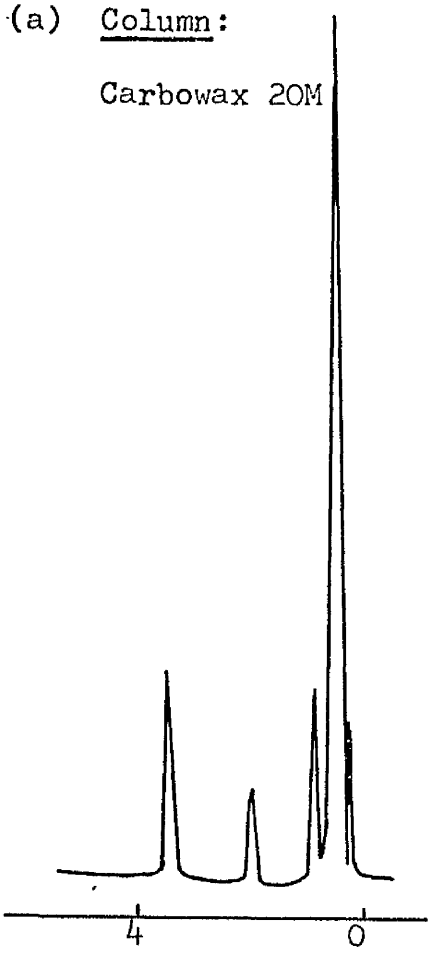


Figure (vi)^2. Pattern V.

Typical G.L.C. traces from 6 week old cultures of F. ulmarius.

(a) Column:

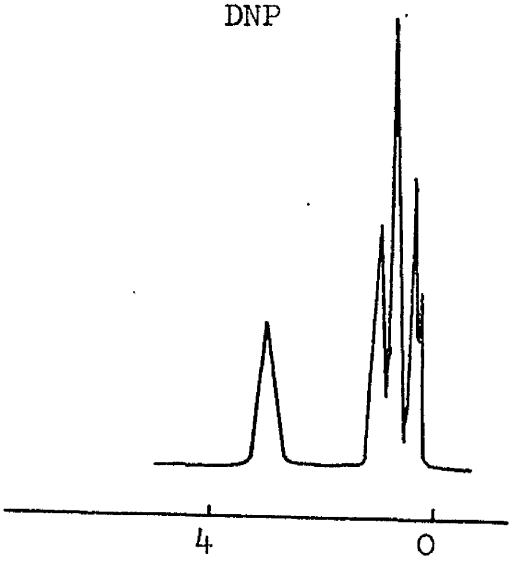
Carbowax 20M



Retention time (min)

(b) Column:

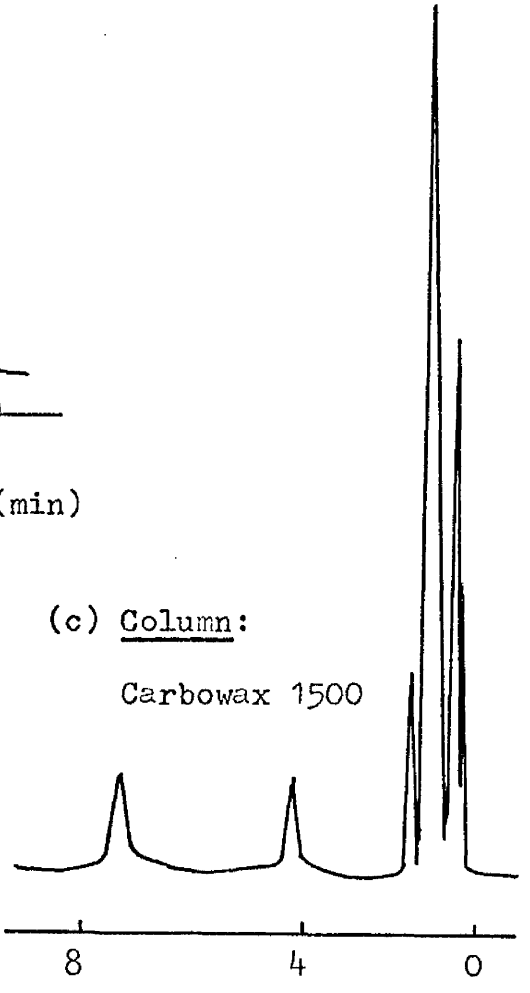
DNP



Retention time (min)

(c) Column:

Carbowax 1500



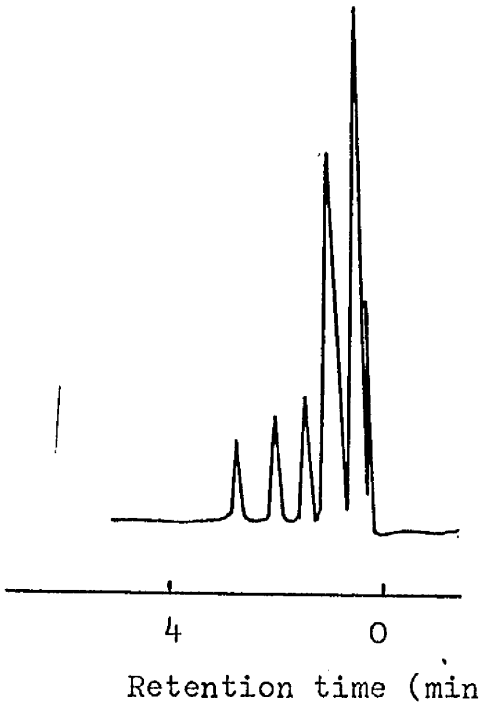
Retention time (min)

Figure (vii). Pattern VI.

Typical G.L.C. traces from 5 week old cultures of F. robustus.

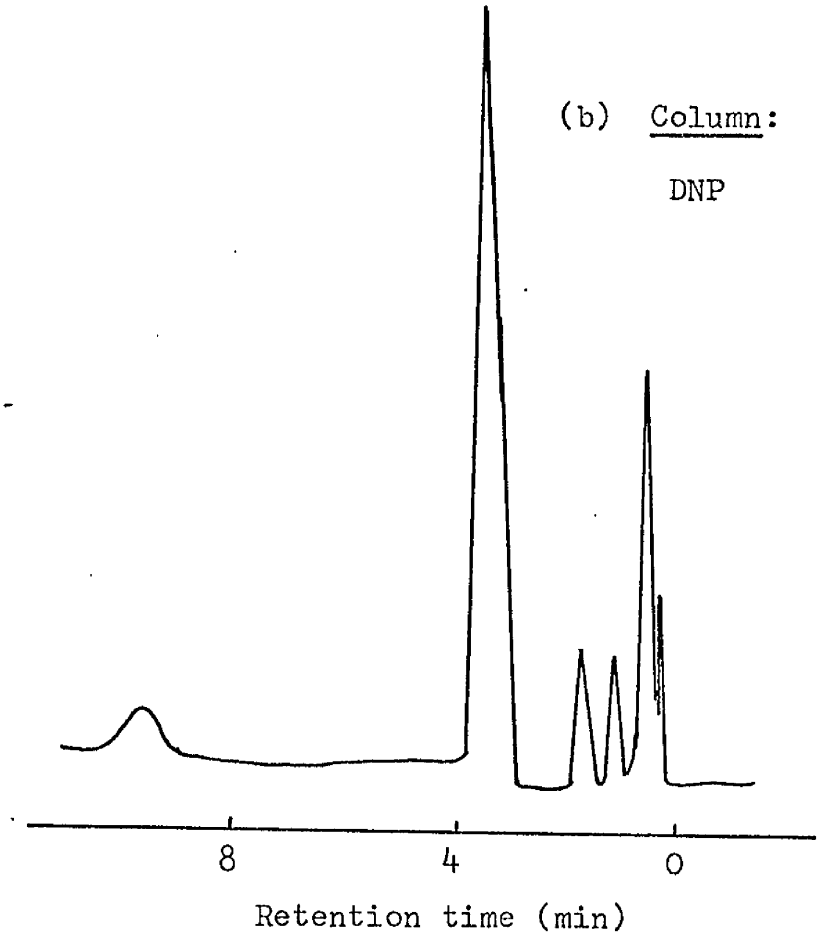
(a) Column:

Carbowax 20M



(b) Column:

DNP



(c) Column:

Carbowax 1500

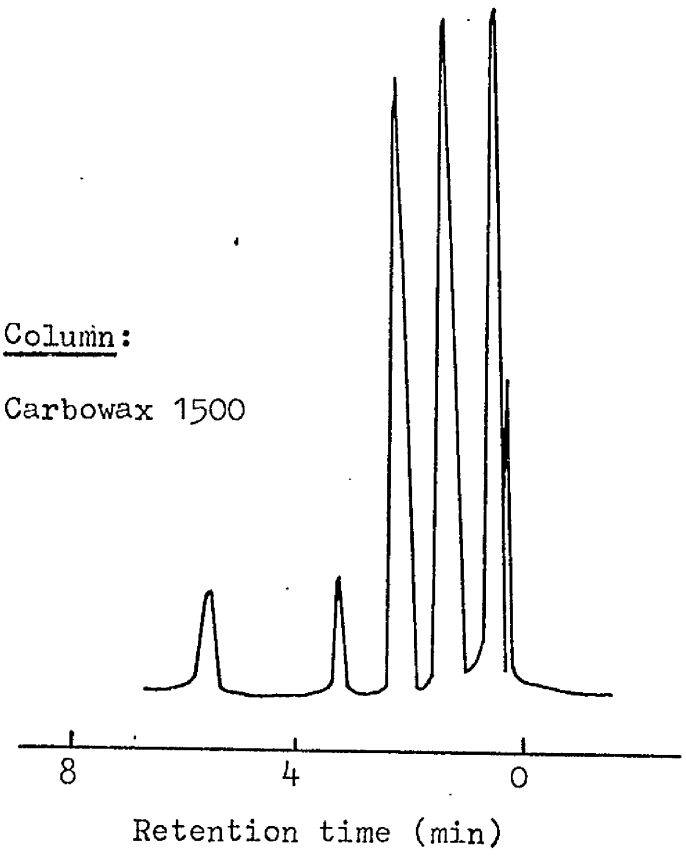
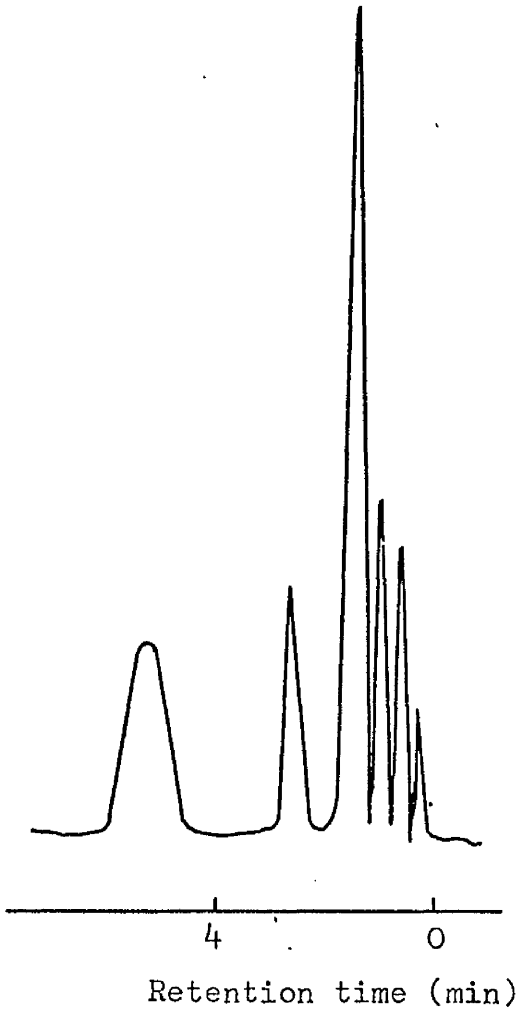


Figure (viii). Pattern VII.

Typical G.L.C. traces from 6 week old cultures of F. noxius 224A.

(a) Column:

Carbowax 20M



(b) Column:

DNP

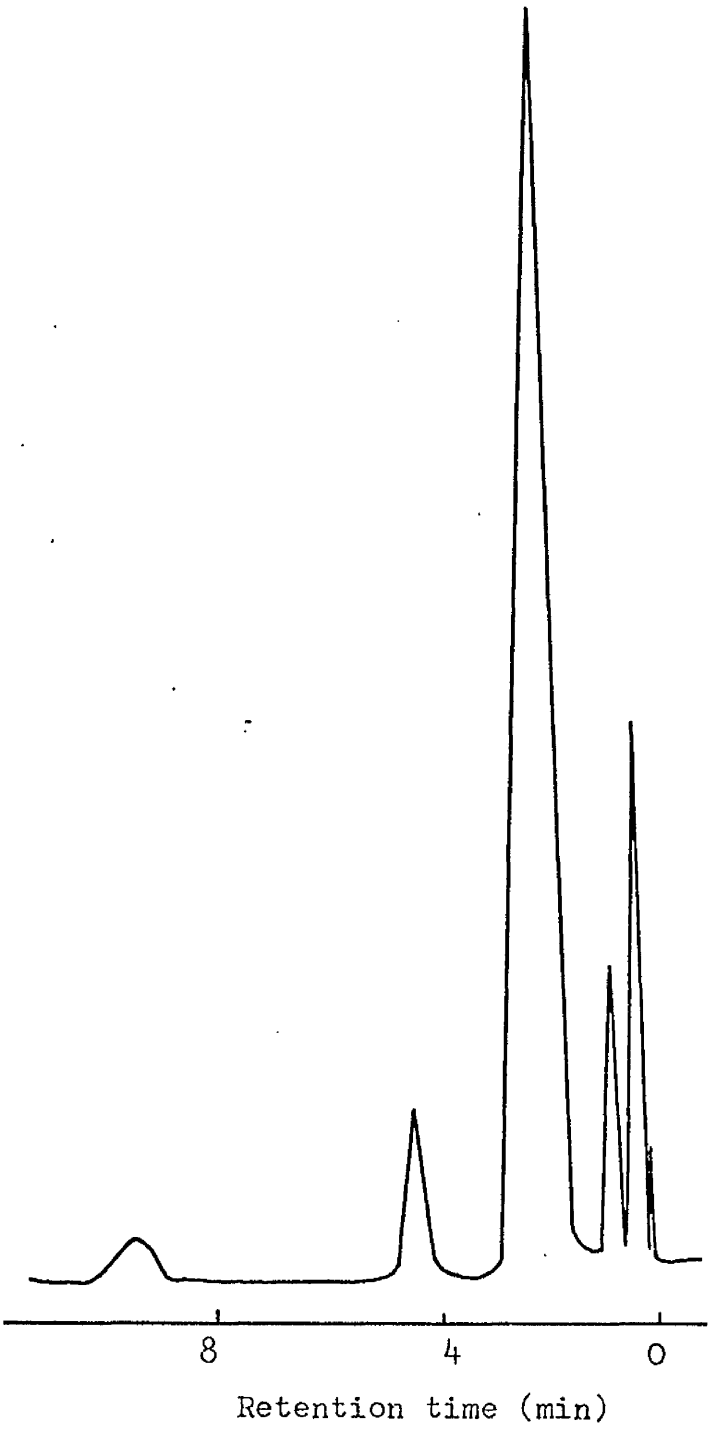


Figure (viii). Pattern VII.

Typical G.L.C. trace from a 6 week old culture of F. noxius 224A.

(C) Column:

Carbowax 1500

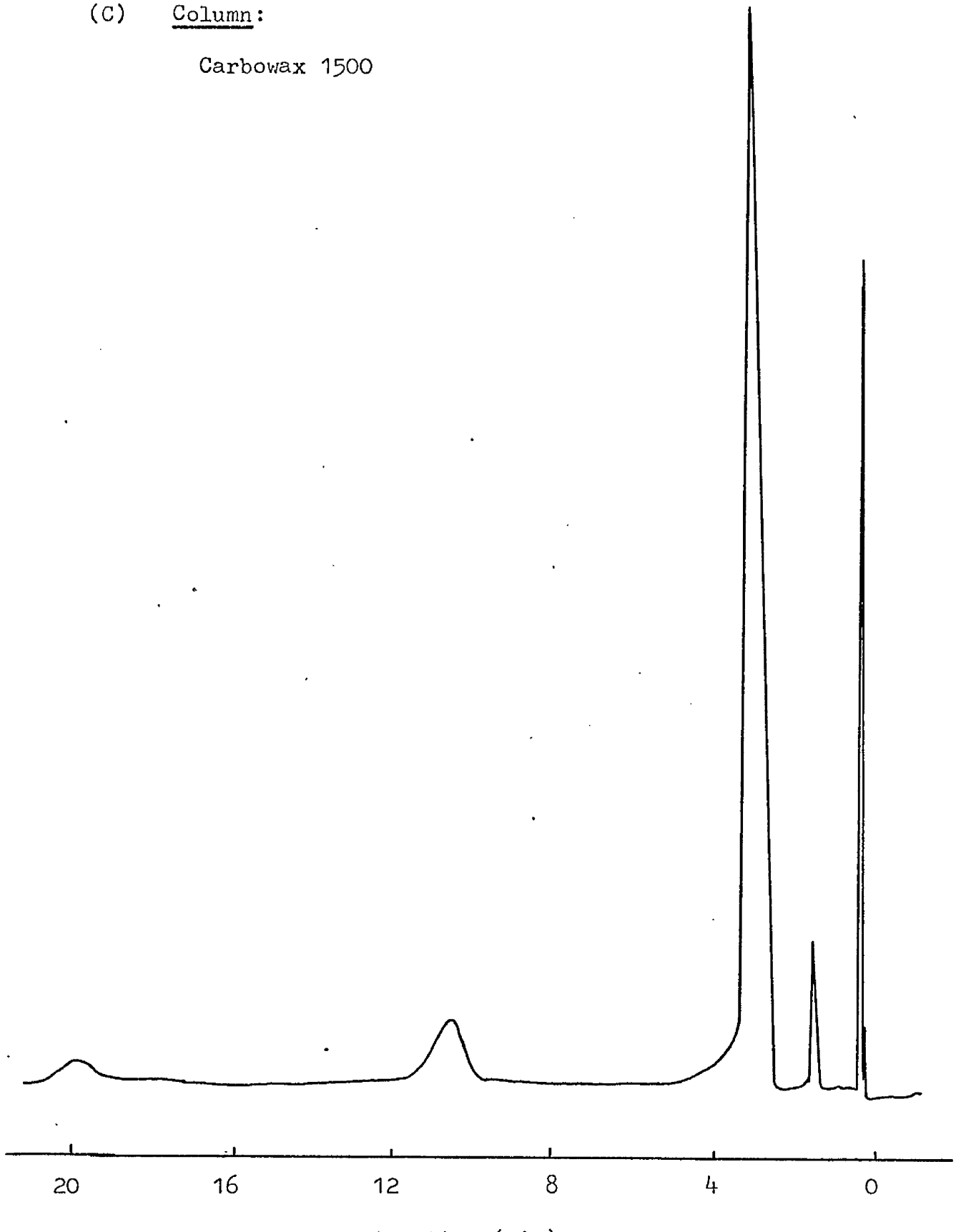
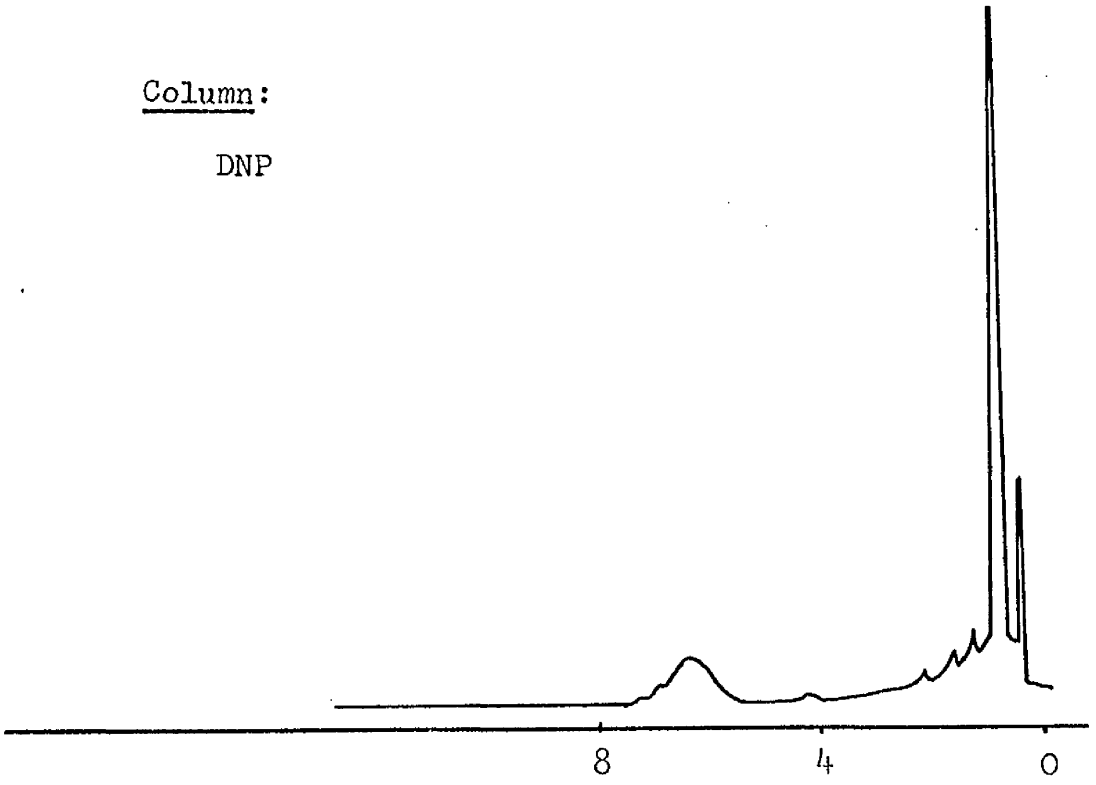


Figure (ix). Pattern VIII.

Typical G.L.C. trace from
6 week old cultures of F. noxius 170.32.

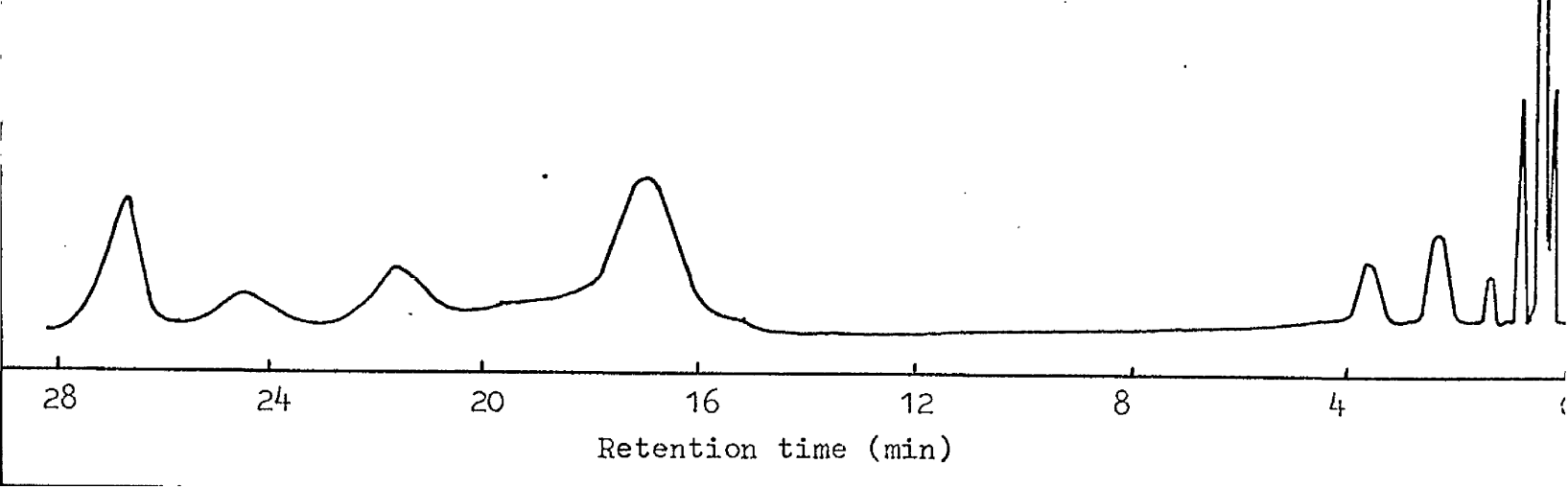
Column:

DNP



Column:

Carbowax 20M



separated peak. The retention times of these peaks are of a similar order to those in Paterns V and VI, but no accurate comparison has been made.

The pattern (Pattern VIII) produced by F. noxius 170.32 (text Figure (ix) (a) and (b)) showed an injection peak followed by eight prominent peaks. No accurate comparison of retention times of these peaks with those produced by other species has yet been made.

3. Comparison of results of survey by biological assay with relation to results of survey by gas chromatographic analysis.

Text Table 4 lists the species producing biological activity and/or producing G.L.C. patterns other than that similar to uninoculated culture medium (group I).

Table 4. Species producing biological activity and/or G.L.C. traces in groups II to VIII

Facet of growth affected / Test species	Lettuce				*A. niger*		Bacteria	Volatile metabolite production (Pattern number)
	% germination	Shoot length	Root length	Shoot + Root length	Colony diameter	sporulation	Streak Growth	
F. Clelandi								III
F. conchatus		+		+				II
F. connatus					-			I
F. fastuosus					-			I
F. fomentarius								III
F. fraxineus						-		I
F. hemitephrus					--		++	III
F. ignarius					-			I
F. lignosus					-			I
F. melanoporus								IV
F. noxius 224A	-		---	--	--	++		VII
F. noxius 170.32			-					VIII
F. occidentalis								II
F. officinalis			+		-			I
F. pinicola 98A		+	-	--				I
F. pinicola 98B			-					I
F. pomaceus								II
F. pseudosenex		+		+				I
F. ribis			-	-	-			I
F. rimosus								II
F. robustus			-	-				VI
F. scutellatus	--	---	---	---	--			I
F. sub-roseus								III
F. torulosus		+	-					I
F. ulmarius								V
F. vinosus								II

Discussion.

1. Biological assay.

In tests with lettuce, shoots and roots responded differently to the presence of the culture gases of several species. F. noxius 224A provides the most conspicuous example of this differential response (cf. Plate 1), and also one test with F. pinicola 98A where shoot growth was stimulated and root growth inhibited. Apart from tests with F. scutellatus, and one with F. noxius all other significant effects reported were significant for one organ only.

F. scutellatus was the only species causing inhibition of shoot growth; the three other significant effects on shoot growth were stimulations. In contrast, only one species, F. officinalis stimulated root growth of lettuce whilst eight species caused inhibition of root growth. Of these effects, only those with F. scutellatus and F. noxius 224A were conspicuous. Other effects did not occur consistently in all 3 trials carried out for each species shown to have activity in the initial test and they were only just significant at 0.05 level of probability in the cases when they were seen.

The greater sinsitivity of roots to the inhibitory compounds produced by the culture gases of F. scutellatus and F. noxius (text Figure (i) (b) and (c)) could be explained by the absorptive function

of roots and the possession of a less well protected meristem compared with that of the shoot.

In tests with A. niger, F. noxius was the only species which affected both the colony diameter and sporulation of this species. This occurred in two of the three replicate experiments. This contrasts with the observations of Martin (1963) who found that three of the four species which caused a decrease in linear growth of A. niger colonies also caused a decrease in sporulation of that species. She suggests that this correlation may indicate that only one volatile metabolite is acting, affecting both facets of growth simultaneously. There are no grounds for making similar predictions in the case of F. noxius although it is possible that one compound might cause both the inhibition of colony growth as well as the stimulation of sporulation. The results of this survey agree with Martin's in that there were no significant stimulations of colony growth compared with several, in this case twelve, instances of significant inhibitions of colony growth (cf. text Figure (ii) (a)). There were two cases of inhibition of sporulation of A. niger compared with one stimulation (cf. text Figure (ii) (b)), but this sample is too small to justify further comment on this proportion.

The only species affecting the growth of bacteria was F. hemitephrus. Cultures of F. conchatus, F. ignarius, F. pomaceus, F. scutellatus,

F. rimosus and F. ribis were reported by Robbins, Hervey, Davidson and Robbins (1945) to inhibit the growth of Staphylococcus aureus. However, this effect could not be observed in tests carried out in this survey with the culture gases of these species. This suggests that the active component was either non-volatile or produced in insufficient quantities under these conditions to affect the growth of bacteria. Sporophore extracts of F. fomentarius, F. ignarius and F. ulmarius were reported by Wilkins and Harris (1944) to have no effect on growth of Staph. aureus or Bacterium coli.

There is no obvious correlation between the habit of species showing biological activity; F. noxius, F. officinalis, F. pinicola and F. robustus are parasitic while the other thirteen active species are mainly saprophytic.

2. Gas chromatographic assay.

Group I.

The observation that species in this group produce traces similar to those given by samples from uninoculated culture medium suggests that further examination of these species would not be profitable at the moment. Obviously, under different conditions, volatile metabolites might be detected from these species. This possibility is discussed in relation to the investigation of the biological activity of F. scutellatus, one of the species in this group (see Section I B).

Groups II and III and remaining species.

The patterns produced by all the species other than those in Group I look worthy of further investigation. In the limited time available, the patterns likely to lead to the most profitable investigations were chosen first for further study.

The most striking pattern in these groups was the characteristic peak produced by the F. pomaceus group of species. This peak was identified by other workers in this Department as being caused by the presence of methyl chloride. This identification was of immediate biological interest since the antibiotic activity of halogen containing metabolites is well known, e.g. griseofulvin, and there is considerable chemical interest in the biochemical pathways of halogen metabolism. These points are receiving attention in this Department and progress will be reported elsewhere.

A closer examination of F. noxius 224A was made. The choice of this species was influenced by the observation of the conspicuous biological activity discussed in the preceding section. The analysis of the gases produced by this species is reported in Section IC of this thesis.

The patterns produced by the other groups have not yet received further attention, hence no further comment on the nature of the metabolites giving these patterns can be made at present. Possible

taxonomic inferences from this work are discussed below.

3. Comparison of results of biological and G.L.C. surveys.

There was no consistent correlation between species producing conspicuous peaks in G.L.C. surveys and those showing biological activity. Only six of the seventeen species producing biological activity also produced prominent peaks on G.L.C. traces. Of these, F. noxius 224A was the only one to produce consistently significant activity. The other species, F. scutellatus, which showed consistently significant activity was one of the species which produced no detectable metabolites.

Of the fifteen species producing interesting G.L.C. traces (i.e. all but species in Group I) only five showed any significant activity. The activity of species in Group II was slight and showed no consistent pattern. The identification of methyl chloride from species of this Group would, however, obviously encourage the widening of the range of assay species used in biological tests. Again, a similar suggestion may be put forward for species in Group III although, here, only F. hemitephrus showed slight activity against bacteria. However, the obvious next step with this group, as with the other groups, is further chemical analysis.

4. Taxonomic implications.

The consistency of G.L.C. pattern produced by the F. pomaceus group could provide a useful character for classifying this difficult group, the present taxonomy of which is under conflict (Bourdot & Galzin, 1927; Cunningham, 1948, 1954; Overholts, 1953; Teixeira, 1962).

The production of hexatriyne, however, does not appear to be consistent in this genus; no species examined in this survey was seen to produce it. Although this observation may be consistent with the opinion held by Donk (personal communication) that this species, F. annosus, is misplaced in the genus Fomes, the irregularity of the appearance of hexatriyne in different strains of this species (reported elsewhere) suggests that this character may not be taxonomically useful in this case.

Similar indications of the inconsistency of the production of particular metabolites within one taxonomic species appear in this survey, in the case of F. noxius 224A and 170.32. Also, this is a character which will obviously be easily affected by environmental conditions, nature of food source, extent of ventilation, etc. Thus, indications are that very little weight could be placed on the use of volatile metabolite production in taxonomic studies.

SECTION IB

INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES FROM CULTURES OF FOMES SCUTELLATUS (SCHW.) COOKE.

SECTION IB. INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES FROM CULTURES OF FOMES SCUTELLATUS (SCHW.) COOKE.

Introduction.

The results in Section IA results paragraph 1 (a), (b) and (c) show that in the conditions examined, gases from cultures of F. scutellatus had no recognisable and consistently significant effect on the germination of lettuce seeds, on the sporulation and colony growth of A. niger, or on the growth of the assay bacteria. They indicate that the growth of lettuce seedlings is significantly reduced by the gases in the conditions examined. The following is a report on an extension of the biological survey by a test of the effects of the gases on the growth of other cultures of the same species, and on an investigation of the factors contributing to the inhibition of growth of lettuce.

1. Extension of biological survey.

a) Test of the effects of culture gases on the growth of other cultures of the same species.

Methods.

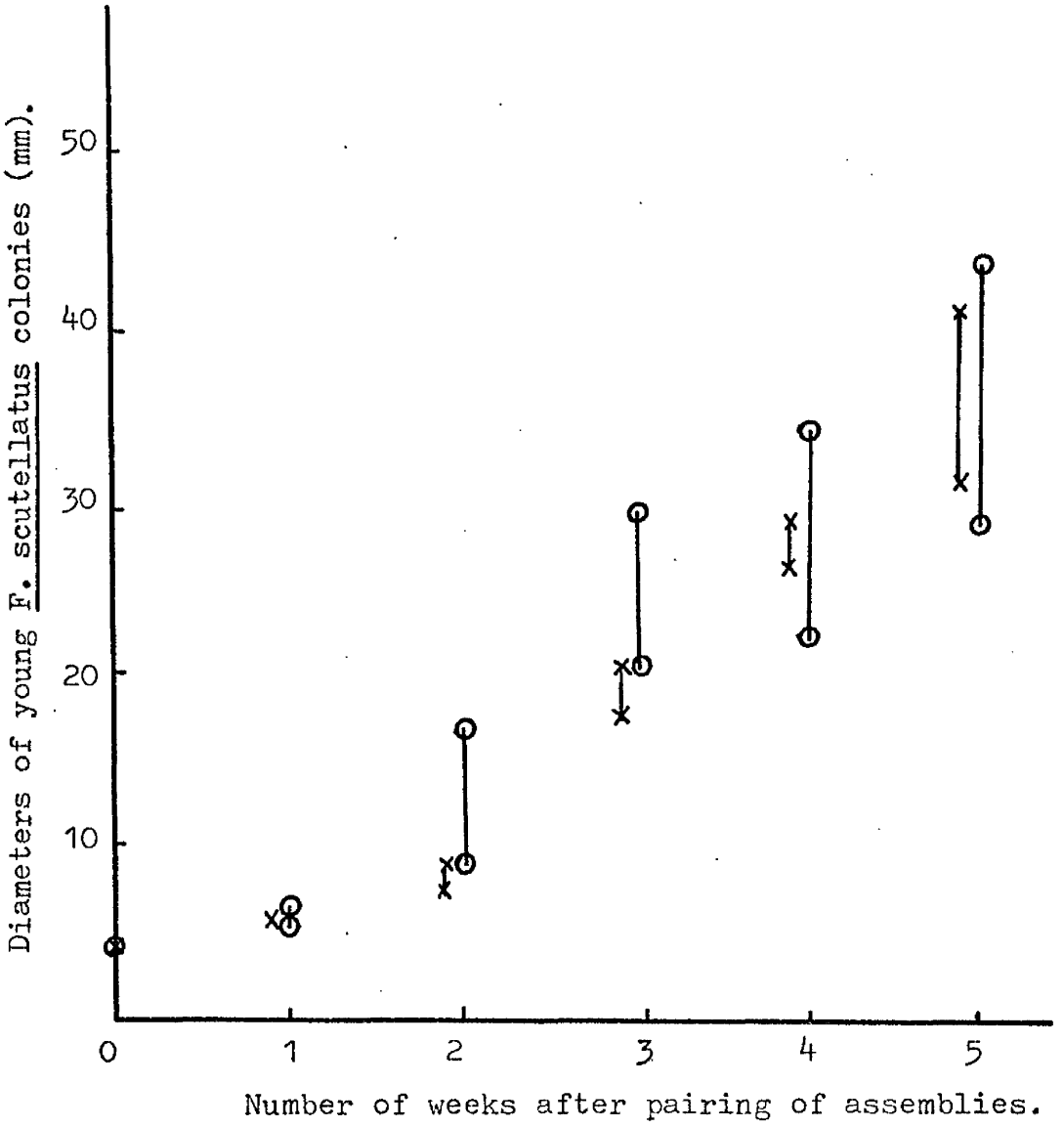
The effect was tested using paired petri dish assemblies (as described in General Methods, paragraph 2 (a)). All cultures were

grown on 5% malt agar and incubated at 24°C in diffuse light. The assay cultures were inoculated centrally with a 4.0 mm disc cut from a petri dish culture. They were paired immediately after inoculation with a culture aged about seven weeks or with a plate of uninoculated 5% malt agar in a control. Paired assemblies were incubated at 24°C in diffuse light. At appropriate time intervals the diameter of each assay colony was recorded as the mean value of two diameters at right angles to each other. Experiments each consisted of at least three test assemblies compared with three controls.

Results.

Text Figure (x) gives the results of one experiment. In this experiment there was no significant difference between the mean of diameters of colonies in assemblies with F. scutellatus and in controls with uninoculated 5% malt agar. In this experiment the variation in the diameters of colonies was slightly greater in the control than in the test treatments, but these differences were considered trivial and were not investigated further. The results of two replicate experiments, given in appendix Table VI, agreed closely with those in Figure (x) except that the variation of measurements in treatments with F. scutellatus and in controls were comparable.

Figure(x). Growth of young colonies of F. scutellatus in paired petri dish assemblies containing young cultures of F. scutellatus paired with mature cultures of the same species and in controls, paired with uninoculated 5% malt agar.



Each line shows the range of readings from 3 assemblies with mature F. scutellatus (x——x) and uninoculated 5% malt agar (o——o). Differences between the mean values of colony diameter for the F. scutellatus and uninoculated agar treatment were not significantly different (P=0.05) at any of the times when measured.

For detailed results see appendix Table VI, expt. I.

Table 5. Carbon dioxide and oxygen concentrations in assemblies testing the effects of gases from F. scutellatus cultures on lettuce.

Treatment		Range of CO_2 concentration as % volume/volume of air space	Range of O_2 concentration as % volume/volume of air space
Paired bottles	Lettuce in presence of F. scutellatus gases	0.02 - 0.18	17.0 - 25.2
	Lettuce without F. scutellatus gases	0.02 - 0.13	15.3 - 23.3
Paired petri dishes	Lettuce in presence of F. scutellatus gases	0.02 - 0.04	20.0 - 20.1
	Lettuce without F. scutellatus gases	0.02 - 0.05	20.0 - 20.5

Conclusion.

It is concluded that these experiments give no evidence to support a hypothesis that the fungus produces volatile metabolites which can inhibit the linear increase in growth of colonies.

2. Analysis of culture gases and tests with authentic components of identified constituents.

a) Carbon dioxide and oxygen.

Methods.

The methods used are described in the General Methods, paragraph 5.

To avoid the effects of pressure changes etc., involved in sampling, any variations in the amounts were not determined during tests. It was obviously not possible to maintain a constant carbon dioxide level in sealed vessels containing living green plants, hence, in tests with authentic carbon dioxide and air mixtures, the initial concentrations were chosen to produce ranges comparable to those found in tests with lettuce and F. scutellatus cultures. The concentrations in each assembly were measured again at the end of each experiment. The concentrations in each assembly did not differ by more than 24% of their average in any assembly.

Results.

Text Table 5 gives the amounts of these metabolites found at the end of representative experiments with paired bottle assemblies and with paired petri dish assemblies.

No greater concentration of carbon dioxide or lower concentration of oxygen was seen in any assembly in measurements made in many other similar experiments after shorter and longer incubation periods.

Text Figure (xi) gives the result of a typical experiment examining the effect of concentrations of authentic carbon dioxide in single bottle assemblies (detailed results in appendix Table VII).

Differences between the number of germinated seeds and between the mean lengths of germinated seedlings are not significant over the range of carbon dioxide concentrations tested ($P=0.05$).

The results of two similar experiments are given in appendix Table VII. They agree closely with those in text Figure (xi).

The changes in oxygen concentration were not more than 20% in assemblies with F. scutellatus cultures, compared with 27% in assemblies with uninoculated 5% malt agar.

The range of carbon dioxide and oxygen concentrations detected in paired bottle assemblies differed little and those in paired petri dish assemblies even less from that of normal laboratory air. The levels of carbon dioxide measured were all below the range shown in tests with authentic mixtures to have no effect on the germination and growth of lettuce.

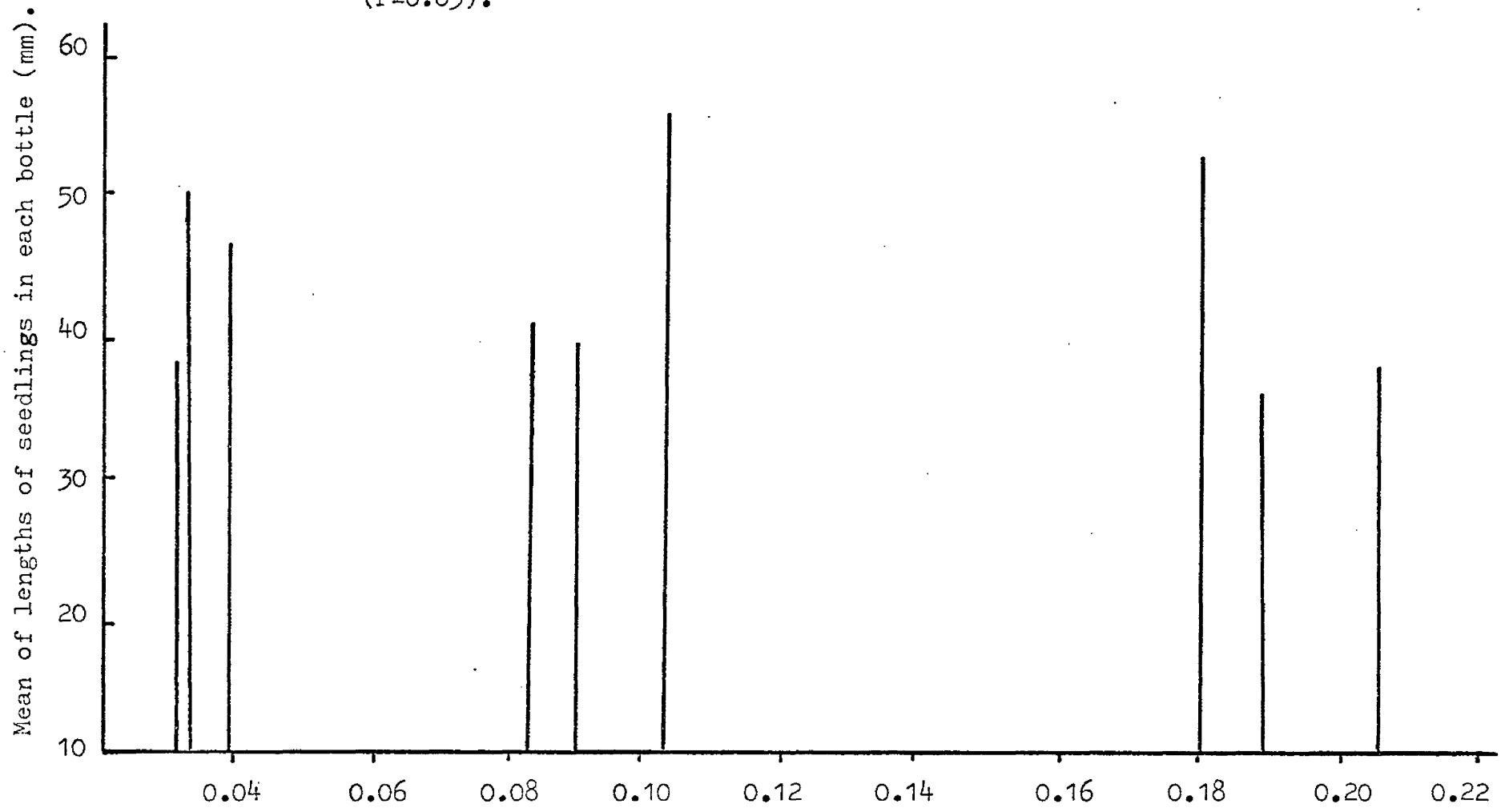
The changes in oxygen concentration were considered to be trivial and the possibility of their having a significant effect was not investigated.

Conclusion

Levels of carbon dioxide and oxygen present are unlikely to contribute significantly to the effects under these conditions.

Figure (xi). Germination of lettuce seeds and length of germinated seedlings in sealed bottles containing known ranges of mixtures of CO_2 and air.

The differences between the means for % germination and lengths of shoots + roots of germinated seedlings are not significant (P=0.05).



Mean of initial and final CO_2 concentration, as vol. of CO_2 per 100 vols. of gas space in each bottle

90 97 90

Mean of records of number of germinated seeds as % of total seeds in the three bottles in the range

Table 6. Operating conditions used for chromatographic analysis of *F. scutellatus* culture gases.

Column					Hydrogen flow rate (ml/min)	Use of columns
Dimensions	Material	Packing	Temperature(°C)	Carrier gas and flow rate(ml/min)		
6ft x 1/8in	Stainless steel	20% 1,2,3-Tris-(2-cyanoethoxy) propane on 60/80 Chromosorb W	65 - 120 and held for 30 min	40	30	Low molecular weight oxygenated compounds
6ft x 1/8in	Stainless steel	10% Apiezon L on 60/80 Chromosorb W	80 - 120 and held for 30 min	38	30	General use
10ft x 1/4in	Stainless steel	Molecular sieve 5A on 60/90 mesh	70	32	30	Permanent gases

b) Other metabolites.

i) Extension of G.L.C. examination.

Introduction.

No compounds other than carbon dioxide and oxygen were detected by G.L.C. analyses in the Fomes survey (Section IA, results paragraph 2.) G.L.C. analyses were extended to include a wider range of conditions of analysis.

Methods.

Text Table 6 gives the additional G.L.C. conditions under which 5 ml samples of gases from the headspace above Roux bottle cultures of F. scutellatus and similar samples were examined.

Results.

No metabolites were detected under these conditions.

Conclusion.

In view of the results in the following section it was not considered profitable to continue this examination at present.

ii) Hydrogen cyanide.

Introduction.

A distinct smell of bitter almonds omitted from the cultures of F. scutellatus suggested that HCN might be present. HCN has been detected by G.L.C. by Woolmington (1961) using a Carbowax 1500 column, by Isbell (1963) using a molecular sieve 5A column in series with a triacetin column, and by Schneider & Freund (1962) using a DNP column for samples trapped on a cooled precolumn. Schneider & Freund said that its high ionization potential made it difficult to detect by G.L.C., even in high concentration. Claeys & Freund (1968) were able to detect it in air bubbled through a solution of HCN (1µg per litre water) using a Porapak Q column, although to obtain detectable concentrations the air from above the solution had to be passed through a cold trap for 30 minutes.

In my investigation, no HCN was detected in headspace gases using Carbowax 1500, DNP or molecular sieve 5A columns using the range of concentrations described laterin this section. No Porapak Q column

was available and the procedure of pretrapping seemd likely to be too inconvenient for this particular work to justify examination of the process. Hence alternative classical techniques were investigated.

Most previous investigations of HCN production by living things has been based on tests with picrate or Gruignard's reagent. A more accurate test is described by Feigl & Anger (1966) and a technique based on a modification of this was developed for this investigation.

In these tests, a solution of 5% cupric ethylacetoacetate plus 5% tetrabase (tetramethyldiaminodiphenylmethane) was made up in chloroform; 1 cm wide strips of filter paper were dipped in this solution and then allowed to dry. In the presence of hydrogen cyanide or dicyanogen the colour of these strips changes from pale green to blue.

In the presence of gases from F. scutellatus cultures, a blue colouration was obtained.

An additional test characteristic for dicyanogen was carried out on the culture gases. This test is described by Feigl & Anger (1966) and was carried out here by saturating a 1 cm wide strip of filter paper in oxine (8-hydroxyquinoline) and then moistening this paper with a drop of 25% potassium cyanide solution. In the presence of dicyanogen, a red colouration is produced.

In the presence of gases from F. scutellatus cultures, no red colour was produced with this test, suggesting that the positive reaction in the cupric ethylacetoacetate plus tetrabase was due to the presence of

hydrogen cyanide only, in the culture gases.

The possibility of adapting this test for quantitative estimates of gaseous HCN was examined. Appendix Table VIII records the time taken by test papers to attain standard shades of blue when placed in the atmosphere above 10 ml aliquots of standard KCN solutions in the concentration range 0.1N to 0.001N contained in sealed 100 ml conical flasks. The colours used were Ridgway's standard colours, pallid violet blue, light violet blue, and smalt blue. This Table shows that the time taken to develop the three standard colours is proportional to the concentration of KCN solution in the flasks. Hydrogen cyanide can be detected in a short time in the air above the 0.001N KCN solution, but similar tests carried out below this order of concentration, showed that the reaction time is slow and too variable to be useful for quantitative estimates. Comparison of the means of times taken to attain the two paler shades of blue, pallid and light violet blue, in these tests (see Table VIII in the appendix) showed that differences between the mean values of time taken to attain these two colours for a 0.1N KCN solution compared with a 0.01N KCN solution and for a 0.01N KCN solution compared with a 0.001N KCN solution was of a similar order and could consistently be measured within this range. Smalt blue was not attained after 24 hours in some tests with low concentrations of KCN and this was not useful in quantitative estimations in this range. Since the palest colour, pallid violet blue was attained most rapidly,

Table 7. Time taken for pallid violet blue colour [Ridgway 53v-BF] to develop in cupric ethylacetoacetate plus tetrabase on filter paper in the tests described in the text.

Concentration of KCN solution [xN]	*Time for colour to develop [min]	Concentration of KCN solution [xN]	*Time for colour to develop [min]
0.001	3.7	0.01	0.85
0.002	3.1	0.02	0.75
0.003	2.5	0.03	0.63
0.004	2.1	0.04	0.52
0.005	2.0	0.05	0.50
0.006	1.9	0.06	0.33
0.007	1.5	0.07	0.32
0.008	1.7	0.08	0.28
0.009	1.5	0.09	0.27
0.01	0.85	0.1	0.18

* each value is the mean of 3 determinations, each of which was carried out on a different replicate flask. The least significant difference between the means of readings for 0.001N and 0.01N = 2.1 min , between 0.01 and 0.1N = 0.48 min.

this shade was used as the standard colour in subsequent estimations. Text Table 7 summarizes the results of the test given in appendix Table VIII for this colour.

To see if this quantitative test could be used for the concentrations of HCN present in test assemblies with F. scutellatus cultures and lettuce, the time for test papers to develop the standard colour in several assemblies was measured at the end of the seven day assay period. Text Table 8 gives the measurements from six paired bottle assemblies.

Table 8.

Assembly no.	* Time taken for standard colour to develop in test strips in paired bottle assemblies (min)
1	5
2	10
3	5
4	15
5	4
6	19

* Readings to the nearest minute.

This Table shows that the times taken for the standard colour to develop in these assemblies was slightly longer than that taken in the flasks with standard KCN solutions shown in appendix Table VIII and in

text Table 7. However the volume of gas space and other factors possibly affecting the concentration of gaseous HCN obviously differed in the two sets of tests.

It was appreciated that the introduction of strips of impregnated paper, and the opening of assemblies involved in this, would introduce many additional variables into the apparatus. Appropriate replication and sampling procedures were therefore devised to allow for this.

In experiments where the daily changes in concentration of HCN were measured in paired bottle assemblies nine assemblies were set up for each treatment; three different ones of each were examined after 18 hours, and on days 1, 2, 3, 5, and 6, and all were examined on day 7. Each assembly was thus tested twice during incubation, and all were tested on the 7th (final) day.

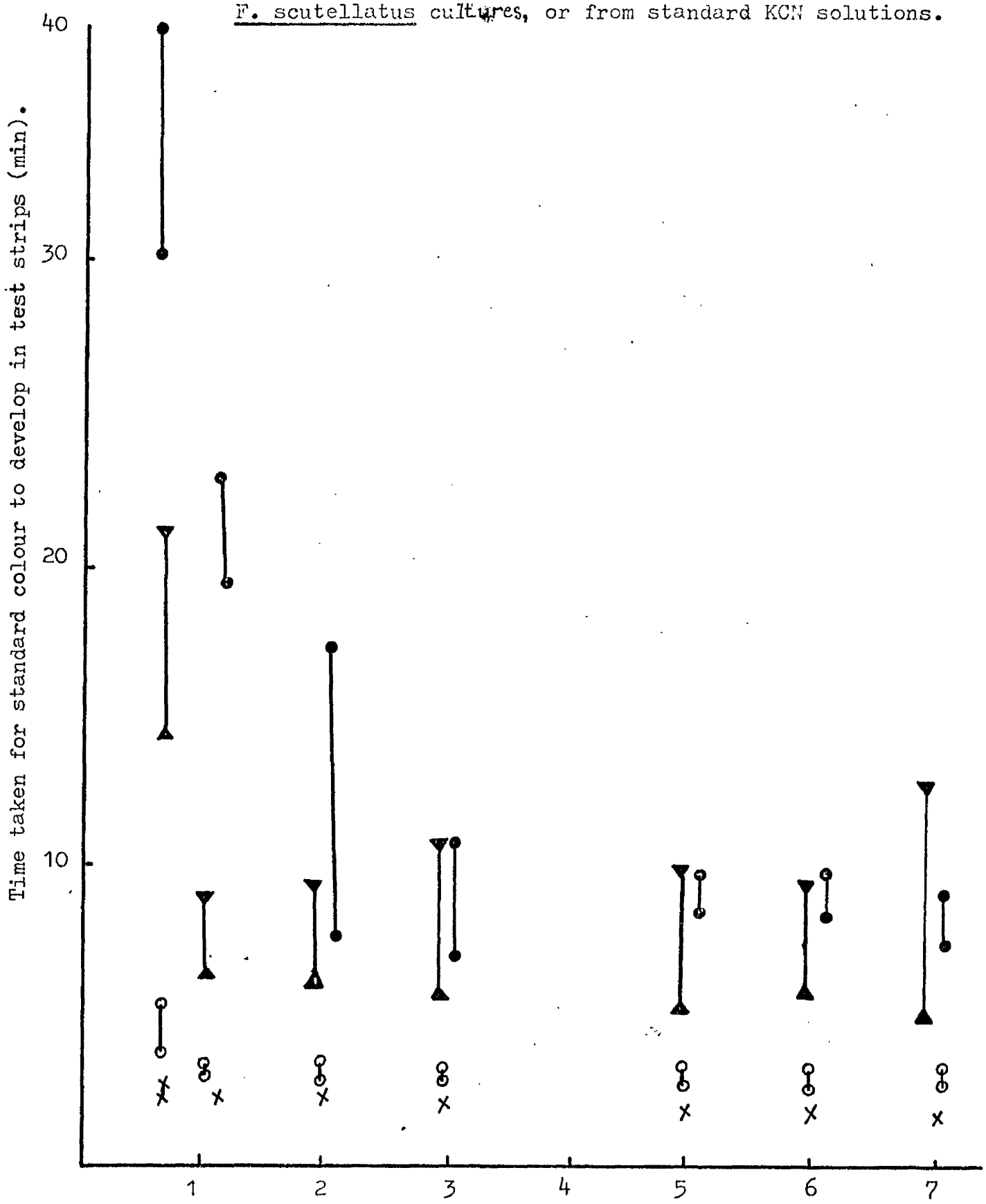
In experiments where the biological effects were examined, the concentrations were measured only at the end of the experiments.

Results.

a) Analysis of HCN in assemblies used in biological test.

Text Figure (xii) gives the daily changes in the concentration of HCN in paired bottle assemblies containing lettuce and F. scutellatus cultures, and HCN concentrations in comparable assemblies containing lettuce and standard authentic solutions of KCN.

Figure(xii). Changes in HCN concentration in paired bottle assemblies containing lettuce seedlings and gases from F. scutellatus cultures, or from standard KCN solutions.



Each line shows the range of the readings from the 3 assemblies using ●—● = F. scutellatus gases, ×—× = 0.1N KCN solution, o—o = 0.01N KCN solution, ▷—◁ = 0.001N KCN solution.

The results were closely consistent, and those for F. scutellatus were confirmed by a replicate experiment, (for detailed results of these experiments and results of the replicate experiment with F. scutellatus, see appendix Table IX)

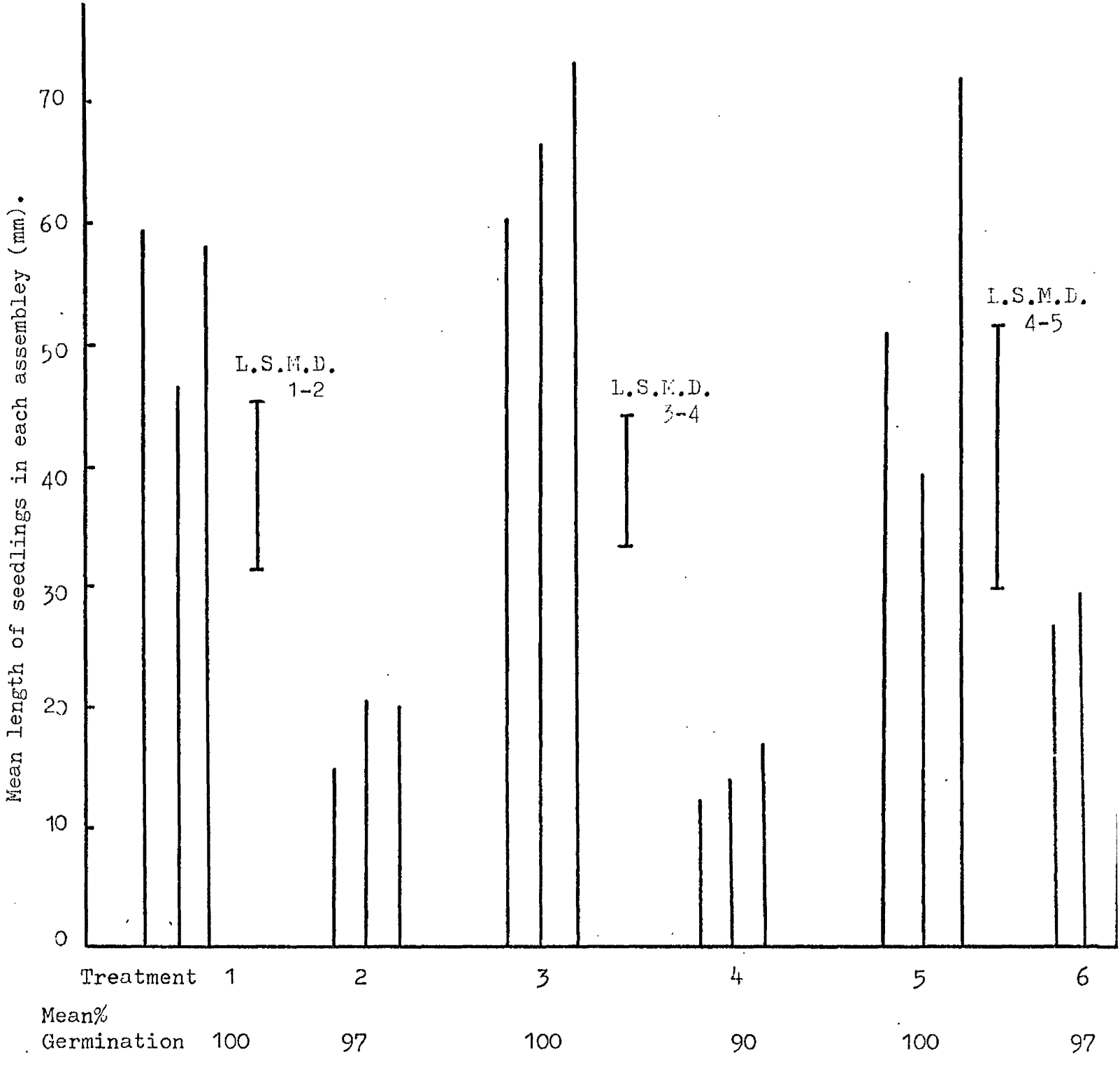
b) Tests with authentic samples of HCN

Text Figure (xiii) gives the results of a typical experiment comparing the effects produced by F. scutellatus culture gases on lettuce with those of authentic HCN/air mixtures of similar concentration. The concentration of KCN used was 0.001N, chosen as the one most likely to give a similar concentration of gaseous HCN to that provided by the culture of F. scutellatus in these assemblies (cf. text Figure (xii)). However, it appears from Figure (xiii) that this concentration may take up to 48 hours to build up in vessels containing F. scutellatus cultures, but only 24 hours in vessels with the solution. To allow for the possible effects of this delay, one set of bottles with KCN solution was paired immediately after the lettuce seeds were planted, and another set was paired with bottles containing lettuce seeds which had been incubated for 24 hours after planting.

The detailed results, including measurements of terminal HCN concentrations and given, for this and two replicate experiments in appendix Tables X, XI, and XII.

The results of all three experiments were closely similar and are summarized in text Table 10.

Figure (xiii). Germination of lettuce seeds and growth of seedlings in paired bottle assemblies containing lettuce paired with F. scutellatus cultures, 0.001N KCN, and in controls paired with uninoculated 5% malt agar.



Three assemblies each containing 10 lettuce seeds were set up for each treatment; the treatments consisted of pairing with (1) sterile 5% malt agar (2) F. scutellatus cultures (3) 10 ml sterile distilled water (4) 10 ml 0.001N aqueous KCN solution. Treatments (1) to (4) were paired immediately after planting the lettuce seeds. (5) 10 ml sterile distilled water (6) 10 ml 0.001N aqueous KCN. Treatments (5) and (6) were paired with lettuce seeds which had been incubated for 24 hrs. after planting. (Measurements were made after 7 days incubation). L.S.M.D. $_{1-2}$ = least significant difference between means for treatments stated.

For detailed results, see appendix Table X.

Table 10. Summary of results of tests in paired bottle assemblies containing lettuce paired with F. scutellatus cultures, 10 ml standard 0.001N KCN solution, compared with control treatments, with uninoculated 5% malt agar, or sterile deionized water.

Expt. no.	Treatment	% reduction compared with control treatments			
		% germination		Mean total seedling length	
		observed	least significant	observed	* least significant
I	F. scutellatus	23	25	83.3	29.8
	0.001N KCN paired immediately	7	9	61.1	18.5
	0.001N KCN paired after 24 hrs	0	0	65.7	23.0
II	F. scutellatus	27	26	66.0	59.5
	0.001N KCN paired immediately	3	8	80.2	21.2
	0.001N KCN paired after 24 hrs	10	16	50.4	41.1
III	F. scutellatus	3	8	67.1	26.0
	0.001N KCN paired immediately	10	16	78.9	16.7
	0.001N KCN paired after 24 hrs	3	8	65.1	33.0

* P = 0.05

Text Table 11 summarizes the results of comparable experiments, three with 0.1N KCN solution and three with 0.01N KCN solution with bottles paired immediately after planting, and one experiment with 0.002N KCN in which one set of bottles was paired immediately after the lettuce seeds were planted and another set paired with bottles containing lettuce seeds which had been incubated for 24 hours after planting. Full results of these experiments are given in appendix Tables XIII and XIV.

Table 11. Summary of results of tests in paired bottle assemblies containing lettuce paired with 10 ml standard 0.1N, 0.01N, 0.002N and 0.001N KCN solutions and in controls, with sterile deionized water.

Expt. no.	Treatment	% reduction compared with control treatments			
		% germination		mean total seedling lengths (mm)	
		observed	*least significant	observed	least significant
I	0.01N KCN	93	33	97.9	30.6
	0.1N KCN	100	31	100	30.6
II	0.01N KCN	100		100	43.5
	0.1N KCN	100		100	43.5
III	0.01N KCN	100	89	100	7.7
	0.1N KCN	100	89	100	7.7
	F. scutellatus	20	32	85.9	24.7
	0.002N KCN paired immediately	67	39	94.0	44.3
	0.002N KCN paired after 24 hrs	26	27	89.6	9.4

* P = 0.05

b) Comparable tests with paired petri dish assemblies.

The results of additional tests with paired petri dish assemblies are detailed in the appendix. These results were all more variable than those obtained with the Roux bottle assemblies. The concentration of HCN fell off rapidly during incubation of petri dish assemblies with KCN solution, while in assemblies with F. scutellatus cultures it rose to a maximum within 24 hours and thereafter changed only slightly although it was still very variable throughout incubation (appendix Table XV). The results of tests with authentic KCN solutions on lettuce in paired petri dish assemblies (appendix Table XVI and XVII) were similar to, but again more variable than, those obtained with the paired bottle assemblies.

Discussion.

There are many previous reports of HCN production by fungi. Reports of HCN production by species of Clitocybe are given in Section IIB of this thesis. Some additional reports are those by Lösecke (1871), Zellner (1907), Bayliss (1911), Müller (1944), Langeron (1945), Heim (1948), Bach (1948), Robbins, Rolnick & Kavanagh (1950), Lebeau & Dickson (1953) and Singer (1962). Locquin (1944), in fact, concludes that HCN is a normal metabolite of fungi.

Most reports have dealt with the production and effects of HCN in solution, but Robbins et al., (1950) reported that several species of fungi were inhibited by gaseous HCN produced by the mycelium of an

unidentified Basidiomycete from their collection. They found that there was "little difference" between the appearance of Aspergillus niger cultures in normal laboratory conditions and those exposed to fungal gases for 2 weeks. They were unable to demonstrate HCN production by actively growing mycelium, and they considered that it was "probably formed by autolysis". Locquin (1947) found that several species of bacteria were inhibited by HCN gas from Marasmius oreades cultures. Volatile HCN is thought to be responsible for the disease symptoms in snow mould of young alfalfa plants (Lebeau & Dickson, 1953, 1955; Lebeau, Cormack & Moffatt, 1959; Ward & Lebeau, 1962; Ward, 1964; and Ward & Thorn, 1966) and in fairy ring disease of grass caused by Marasmius oreades (Bayliss, 1911; Lebeau & Hawn, 1963; Filer, 1965, 1966). These reports are mostly, however, based on the analysis of culture filtrates or mycelial extracts and the study of their effects on the hosts, not on the direct study of the effect of culture gases, although Filer (1966) did demonstrate the evolution of gaseous HCN by one isolate of Marasmius oreades in sufficient quantities to inhibit the growth of seedlings of Poa pratensis, Agrostis tenuis and Festuca rubra.

The results reported here support the hypothesis that in these test conditions Fomes scutellatus cultures can build up a concentration of gaseous HCN equivalent to that formed above an aqueous solution of KCN of the order of 0.001N, and that this could account by itself for

all the inhibitory effects seen. In particular the results in text Figure (xii) show that the HCN concentration built up to an inhibiting level within 24-48 hours of pairing the assemblies, and that this concentration changed only slightly during the remainder of the test period. The results in text Figure(xiii) treatments 4 & 6, show that a delay of 24 hours in the building up of an inhibiting concentration had no significant effect on inhibition compared with that in controls. The changes in carbon dioxide concentration are slight, and comparison with the effects of similar concentrations of authentic carbon dioxide suggest that it is unlikely to have had any significant effect in these conditions. It may, of course, have effects in other conditions. At the moment, however, it does not seem justifiable to continue the study either of this possibility or that of some other unidentified metabolite affecting the interaction in these or other conditions. The possibility of hydrogen cyanide affecting interactions in nature is obviously dependent on the particular conditions which may affect production, the build up of active concentrations, on the sensitivity of organisms to it, etc. This is particularly well illustrated by the variability of the records of germination of seeds and inhibition of seedling growth especially in paired petri dishes, but it will contribute to most of the other variation seen throughout the investigations. In most case the slightly lower% germination recorded in the presence of F. scutellatus

gases is not significantly different from that in pure culture, but in a few cases very significant reductions were recorded. In the paired bottles the record of germination in the presence of 0.001N KCN solution is not significantly different from that in assemblies with water only, while germination was significantly reduced in assemblies with 0.002N KCN solution; almost complete inhibition of germination was recorded in assemblies with 0.01N KCN and 0.1N KCN solution. Such relatively slight changes in HCN concentration could account for the variation in germination in all assemblies and greater variation and reduction in germination in the petri dishes.

It seems likely that the effect of HCN is primarily on the cytochrome system in aerobic respiration. If this is so then the flexibility of the anaerobic respiratory pathways of many bacteria and fungi would suggest that they would be unlikely to be affected. In this connection Iwanoff & Osnizkaja (1934) showed that A. niger is not inhibited by, and can even utilize cyanide and Chughtai & Walker (1956), who showed that concentrations of 0.02N were optimum for mycelial growth. The results presented here show that F. scutellatus is itself able to grow apparently normally in mixtures with a high HCN concentration, and hence that this is unlikely to be self-inhibitory in most conditions.

It is obviously possible to suggest many niches in soil etc. where the product may have a significant ecological effect and the results of a brief investigation are described in Section IV of this thesis.

SECTION IC

INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES FROM CULTURES OF FOMES NOXIUS CORNER.

SECTION IC. INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES FROM CULTURES OF FOMES NOXIUS CORNER.

Introduction.

The results in Section IA paragraph 1 (a), (b) and (c) show that in the conditions examined, gases from cultures of F. noxius inhibited the root growth of lettuce, but had no significantly consistent effect on the germination or shoot growth of lettuce seeds, or on the sporulation and colony growth of A. niger, or on the growth of assay bacteria. The results in Section IA paragraph 2 show that the culture gases of this species give four distinct peaks on G.L.C. traces. For these two reasons the activity of this species seemed likely to be a suitable subject for further investigation.

1. Extension of biological survey.

a) Further examination of the characteristic effects on lettuce.

i) Description of symptoms.

Results.

The gross characteristic appearance of seedlings grown in the presence of F. noxius culture gases has already been reported and illustrated in Section IA, results paragraph 1 (a). Microscopic examination showed that cell walls in affected parts were brown and mucilaginous and that in more severely affected parts, the roots were completely dried up and shrivelled. Roots were affected from the tips

upwards to the shoot. The extent of damage was of the same order within individual assemblies, but, as can be seen from the lengths of roots measured in different assemblies recorded in appendix Table II, the variation in extent of inhibition in different assemblies was considerable. The reasons for this variation were not investigated at this stage.

ii) Examination of ability of inhibited seedlings to continue growth when removed to atmospheres free from F. noxius gases.

Methods.

Paired petri dish assemblies were used as described in Section IA, methods paragraph 2 (a). After incubation for seven days, the lettuce seedlings were examined in the usual way. Examination was carried out as rapidly as possible and with the minimum of disturbance to the seedlings. Immediately after examination, half of the dishes containing lettuce which had been paired with F. noxius cultures and half of those which had been paired with uninoculated 5% malt agar were reassembled with fresh uninoculated 5% malt agar. The remaining plates were reassembled with the same plates as before. All assemblies were incubated for a further seven days after which the lettuce seedlings were re-examined. Other experimental details and number of replicates etc., are given in the tables and illustration of results.

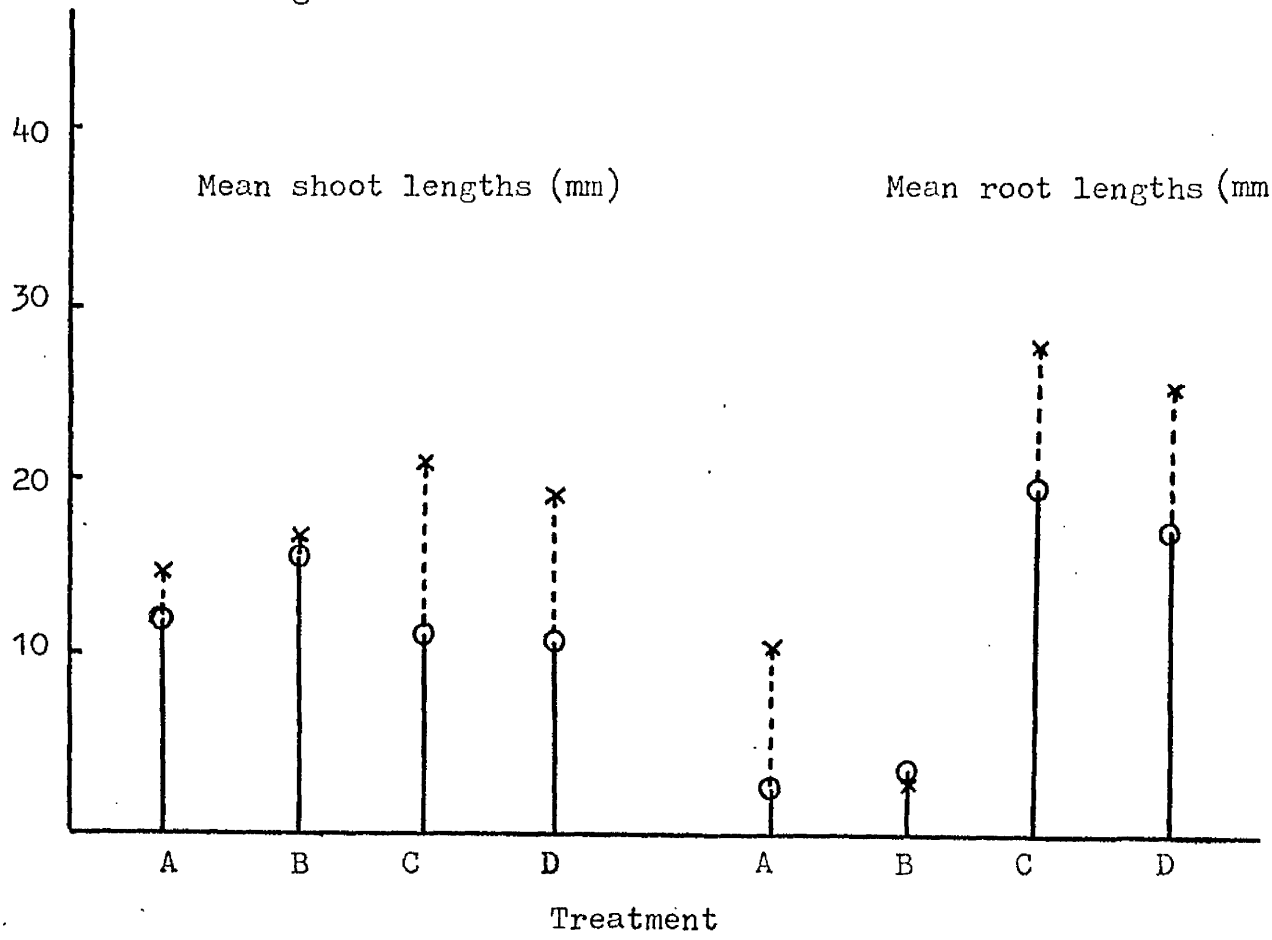
Results.

Appendix Tables XVIII, XIX and XX give the measurements of germination and of seedling length made on days 7 and 14 in three replicate experiments. Text Figure (xiv) illustrates the results of a typical experiment.

In two of three replicate experiments, seedlings remaining in the presence of F. noxius gases between days 7 and 14 showed a significant reduction in length over this period. Where seedlings remained in the presence of unchanged uninoculated 5% malt agar extension of roots and shoots was recorded, similar in extent to that in assemblies where dishes containing lettuce were reassembled with fresh uninoculated 5% malt agar. Similarly, in assemblies where F. noxius cultures were replaced with uninoculated 5% malt agar on day 7, root elongation between days 7 and 14 was similar to that in control assemblies, paired with uninoculated 5% malt agar for the whole incubation period in all three trials. Shoot elongation in these was comparable in two of the three trials and only just significantly different in the third.

In assemblies where F. noxius cultures were exchanged for uninoculated 5% malt agar after seven days, there was a tendency for growth to be resumed. They formed new lateral roots and adventitious roots and the primary root did not develop further. In control treatments with uninoculated agar only, lateral roots remained short

Figure (xiv). Shoot and root lengths of germinated lettuce seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures for 7 and 14 days and in controls, paired with uninoculated 5% malt agar.



Treatment A = In presence of F. noxius from days 1-7
" " " " 5% MA " " 7-14
" B = In presence of F. noxius from days 1-14
" C = In presence of 5% M A from days 1-14
(5% MA replaced with fresh medium from days 7-14)
" D = In presence of (unchanged) 5% m A from days 1-14

O = Mean of length of seedlings in three assemblies after 7 days incubation
x = " " " " " " " " " 14 days "
___ = Elongation between days 1 to 7
--- = " " " 7 to 14

Treatments	Differences between mean shoot lengths (mm) extension between days 7 and 14 (mm) Observed	L.S.M.D.	Differences between mean root (mm) extension between days 7 and 14 Observed	L.S.M.D.
A & B	2.2	5.4	8.6	3.5
A & C	8.1	8.7	1.1	6.3
B & D	8.6	2.1	10.0	6.8
C & D	1.9	9.1	0.3	8.6

For detailed results, see appendix Table XVIII.

and there were few adventitous roots. Where inhibition by F. noxius gases after seven days was slight, elongation was continued mainly by the primary root when seedlings were subsequently paired with uninoculated 5% malt agar.

Discussion.

These results suggest that the effect was mostly on the active apical meristem, that it was permanently killed, and that lateral dormant meristems were not killed. These could begin to grow as soon as they were removed from the culture gases.

These observations were not further investigated here, but it is appreciated that they may have some relevance in connection with the pathogenicity of F. noxius discussed at the end of this section.

b) Examination of the effects of F. noxius culture gases on cress.

Methods.

Paired petri dish assemblies as described in the General Methods paragraph 2 (a) were used in these tests. Cress seeds were sterilized, planted and incubated as for lettuce.

Results.

Appendix Table XXI gives the results and experimental detail of three replicate experiments. In these tests, there were no significant differences between the means of numbers of germinated seeds, or of the mean lengths of germinated seedlings, in assemblies containing cress paired with F. noxius cultures and in controls

Table 12. Growth of roots of lettuce and cress in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures compared with growth in controls paired with uninoculated 5% malt agar.

Expt. no.	Lengths of roots of lettuce		Lengths of roots of cress	
	Mean % inhibition	Mean inhibition (x value required for significance (P=0.05))	Mean % inhibition	Mean inhibition (x value required for significance (P=0.05))
I	62.2	3.9	64.8	3.5
II	30.5	2.9	60.2	4.0
III	78.7	5.4	45.4	5.5

paired with uninoculated 5% malt agar. In all three experiments, root growth was significantly less in assemblies with F. noxius cultures compared with controls.

The appearance of the cress seedlings in assemblies with F. noxius cultures was closely similar to that of affected lettuce seedlings in similar tests.

Text Table 12 gives the mean inhibition of growth of roots of cress and lettuce seedlings in assemblies with F. noxius gases compared with control treatments in similar tests, in the three experiments already described for each species. In these tests, lettuce and cress are inhibited to a similar extent.

Discussion.

Lettuce seeds were found to be easier to handle than cress and since there was no evident difference in the behaviour of these two species, this investigation was continued using only lettuce as an assay organism.

2. Analysis of culture gases and tests with authentic samples of identified constituents.

Introduction.

This section is presented in three sub-sections:-

a) Analysis of carbon dioxide and oxygen and tests with authentic samples of these constituents.

b) Analysis of other gaseous metabolites by G.L.C. and tests with authentic samples of identified constituents.

c) Examination of culture gases by other techniques.

Note.

In all subsequent tests with lettuce reported in this section only % germination and root growth were analysed statistically since F. noxius culture gases have been shown to consistently affect root and not shoot growth in the preliminary tests.

a) Analysis of carbon dioxide and oxygen and tests with authentic samples of these constituents.

Methods.

Carbon dioxide and oxygen levels were determined and authentic carbon dioxide was tested by the methods described in the General Methods, paragraphs 5 and 2(d) respectively. Both paired bottle and paired petri dish assemblies were used. The weakness of the paired petri dish assemblies, in some experiments was appreciated, for reasons given in the appropriate places below. Carbon dioxide and oxygen levels were measured only at the end of biological tests, except in one experiment with each type of assembly. This was set up to measure changes during incubation, using a sufficiently large number of replicates to permit daily sampling, as described in the F. scutellatus investigation, Section IB, paragraph 2 (a). In this examination, however, test assemblies were each examined on every occasion since the introduction of variables by sampling with a syringe was considered to be small.

Results.

i) Records of terminal levels of carbon dioxide and oxygen in assemblies where biological effects were seen.

In paired bottle assemblies.

Appendix Table XXII gives the terminal concentrations of carbon dioxide and oxygen found in paired bottle assemblies containing lettuce paired with F. noxius cultures and in controls, paired with uninoculated 5% malt agar. This Table also gives the germination and growth of lettuce in these assemblies in the three replicate experiments carried out. The range of carbon dioxide and oxygen concentrations detected in these experiments is recorded in text Table 13. In all three experiments, the germination of the lettuce seeds and growth of germinated seedlings was significantly less in the presence of F. noxius than in controls. Inhibited seedlings showed general stunting and did not show the symptoms characteristic of inhibition by F. noxius. The symptoms found in these tests are discussed below in relation to those recorded in assemblies with high authentic carbon dioxide concentrations.

In paired petri dish assemblies.

Terminal carbon dioxide and oxygen concentrations were measured in assemblies set up to examine the biological effects of F. noxius gases on lettuce reported in Section IA. The range of concentrations in these experiments is recorded in text Table 13. It differs little from that of the atmosphere.

The biological effects recorded in these experiments are reported in Section IA.

The symptoms were the characteristic ones reported on page 38 above.

Table 13. Range of carbon dioxide and oxygen concentrations recorded in assemblies containing lettuce paired with F. noxius cultures and in controls, paired with uninoculated 5% malt agar.

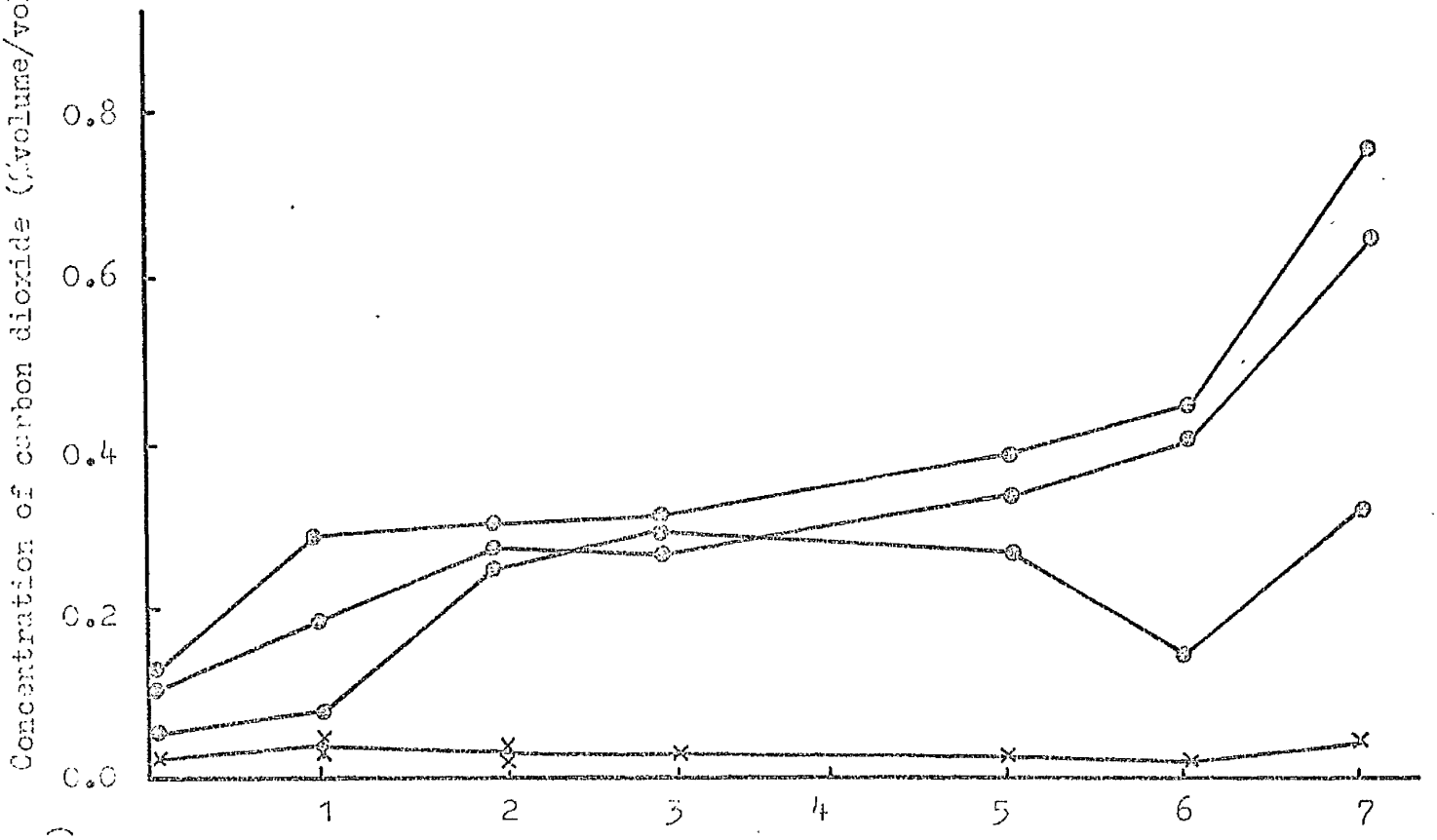
Treatment		Range of CO_2 concentrations as % volume/volume of air space	Range of O_2 concentrations as % volume/volume of air space
Paired bottles	Lettuce in presence of F. noxius gases	0.270-0.990	18.31-20.90
	Lettuce without F. noxius gases	0.025-0.042	17.60-22.23
Paired petri dishes	Lettuce in presence of F. noxius gases	0.021-0.049	18.40-21.20
	Lettuce without F. noxius gases	0.029-0.046	18.00-20.82

ii) Records of changes in carbon dioxide and oxygen concentrations during incubation.

In paired bottle assemblies.

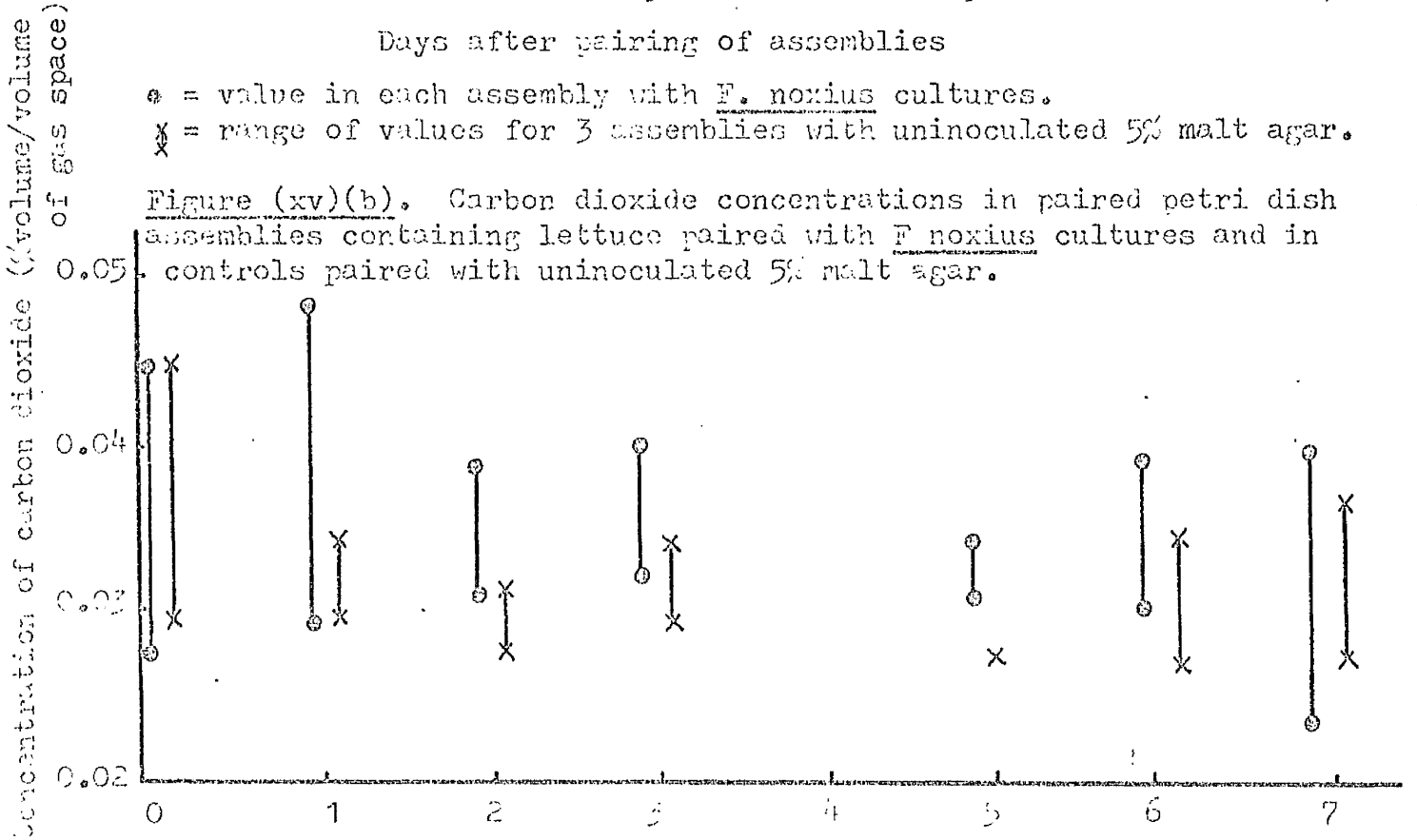
Text Figure (xv)(a) gives the concentrations of carbon dioxide measured in paired bottle assemblies of similar composition to those used in the biological tests described above.

Figure(xv)(a). Carbon dioxide concentrations in paired bottle assemblies containing lettuce paired with F. noxius cultures and in controls, paired with uninoculated 5% malt agar.



● = value in each assembly with F. noxius cultures.
⊗ = range of values for 3 assemblies with uninoculated 5% malt agar.

Figure (xv)(b). Carbon dioxide concentrations in paired petri dish assemblies containing lettuce paired with F noxius cultures and in controls paired with uninoculated 5% malt agar.



⊗ = range of values for 4 assemblies with F. noxius cultures examined daily and then discarded. ⊗ = range of values from control assemblies.

In this experiment, the concentrations in the assemblies with F. noxius rose steadily during incubation. The concentration drop in one bottle on one day was not examined further; it is likely that it may have been affected by a leak or other error in sampling. The values measured in control assemblies were all closely similar to one another and differed little from the atmosphere.

In paired petri dish assemblies.

Text Figure (xv)(b) gives the range of concentrations of carbon dioxide found in paired petri dish assemblies of similar composition to those used in the biological tests described above.

In this experiment, concentrations measured each day differed little from that of the atmosphere.

iii) Tests with authentic carbon dioxide at levels found in i) and ii). Results.

The effects of concentrations of carbon dioxide in the range 0.03 to 0.2% volume/volume on the germination and growth of lettuce are given in Section IB paragraph 2(a). In these tests, concentrations of this order were shown to have no effect on the germination of lettuce seeds or growth of germinated seedlings. The values found in paired petri dish assemblies with F. noxius were all within this range.

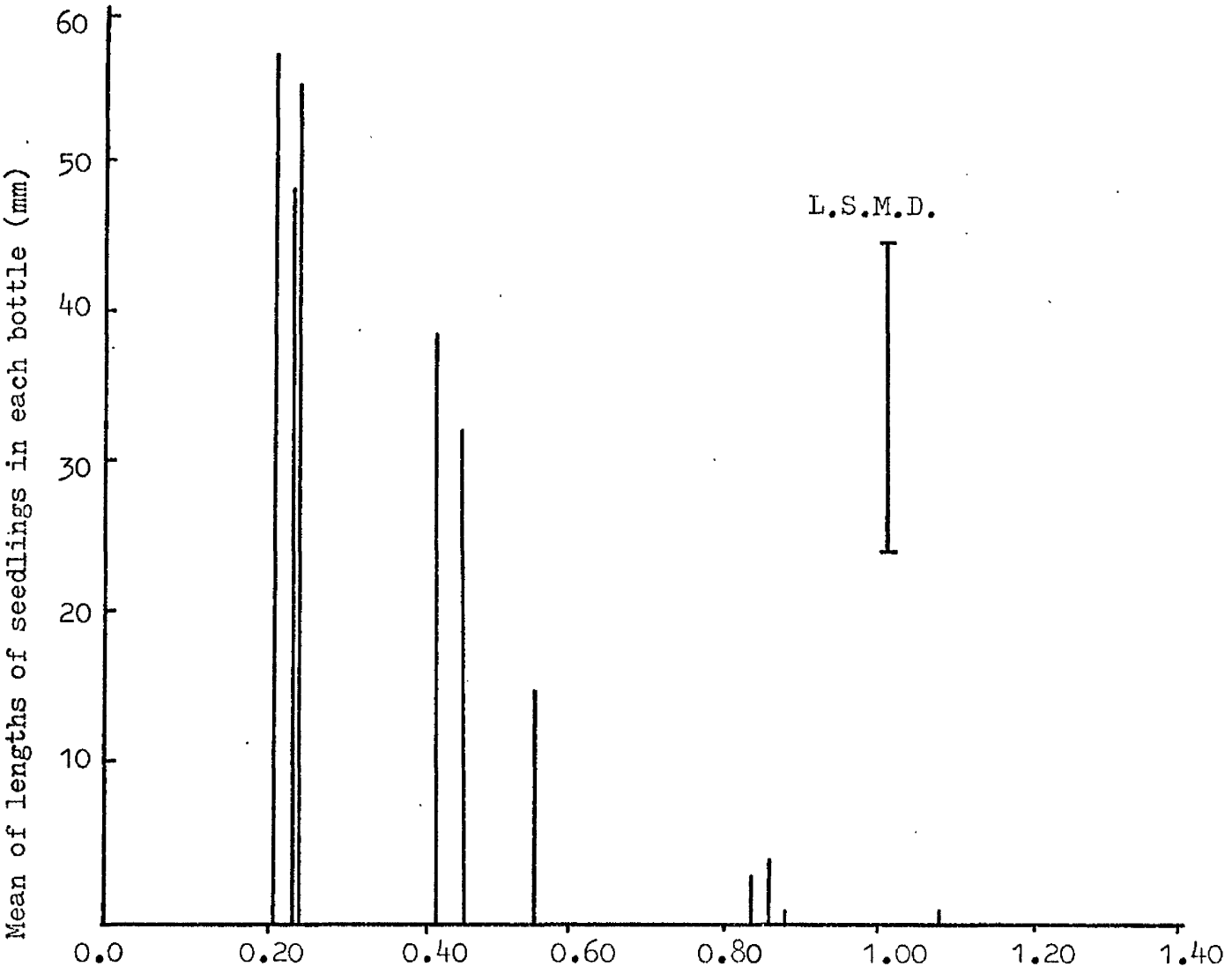
Text Figure (xvi) gives the germination of lettuce seeds and growth of germinated seedlings in similar tests with concentrations of carbon dioxide covering the range detected in paired bottle assemblies with F. noxius cultures.

In this experiment, the numbers of germinated seeds was significantly reduced in treatments with the highest range tested. The lengths of the germinated seedlings were significantly less than those in control treatments in all the ranges tested in this experiment.

The results of this and two similar experiments are given in appendix Table XXIII. They agree closely with those in Figure (xv). Only one treatment in all of the three experiments did not produce significant reduction in seedling growth; the range 0.405% to 0.591% volume/volume in experiment II. The lowest concentration range found to inhibit lettuce seed germination was 0.812% to 1.009% in experiment II. Concentrations of carbon dioxide measured at the beginning and end of these experiments did not differ by more than 57% of their average in any assembly (see Section IIA, paragraph 2(a)).

The symptom of inhibition observed in these treatments was of general stunting and compared closely with that described in tests with F. noxius cultures in paired bottle assemblies described above, in paragraph 1 of this section.

Figure (xvi). Germination of lettuce seeds and length of germinated seedlings in sealed bottles containing known ranges of mixtures of authentic CO_2 and air.



Mean of initial and final CO_2 concentration, as vol. of CO_2 per 100 vols. of gas space in each bottle.

100 100 100 | 100 100 100 | 90 80 0 | 3 0 | 0 | 0

Mean of records of number of germinated seeds as % of total of 10 seeds in each bottle in the range shown.

L.S.M.D. = Least significant difference between means of lengths of seedlings in the 3 assemblies in each treatment in the range shown.
Least significant difference between the mean numbers of germinated seeds for each treatment = 56%

For detailed results see appendix Table XXIII, expt. I.

The changes in oxygen concentration found in section (i) and (ii) (see text Table 13) were not more than 8% in paired petri dish assemblies and 9% in paired bottle assemblies with F. noxius cultures compared with 10% in paired petri dish assemblies and 12% in paired bottle assemblies with uninoculated 5% malt agar.

Discussion and conclusions.

Concentrations of carbon dioxide detected in paired bottle assemblies with F. noxius cultures were as much as x 6.5 the maximum detected in paired petri dish assemblies. The values in paired petri dish assemblies, however, differed little from atmospheric concentrations. The changes in oxygen concentration were considered trivial and the possibility of changes in oxygen concentration producing significant effects in tests with F. noxius was not investigated further.

The symptoms in paired bottle and paired petri dish assemblies with F. noxius cultures also differed. Those in paired petri dish assemblies, with carbon dioxide levels below levels shown to be inhibitory, showed characteristic symptoms of F. noxius inhibition, while those in paired bottle assemblies showed general stunting comparable to the stunting observed in assemblies with similar concentrations of authentic carbon dioxide. This suggests that the characteristic inhibition observed in paired petri dish assemblies cannot be attributed to the presence of inhibitory levels of carbon dioxide. In the paired bottle assemblies,

however, the general stunting is attributable to the presence of high carbon dioxide levels.

b) Analysis of other metabolites by G.L.C. and examination of effects of identified constituents.

Method.

Cultures were sampled and G.L.C. analyses were carried out on the samples by the methods described in the General Methods, paragraph 5.

The initial survey work was carried out using columns containing Carbowax 1500, Carbowax 20M, D N P, and Porapak R (see Section IA methods paragraph 2). The following columns were used for the reasons stated in text Table 14 in addition to those used for the examination of F. scutellatus in Section IB.

Table 14. Operating conditions used for chromatographic analysis of F. noxius culture gases.

Column				Carrier gas and flow rate (ml/min)	Hydrogen flow rate (ml/min)	Use of Columns
Dimensions	Material	Packing	Temperature(°C)			
6ft x 1/8in	Stainless steel	*15% Acetyl tributyl citrate (Citroflex A-4)	75°C and 75 - 120 and held for 30 min	40	30	Carbonyl compounds, hydrocarbons
18in x 1/4in	Stainless steel	*Davison 08 grade Silica gel 30/60 mesh	70 - 130 and held for 30 min	38	30	Gases
6ft x 1/4in	Copper	*25% glycerol on 60/80 mesh	60	36	30	Separates iso-amyl alcohols
5ft x 1/8in	Stainless steel	* 5% SE-30	70 and 70 - 120 and held for 30 min	30	30	High molecular weight compounds, general Use

*60/80 mesh Chromosorb W used as a support in these columns.

Peaks which were seen consistently were identified by comparison of their retention times with those of authentic pure compounds on Carbowax 20M and D N P columns. Identification of the alcohols was confirmed by syringe reactions (Hoff & Feit, 1964).

The concentrations of identified constituents were estimated by comparisons of peak heights of samples of culture gases and of gases above aqueous solutions of authentic compounds in similar assemblies, or of gases in sealed bottles containing air/gas mixtures of authentic compounds.

The biological effects of identified constituents were judged by comparing the effects of total culture gases with those of similar concentrations of authentic compounds measured under standard G.L.C. conditions. All quantitative work would from choice have been carried out in a controlled environment, using paired bottle assemblies. However, in these assemblies, carbon dioxide concentrations build up to inhibitory concentrations when F. noxius cultures are present as described above. Direct comparison of tests with authentic samples and tests with F. noxius cultures in these assemblies was thus not possible. Hence a compromise was used, in which tests with authentic material were carried out with paired bottle assemblies. In these, the lettuce plants could be exposed to a chosen level of authentic material for a determined time. The results were then used as a

standard for the interpretation of possible effects of the lower concentrations of the substances identified in paired petri dish assemblies containing F. noxius cultures.

Additional tests were carried out with solutions of authentic material in paired petri dish assemblies. In these assemblies the effects of authentic material can be compared directly with those of F. noxius gases under comparable conditions. Quantitative comparisons were not possible, however, in some tests, as the concentrations were much affected by the gas exchange with the atmosphere, unavoidable in these assemblies.

Results.

i) Analysis of culture gases by G.L.C..

Four major peaks were found in gas chromatograms of samples taken during the survey work recorded in Section IA results paragraph 2. They were identified as being the result of the presence of acetaldehyde, ethanol, isobutanol and a mixture of pentanols. Comparison of the retention times of 2-methyl-butan-1-ol and 3-methyl-butan-1-ol showed that the pentanol peak was due to the presence of both of these in approximately equal proportions. This mixture is referred to as 'pentanol' throughout this Section.

The peak heights of these constituents given by samples of F. noxius culture gases changed greatly during the 30 month period of

experimentation. In the earlier samples, taken during the preliminary survey work, standardized peak heights were recorded of over 60 mm (acetaldehyde), 150 mm (ethanol) 30 mm (isobutanol), and 30 mm (pentanol). These values may be compared with those of authentic material given in text Table 15 below. In the great majority of later experiments these peaks were of the order of x 10 lower. Text Table 15 gives typical terminal peak heights of samples from paired petri dish assemblies containing lettuce paired with F. noxius cultures, the peak heights given by samples taken from above standard aqueous solutions of the identified components, and the peak heights given by samples from standard air/gas mixtures of each constituent.

Table 15. Peak heights of constituents in F. noxius culture gases and peak heights of authentic samples of identified constituents under uniform conditions.

Source of sample	Authentic compound	Concentration added (µl)	Peak height (mm)
Headspace above 20 ml aqueous solution in 1 l bottle	Acetaldehyde	10	17536
		1	1888
		0.1	752
	Ethanol	10	116
		1	78
		0.1	26
	Isobutanol	10	226
		1	66
		0.1	45
	Pentanol	10	976
		1	312
		0.1	53
Air-gas mixture in 1 l bottle	Acetaldehyde	1	2333
	Ethanol	1	2853
		0.1	119
	Isobutanol	1	2622
		0.1	199
	Pentanol	1	3077
		0.1	59
F. noxius culture gases	Acetaldehyde		12
	Ethanol		10
	Isobutanol		7
	Pentanol		2

Biological effects were, however, seen in assemblies with these low levels of the four identified components, and in some cases where only traces of them were found. An experiment was set up to investigate the daily changes in concentration of these constituents in one of these cases. It also revealed only traces of each constituent throughout the assay period (text Figure (xvii) and appendix Table XXIV.

b) Examination of effects of authentic samples of identified constituents.

i) Tests with samples of individual authentic constituents.

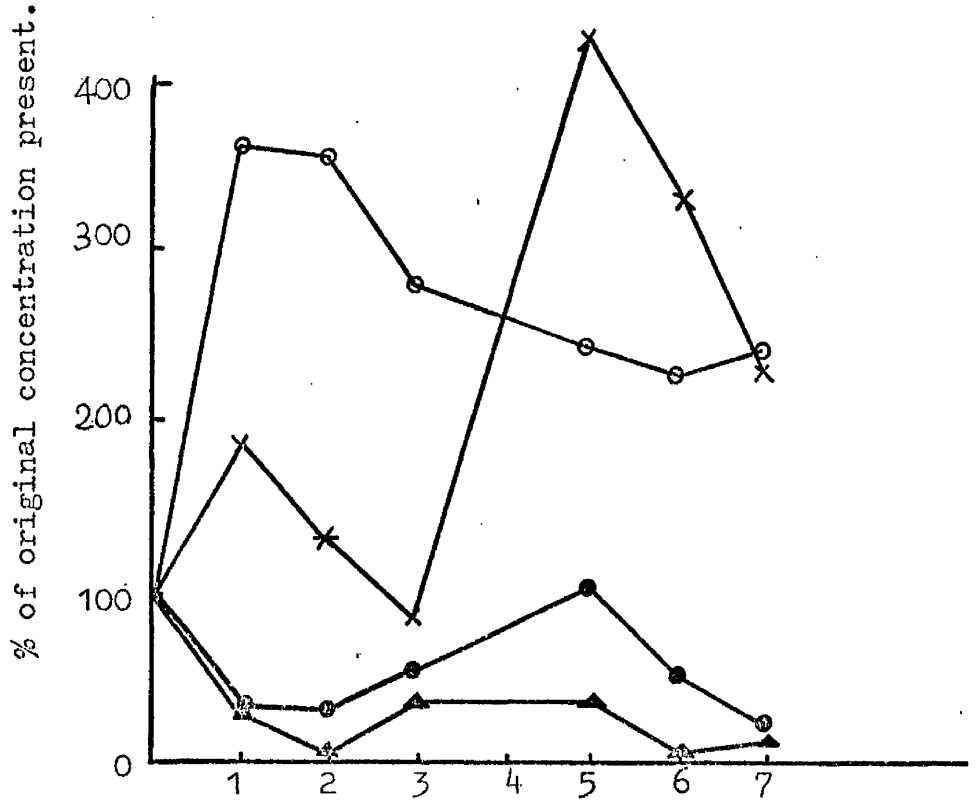
Particular methods.

Only terminal concentrations of authentic metabolites were measured in assemblies testing the biological effects of these gases. Initial concentrations were estimated from samples from a suitable number of similar bottles examined 20 min after pairing and then discarded.

Results.

Appendix Tables XXV, XXVI and XXVII give the germination of lettuce seeds and growth of germinated seedlings in assemblies, containing lettuce paired with 20 ml standard 0.05% aqueous solutions of each authentic constituent of F. noxius gases and in controls, with sterile deionized water. Terminal concentrations of the authentic constituents are also given in these Tables. In the three experiments carried out,

Figure(xvii). Changes in concentrations of acetaldehyde, ethanol, isobutanol, and pentanol in paired petri dish assemblies containing lettuce paired with F. noxius cultures.



Values plotted are the means of measurements made from 3 assemblies examined under standard G.L.C. conditions.

Initial peak height	acetaldehyde	=	5 mm
" " "	ethanol	=	8 mm
" " "	isobutanol	=	2 mm
" " "	pentanol	=	2 mm

the differences between the number of germinated seeds and between the lengths of roots of germinated seedlings in assemblies with test solutions compared with controls, were not significant ($P = 0.05$).

There were large and irregularly distributed differences between the measurements of the terminal concentrations; these ranged from 8% to 518% of the average for acetaldehyde, 7% to 398% for ethanol, 16% to 291% for isobutanol and 19% to 269% for pentanol.

Appendix Table XXVIII gives the range of peak heights of authentic volatiles in the initial tests.

Discussion.

The results these Tables show that there is a large experimental error in this technique. This may be in amounts of authentic material introduced, or errors in determinations, or to a combination of the two. The differences between records of initial concentration are less than between records of final concentration. This shows that further experimental errors are introduced at this stage. In all cases, however, the concentrations examined were considerably greater than those seen in F. noxius experiments. It did not therefore seem justifiable to seek to increase the accuracy of the technique at this stage.

Conclusion.

The characteristic inhibition produced by F. noxius gases cannot be attributed to the presence of any one identified constituent at concentrations detected in the culture gases.

ii) Tests with mixtures of authentic samples of identified constituents. Particular methods.

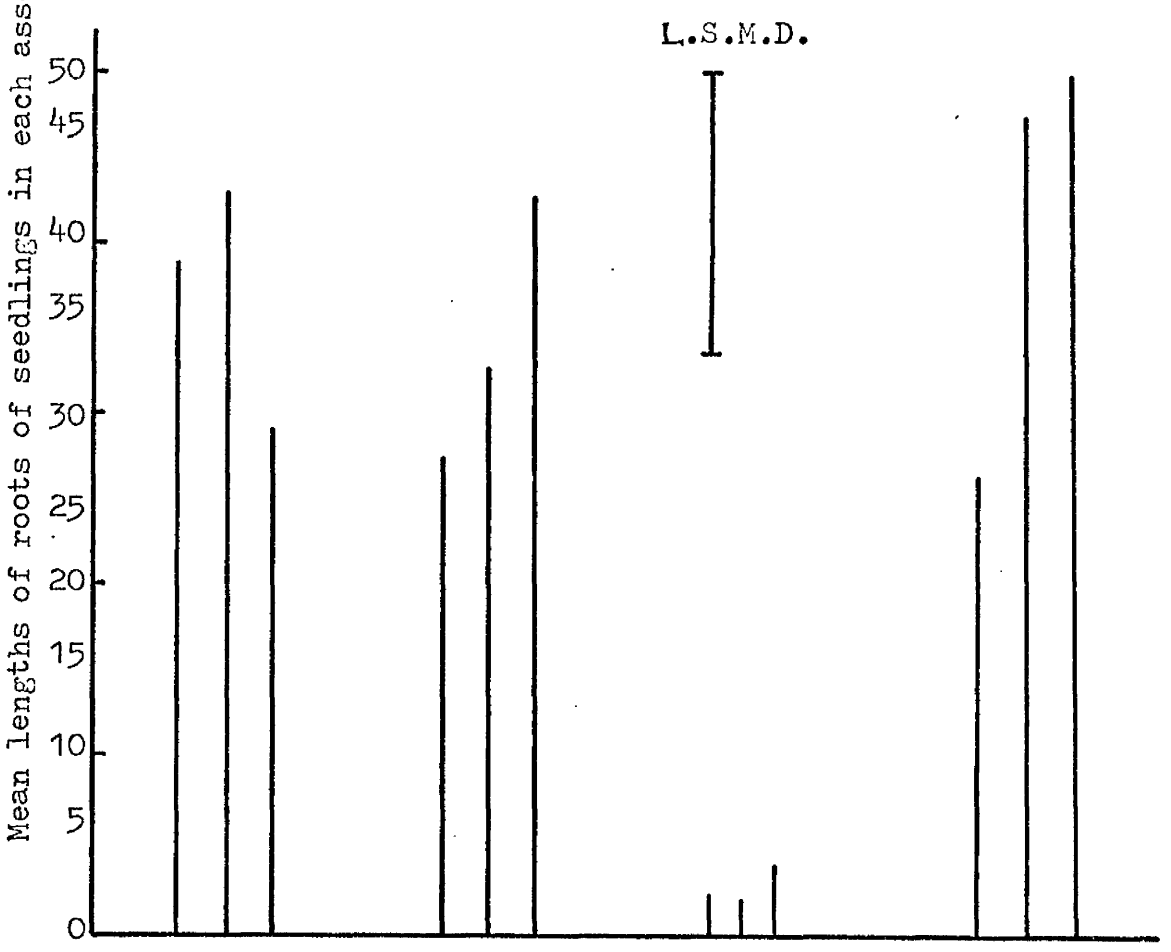
The opportunity for greater variability in producing standard initial concentrations of mixtures as opposed to individual authentic constituents was appreciated. Hence both initial and final concentrations of authentic constituents were measured in the biological test assemblies. Tests assessing the loss of volatiles from assemblies during incubation were carried out separately from the biological tests with a suitable number of replicates.

Results.

Text Figure (xviii) gives the germination of lettuce seeds and root growth of germinated seedlings in a typical experiment with lettuce paired with each of three authentic mixtures of the identified constituent and in controls with sterile deionized water. The composition of these mixtures is detailed on this Figure.

In this experiment, the germination and growth of lettuce were not significantly reduced in treatments with mixtures I and II. Germination was not affected by mixture III, but the growth of the seedlings was severely inhibited. Inhibited seedlings showed severe general stunting and not the symptoms characteristic of inhibition by F. noxius cultures. Initial and final concentrations of authentic constituents were again

Figure (xviii). Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with standard solutions of acetaldehyde, ethanol, isobutanol and pentanol and in controls paired with 20 ml sterile deionized water.



Treatment	I	II	III	H_2O
Mean % Germination	93	93	80	100

Each treatment consisted of 3 assemblies each containing 10 lettuce seeds paired with 20 ml of one of the standard aqueous mixtures I, II and III given below.

Mixture No.	Volume in 20 ml sterile deionized water (µl)			
	Acetaldehyde	Ethanol	Isobutanol	Pentanol
I	1.0	1.0	0.1	0.1
II	10.	10	1.0	1.0
III	100	100	10	10

L.S.M.D. = Least significant difference between means of lengths of roots for each treatment. (P=0.05)
Differences between means of numbers of germinated seeds in each treatment were not significant. (P=0.05)

For detailed results see appendix Table XXIX, expt. I.

variable, but terminal concentrations were at least as high as, and in mixtures II and III were greatly in excess of, concentrations found in the culture gases. The detailed results of this experiment and the results of two replicate experiments are given in appendix Tables XXIX, XXX and XXXI. The results of the replicate experiments were closely comparable to those described above.

Text Figure (xix) gives the results of an experiment set up to examine the variability in concentrations of authentic constituents in these experiments.

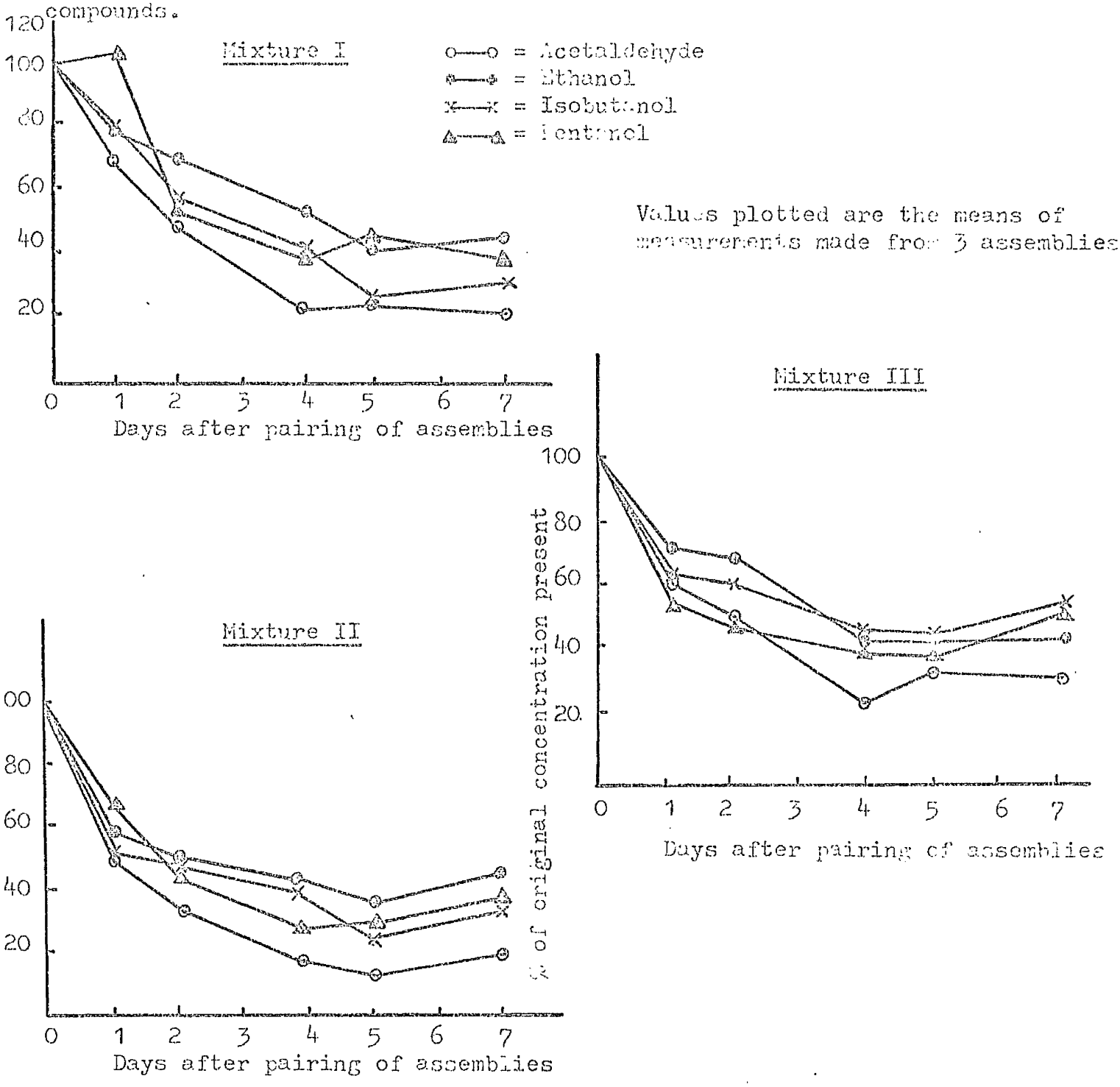
Detailed results of this test are given in appendix Table XXXII.

In this experiment levels of acetaldehyde fell rapidly throughout incubation in mixtures II and III, whilst concentrations of the other constituents fell more gradually. They fell in all mixtures to levels about 1/3 of the original. This fall was slightly greater than that observed in the biological tests. The effect of daily sampling in this test was thought to be a factor contributing to this, but this possibility was not further investigated.

Discussion.

Experimental errors in this phase of the investigation appear to be of a similar order to those in the tests of individual compounds. Since the levels of all remained above those found in assemblies with F. noxius gases, it did not seem profitable to seek to increase the accuracy of the

Figure (xix). Changes in concentrations of identified volatile metabolites in paired bottle assemblies containing lettuce paired with standard aqueous mixtures of these compounds.



Vertical axes = % of original concentration present.

For detailed results see appendix Table XXXII.

technique.

Conclusion.

The characteristic inhibition produced by F. noxius gases cannot be attributed to the presence of mixtures of the constituents identified at concentrations detected in the culture gases.

ii) Tests with authentic samples of identified constituents using paired petri dish assemblies.

Particular methods.

The effects of a wider range of concentrations of individual authentic constituents were examined to compensate for the difficulty in obtaining standard concentrations in these less controlled environments.

In all tests a sufficiently large number of replicates was set up to allow sampled petri dishes to be discarded after sampling.

Comparable assemblies containing lettuce paired with F. noxius and controls paired with uninoculated 5% malt agar were set up at the same time as the biological tests with authentic components.

Results.

The results of tests with individual authentic components are given in appendix Tables XXXIII, XXXIV, XXV and XXXVI and of tests examining the consistency of obtaining standard initial concentrations

in appendix Tables XXXVII.

The results of tests with mixtures of authentic components are given in appendix Tables XXXVIII, XXXIX and XL. Appendix Table XLI gives the results of the experiment examining the change in concentrations of authentic volatiles during incubation.

Discussion.

The results of all these tests were closely comparable to those obtained using paired bottle assemblies. As was expected, there was greater variation in the levels of authentic components in tests using petri dishes.

Conclusion.

These tests support the conclusions drawn from tests with paired bottle assemblies.

3. Examination of F. noxius culture gases by other techniques.

Introduction

Since none of the constituents so far identified could be shown to produce the inhibition observed with F. noxius gases, further examination for additional volatile constituents was made using tests chosen on an ad hoc basis.

a) Examination for hydrogen cyanide production.

Introduction.

Reports of hydrogen cyanide production in many fungi including F. scutellatus and the failure to detect it by G.L.C. analysis (see Section IB) suggested that F. noxius gases should be examined for the production of this compound.

Methods.

The methods used were those described in Section IB paragraph 2 (b).

Results.

No hydrogen cyanide could be detected in the culture gases of this species.

b) Smell.

Introduction.

Indications of the types of compounds present can often be gained from the smell produced by fungal cultures. In this case, cultures of the age used in biological tests (4 to 6 weeks old), emitted a characteristically sweet, sickly odour. Younger cultures (aged 0 to 4 weeks) had a weakly alcoholic smell. This odour resembled that produced by an amine and/or sulphur containing compound, but it could not be identified more precisely. The relationship between the presence of this smell and biological activity was investigated in the following experiments.

Germination and growth of lettuce in the presence of gases produced by young cultures of F. noxius.

Methods.

Biological tests with lettuce were set up in paired petri dish assemblies with young cultures of F. noxius, which were not emitting the characteristic smell. Routine G.L.C. examination of gases in assemblies was made.

Results.

Table XLII of the appendix gives the germination of lettuce seeds and growth of lettuce seedlings in three replicate experiments each comprising 6 assemblies containing lettuce paired with F. noxius cultures and 6 control assemblies with lettuce and uninoculated 5% malt agar. No significant inhibition of root length was observed in any of these tests. Carbon dioxide, oxygen, acetaldehyde, ethanol, isobutanol and pentanol were found in concentrations comparable to these detected in paired petri dish assemblies with mature cultures of F. noxius.

Conclusion.

These results support the hypothesis that an additional compound which has a characteristically sweet and sickly odour and not one of the components identified by G.L.C. contributes to the inhibitory effects produced by F. noxius gases on lettuce.

It was considered unprofitable to carry this investigation further until more chemical information became available.

c) Examination for the production of volatile amines.

Introduction.

Tests of the pH of the culture gases with B.D.H. indicator papers showed that those of cultures of the age used in biological tests, (4-6 weeks old) frequently had a pH of 8.8-9.1. This was not always the case, however, pH values of 4.8-5.1 were also found. This together with the unusual smell suggested the possibility of amines being present. To test this, the first step taken was to carry out biological tests with F. noxius with oxalic acid present in test assemblies to absorb basic components of the culture gases. This was followed by a G.L.C. and preliminary chemical examination for amines. The methods and results and outcome of these experiments are reported below.

(i) Biological tests with oxalic acid present in assemblies.

Methods.

Paired petri dish assemblies containing lettuce paired with F. noxius cultures or 5% malt agar were used in these tests. Standard amounts (2 ml), of 1N oxalic acid were added to two glass wells, each with a 1 ml capacity, placed in the assemblies with F. noxius cultures and with uninoculated agar. To fix the wells into the assemblies, plugs

of mycelium on agar or agar alone, in the case of controls, were removed from the plates to be used in the tests and the wells inserted into the holes left by the removal of these plugs. A similar number of control treatments with wells containing sterile deionized water were also assembled and examined. A routine G. L. C. examination was also made.

Results

In 3 replicate experiments, no consistent reduction of inhibition was recorded in assemblies with F. noxius gases and oxalic acid (see appendix Tables XLIII, XLIV and XLV) in one experiment (appendix Table XLIII) reduction in inhibition was significant while in another experiment (appendix Table XLIV) root length was significantly less in controls with oxalic acid.

G.L.C. traces from assemblies with different treatments did not differ, traces of the already identified metabolites were recorded from all treatments.

Conclusion.

These results are inconclusive, but are sufficient to indicate that a further examination could be worth while.

ii) Examination by G.L.C. and classical chemical techniques.

Methods.

A wide range of column types have been used. The ones most recommended are alkali-coated Carbowax 20M (e.g. Cieplinski, 1966) and polyimine columns (e.g. Sze, Borke and Ottenstein, 1963). Since neither of these was available at this time, I used a stainless steel column (6ft x 1/4in) packed with 20% amino 220, on 60/90 mesh Chromosorb W recomended by Glen, (personal communication) for the detection and separation of volatile amines.

The other G.L.C. conditions under which examination was carried out were as follows:- column temperature = 65°C, carried gas, helium, at a flow rate of 38 ml/min, and hydrogen flow rate of 40 ml/min.

In preliminary tests it was found that samples of authentic ammonia, methylamine, ethylamine and butylamine could be identified in these conditions although retention times were short and separation was poor.

The following samples from F. noxius gases were examined:-

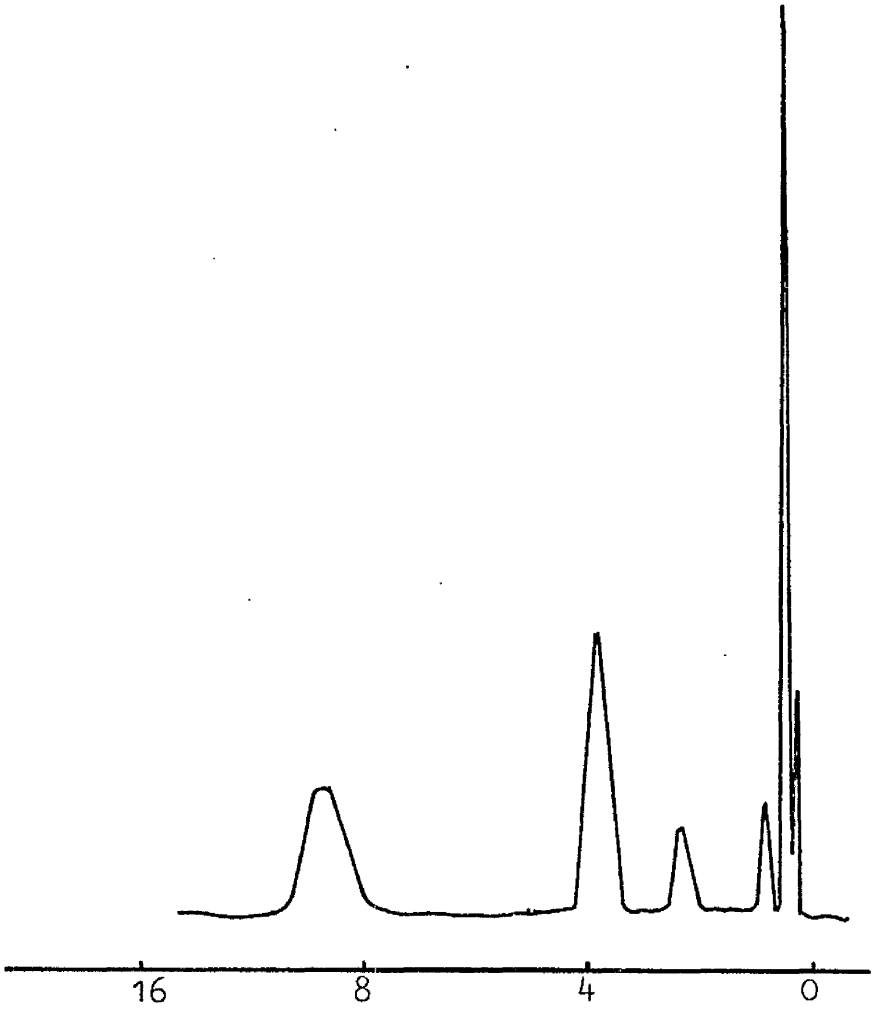
i) Samples from the headspace above Roux bottle cultures of F. noxius;

ii) Samples from paired petri dish assemblies containing lettuce and F. noxius cultures;

iii) Samples of condensed culture gases collected in solid carbon dioxide;

Figure (xx). Typical G.L.C. trace from a 6 week old culture of *F. noxius* 224A.

Column: Amino 220



iv) Similar samples bubbled through deionized water;

v) Samples of the air above dried residues of dilute HCl through which F. noxius gases had been bubbled for 8 hours, examined before and after addition of NaOH. Control samples of HCl alone were examined in the same way.

In addition, the pH of sample (iii) and of the solution of sample (iv) were measured. The melting points of the dried residues of sample (v) were also examined.

Compounds were identified on the G.L.C. by comparison of retention times with those of samples of authentic compounds as described in the General Methods, paragraph 5.

Identification was also made using syringe reactions (Hoff & Feit 1964).

Results.

Text figure (xx) gives a typical chromatogram obtained using this column. The three major peaks shown have been identified as resulting from the presence of ethanol, isobutanol and pentanol. Acetaldehyde was not detectable on this column. A variable number of smaller peaks with short retention times were seen. These are discussed below.

The pH of the gases collected in solid carbon dioxide was in the range 3.5-4.6 and in the solution of F. noxius gases in deionized water was 3.0.

Crystals were obtained from F. noxius and control samples collected in dilute HCl. These were initially colourless, but changed to yellow on standing. They did not melt at temperatures up to 362°C i.e. at temperatures higher than the melting points of amine salts. Their nature was not investigated further at this stage.

Discussion.

The results suggest that an investigation of the small unidentified peaks might be profitable. However, since the pH of the samples examined was low and no amine salts were collected, this examination was discontinued.

d) Examination for the production of volatile sulphur compounds.

Introduction.

In a preliminary test, a black precipitate formed when F. noxius culture gases were drawn through a syringe containing 0.1N Ag NO_3.

This investigation was continued with the following tests:-

(i) Biological tests with silver nitrate present in assemblies.

Methods.

Methods were those described for oxalic acid in the previous section, except that additional tests where only 1 ml Ag NO_3 or 1 ml water was included in assemblies were carried out.

Results.

Text Figure (xxi) and appendix Table XLVI give the results of an experiment with two wells, each containing 1 ml 0.1N silver nitrate, included in half the assemblies containing lettuce paired with F. noxius cultures and in half the control assemblies with lettuce and uninoculated 5% malt agar. In the remaining half, similar wells containing sterile deionized water were inserted.

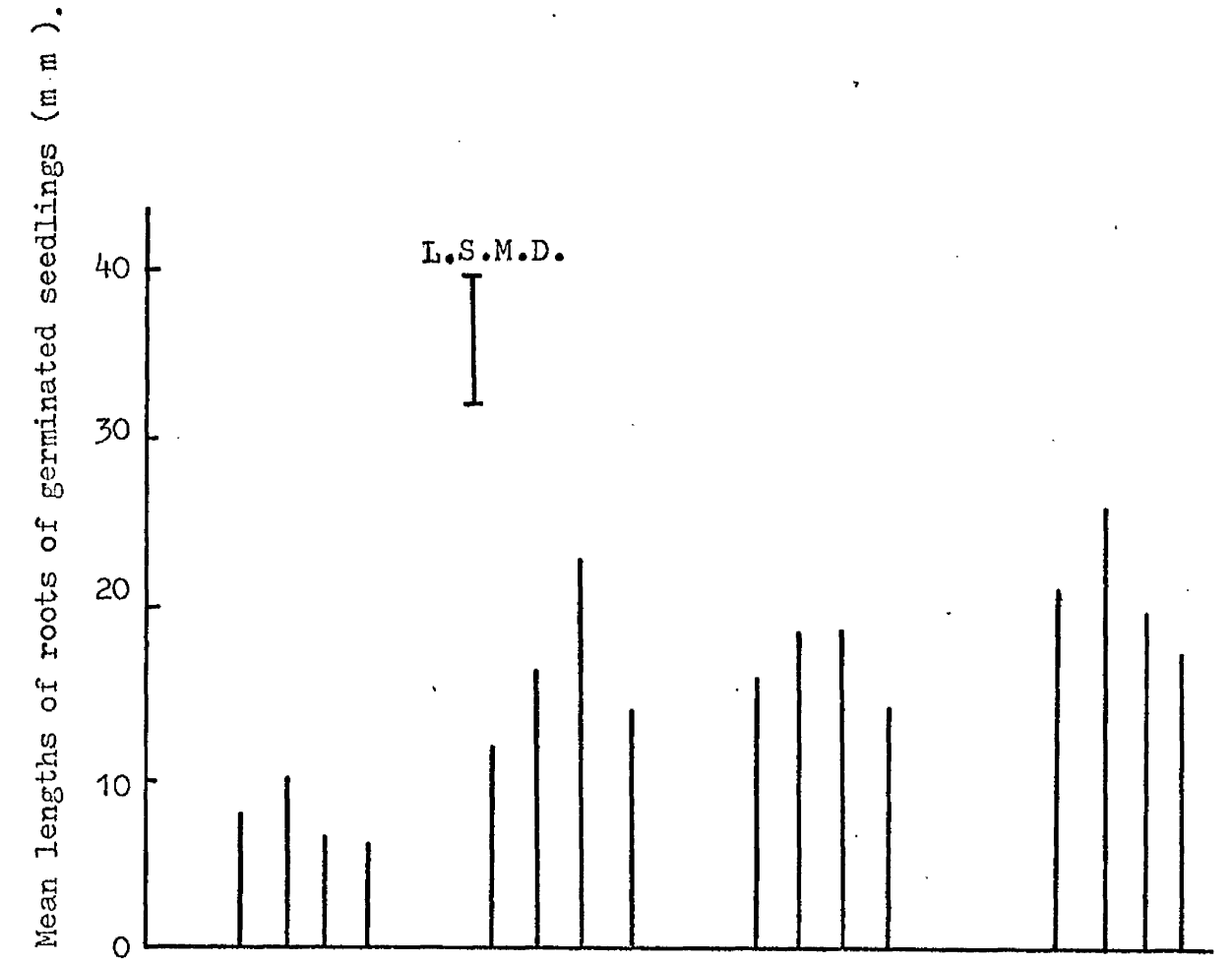
In this experiment and in one replicate experiment (see appendix Table XLVII) the gases from F. noxius did not inhibit the root growth of the seedlings in the presence of silver nitrate. In another replicate experiment (appendix Table XLVIII) root growth was greater in assemblies containing F. noxius cultures plus silver nitrate than in assemblies with F. noxius alone, but was still less than in controls in both these treatments.

In similar experiments with 1 ml 0.1N silver nitrate, the same trends were observed in two of three replicate experiments (see appendix Tables XLIX and L). In the third experiment (appendix Table LI) root growth was inhibited to a similar extent in all assemblies containing F. noxius cultures.

Discussion.

The results of these tests suggest that more work on this line might be profitable. The continuation has been postponed, however, in favour of following more promising lines.

Figure (xxi). Germination of lettuce seeds and growth of germinated seedlings in the presence of gases from F. noxius cultures and in controls with uninoculated 5% malt agar in paired petri dish assemblies with and without Ag NO_3.



Values are the means of measurements from each of 4 assemblies which comprised each treatment.

Treatment (1) Lettuce with F. noxius cultures.
" (2) " " uninoculated 5% malt agar.
" (3) " " F. noxius cultures + 2 ml 0.1M Ag NO_3.
" (4) " " uninoculated 5% malt agar + 2 ml 0.1M Ag NO_3.

For detailed results see appendix Table XLVII .

L.S.M.D. = Least significant difference between the means of lengths of roots for each treatment (m.m).

L.S.M.D. = not significant for numbers of germinated seeds.

ii) Examination by G.L.C. and classical chemical techniques.

Introduction.

There are reports of G.L.C. analyses of sulphur containing compounds using a wide range of column types. These include two of the columns already used to examine F. noxius gases in this investigation i.e. Carbowax 1500 (e.g. Salvadeo, Catenacci & Maugeri, 1966; Ronald & Thomson, 1966), Carbowax 20M (Oaks, Hartmann & Dimick, 1965; King & Coley-Smith, 1969 (b) and (c)). Further examinations of F. noxius gases using these columns was made using modifications of the syringe reaction techniques described by Hoff & Feit (1964) and some other classical chemical tests.

Examination by G.L.C.

Methods.

Syringe reactions were carried out as follows:-

α) Samples of F. noxius culture gases were drawn into a 5 ml syringe containing various amounts (1µl to 2.5 ml) of 0.1N Ag NO_3.

β) Samples of F. noxius culture gases and of authentic compounds were mixed with standard gas samples of acetyl chloride in a 5 ml syringe.

In each case, comparison was made with control samples of gases from above uninoculated 5% malt agar.

Peaks were identified by comparison of retention times with those of authentic samples as described in the General Methods paragraph 5.

Results.

α) Traces were not altered by treatment of the gases with Ag NO_3.

β) With authentic samples of ethanol, isobutanol, pentanol, hydrogen sulphide, and ammonium sulphide reaction with acetyl chloride could be followed. However, patterns of peaks given by acetyl chloride and acetyl chloride plus authentic compounds did not produce a consistent pattern in all tests. Similarly, in some tests with F. noxius culture gases, the pattern of peaks was not consistant. The presence of ethanol, isobutanol and pentanol could be confirmed, however, although neither hydrogen sulphide nor ammonium sulphide could be detected.

Discussion.

The extreme reactivity of acetyl chloride is thought to be the main factor contributing to the variation in G.L.C. pattern produced in these tests. Thus further investigations of these reactions was not considered worthwhile at this stage. However, the results indicate that similar tests with less reactive compounds could be useful in this investigation.

Further chemical tests.

Methods.

α) With Ag NO_3 F. noxius gases were bubbled through 0.1N Ag NO_3 for eight hours on several different occassions. Controls were also

run, with gases from above uninoculated 5% malt agar.

β) Routine spot tests with sodium nitroprusside and lead acetate were made on F. noxius gases.

Results.

α) A black granular precipitate formed when F. noxius gases were passed through the solution awaiting analysis.

β) No positive reaction could be obtained in either of these tests.

Discussion.

It is appreciated that the failure to obtain positive reactions in tests with lead acetate and sodium nitroprusside may be due to the presence of very small quantitites of the unknown compound. Further comment on these reactions and on the nature of the precipitate produced in reaction with $AgNO_3$ is being withheld until further analysis of this precipitate has been made.

General discussion.

So far, only common respiratory metabolites have been identified in the culture gases of this species. However, the characteristic inhibitory effect of the total culture gases cannot be attributed to the action of any one of these metabolites, nor to a mixture of these compounds.

These results contrast with those of Glen & Hutchinson (1969) who showed that the inhibitory active of yeast gases on A. niger and cress could be accounted for by the presence of similar repiratory products. The concentrations of the comparable metabolites found in yeast gases were, however, very much greater (approximately x 100) than concentrations found in similar assay assemblies with F. noxius cultures.

The possibility that the biological activity of F. noxius is due to the presence of a volatile amine or a volatile sulphur compound is strengthend by the number of references to the production of either of these types of compounds by fungi and other living things. The production of nitrogen-containing metabolites by fungi was revieved by Birkenshaw & Stickings (1962), and this review contains accounts of many potentially volatile basic compounds. No reports of volatile amine production by species of Fomes are reported, but there are reports from many other Basidiomycete genera. Several sulphur-containing nitrogenous compounds are also included in this survey.

That these compounds may interfere with enzyme action in living things and in some cases may be toxic to higher plants (Lutz, 1898; Kobyl-skii, Polevoi, Yuzonasov & Vozilova, 1966), does not preclude the possibility of their being active constituent(s) in the culture gases of F. noxius.

Reports of the production of sulphur-containing metabolites are also abundant, especially hydrogen sulphide by fungi (Cugini, 1876; Selmi, 1876; March, 1929; Katta & Lynd, 1965), and by bacteria (reviewed by Clark, 1953), and by higher plants (reviewed by Virtaken, 1965). The biological action of sulphur compounds is also well known. Walker, Morell & Foster (1937) showed that a wide range of low molecular weight sulphur compounds were toxic to several species of fungus, including A. niger. Ethionine and methionine were reported to affect the virulence and sporulation of Fusarium levi (Jones & Woltz, 1969). Again in relation to the pathogenicity of species is the large volume of work on the effects of volatile sulphur-containing exudates of onion (Allium) on the stimulation of germination of the onion parasite, Sclerotium cepivorum, overcoming soil fungistasis (Coley-Smith, 1960; Coley-Smith & Holt, 1966; Coley-Smith, King, Dickinson & Holt, 1967; Coley-Smith, Dickinson, King & Holt, 1968; King & Coley-Smith, 1968; Allen & Young, 1968; King & Coley-Smith, 1969 (a), (b) and (c); Keyworth & Milne, 1969). The antibacterial action of the exudates of onion were known before this time (Cavillito, Buck & Suter, 1944; Stoll & Seebeck, 1951; Virtaken & Matikkala, 1959).

Many sulphur compounds are, of course, used as fungicides. The toxicity of such compounds is thought to be as a result of the action of elemental sulphur. It is outwith the bounds of reasonable relevance to

review this topic here.

It thus seems possible that sulphur compounds could be contributing to the biological activity of the gases of F. noxius. The experiments in this investigation where silver nitrate was included in the assay assemblies provided evidence for this possibility, although it is felt that the next step in this investigation should be to examine the chemical nature of the compounds produced by F. noxius more rigorously. It should be possible to obtain information from the precipitated product of the reaction with silver nitrate or by examination of the culture filtrates and homogenized mycelium of this species should characterization of the precipitate be uninformative.

The biological activity of this species is of particular interest since F. noxius is a well known parasite of tropical crops, causing stem rot of oil palm and root rot of tea (Corner, 1932), root rot of hoop pine (Araucaria cunninghamii) (Rep. Dip. Agric. Papua and New Guinea, 1963), root rot of Pinus elliattii and Cedrella sp. (Zondag & Gilmour, 1963), root rot of cacao (Voelckera, 1953; Shaw, 1963) and of citrus seedlings (Hopkins, 1957) and , of greatest economic importance, root rot of rubber (Hevea brassiliensis) (Pichel, 1956) especially in nurseries and in young trees (Ramakrishnan & Radhakrishna Pillay, 1962 and 1964).

The symptoms of this root rot, described by Thrower (1965) in cacao and by Ramakrishnan & Radhakrishna Pilley (1962 and 1964) in rubber are

only partly similar to observations made in this investigation. The formation of a crust of mycelium on the stem or trunk of the infected plant is one of the most characteristic symptoms in the field. It obviously cannot occur under the conditions of these tests. Other symptoms, however, correspond in as far as affected plants in nature and in this investigation both showed browning of the roots, especially of the tap root, in seedlings and in many seedlings, the excessive production of lateral and adventitous roots. (Ramakrishnan & Radhakrishna Pillay, 1964). It is thought that effect of gaseous metabolites in the production may be of relevance to disease symptoms in natural host plants, but no further investigations were carried out at this stage apart from a brief study of the effects of culture gases acting through soil, described in Section IV of this thesis.

SECTION IIA

A SURVEY OF THE GENUS CLITOCYBE FOR THE OCCURRENCE OF VOLATILE METABOLITES.

Table 16. List of species of Clitocybe known to produce hydrogen cyanide.

Species	Reference
C. alexandri	Heim (1928)
C. candida	Locquin (1947)
C. clavicops	Bousset (1941)
C. cyanthiformis	Renard (1912)
C. fragrans	Mirande (1932)
C. geotropa	Maire (1926)
C. gibba	Singer (1962)
C. gigantea	Josserand (1932)
C. infundibuliformis	Groshoff (1909)
C. nebularis	Heinemann (1942)
C. parilis	Josserand (1938)
C. suaveolens	Heinemann (1942)

Table 17. List of species of Clitocybe reported to produce antibiotics.

Species	Compound (where identified)	Reference
C. aurantiaca		Wilkins & Harris (1944)
C. brumalis		" " " "
C. candicans		" " " "
C. candida	clitocybin	Hollande (1947)
C. claviceps		Wilkins & Harris (1944)
C. compressipes		Ford (1911)
C. connata		Wilkins & Harris (1944)
C. cyanthiformis		Elliott (1926; Wilkins & Harris (1944)
C. dealbata	muscarine	Jeliffe (1937); Clarke & Smith (1913)
C. diatetra	diatretyne	Anchel (1952), (1953), (1955).
C. ditopus		Wilkins & Harris (1944)
C. flaccida		" " " "
C. galinacea		" " " "
C. gangraenosa		" " " "
C. infundibuliformis		" " " "
C. illudens	muscarine	Farlow (1899); Clarke & Smith (1913); Ford (1911)
C. inornata		Wilkins & Harris (1944)
C. investiens		Robbins, Hervey, Davidson & Robbins (1945)
C. inversa		Wilkins & Harris (1944)
C. metachroa		" " " "
C. morbifera		Jeliffe (1937)
C. multiceps		Clarke & Smith (1913); Ford (1911)
C. obsoleta		Wilkins & Harris (1944)
C. olearia	muscarine	Maretic (1967)
C. pruinosa		Wilkins & Harris (1944)
C. rivulosa	muscarine	Ramsbottom (1953)
C. sudorifica		Ford & Sherrick (1911)
C. tabescens		Robbins, Hervey, Davidson & Robbins (1945)
C. vibescina		Wilkins & Harris (1944)

SECTION IIA. A SURVEY OF THE GENUS CLITOCYBE FOR THE OCCURRENCE OF VOLATILE METABOLITES.

Introduction.

The genus Clitocybe was chosen for the extension of survey work for several reasons. Firstly, reports of hydrogen cyanide production by several species in this genus (listed in text Table 16) suggested that investigation of this genus might make an interesting follow-up to the observations reported in the Section (IB) on F. scutellatus in this thesis. Secondly, there are reports of antibiotic production in this genus (listed in text Table 17) and, thirdly, descriptions of many of these species for use in their identification mention the characteristic smells produced by sporophores e.g. Lange (1935-40).

Methods.

The survey was carried out by biological assay and by chemical assay and, in addition, all species were tested for HCN production.

Appendix Table LII lists the 12 species which were examined.

1. Biological assay.

Methods were as for the Fomes survey except that all cultures were inoculated with one single central inoculum. This was done because the reports of antibiotic production by this group suggested that effects similar to those described in F. scutellatus were likely to occur. Also, a species of Rhizobium was included in the range of assay species in all tests.

2. Gas chromatographic survey.

As for Fomes survey.

3. Examination for the presence of gaseous hydrogen cyanide.

This was carried out with all species by the cupric ethylacetoacetate plus tetrabase test described in Section IB of this thesis.

Results.

1. Biological survey.

a) The results are recorded in appendix Table LIII; they are summarized and analysed in appendix Table LVI, and illustrated in text Figures(xxii) (a), (b), (c) and (d).

In all cases where inhibition occurred, the effects were of stunting of growth; no other characteristic morphological differences were seen between seedlings paired with the test fungus and those in control treatments.

b) Interactions with Aspergillus niger.

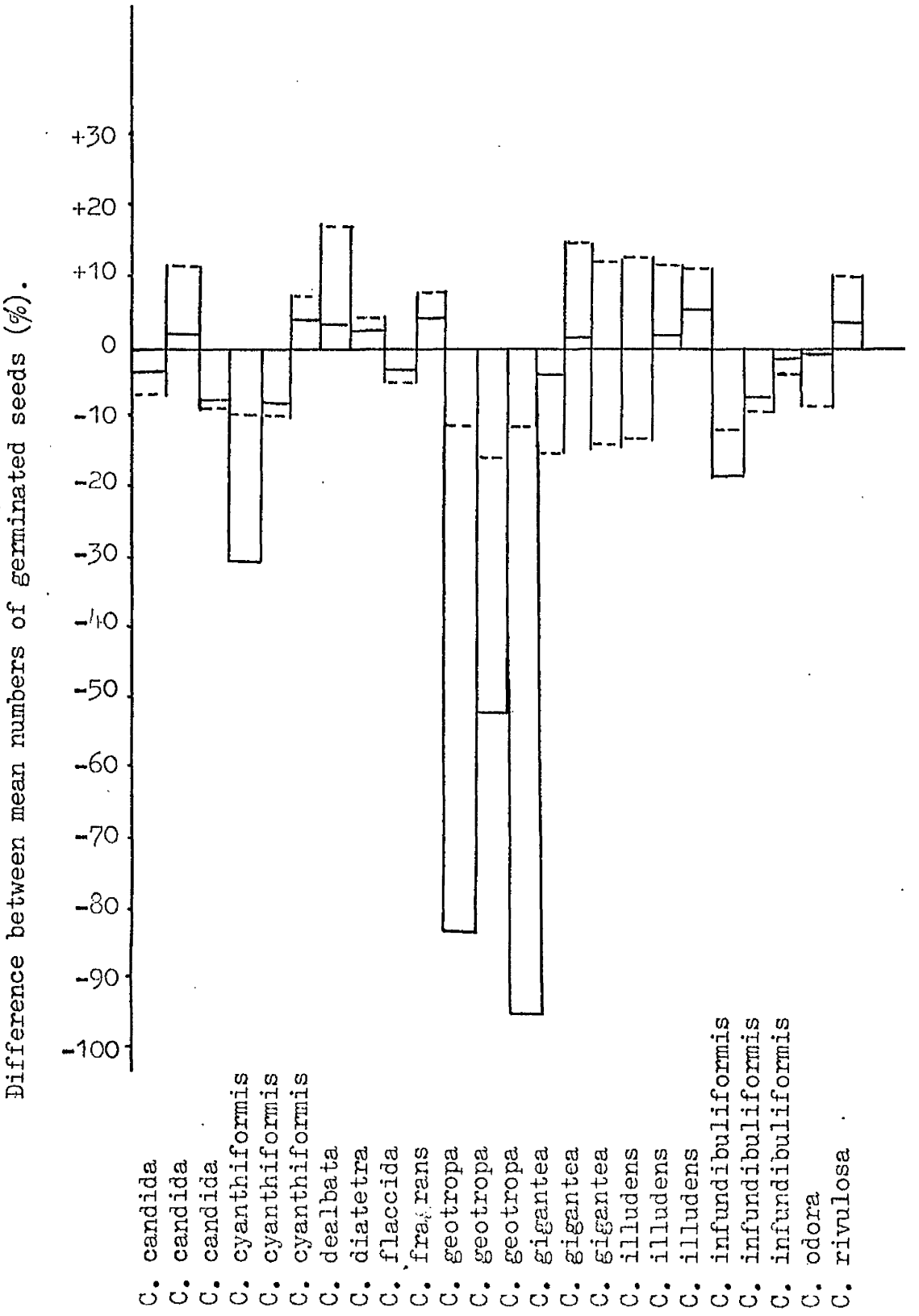
The results are recorded in appendix Table LIV; they are summarized and analysed in appendix Table LVI, and are illustrated in text Figures (xxiii) (a) and (b).

c) Interactions with bacteria.

The results are recorded in appendix Table LV.

Text Table 18 summarizes the results of all tests in which a significant biological effect was recorded.

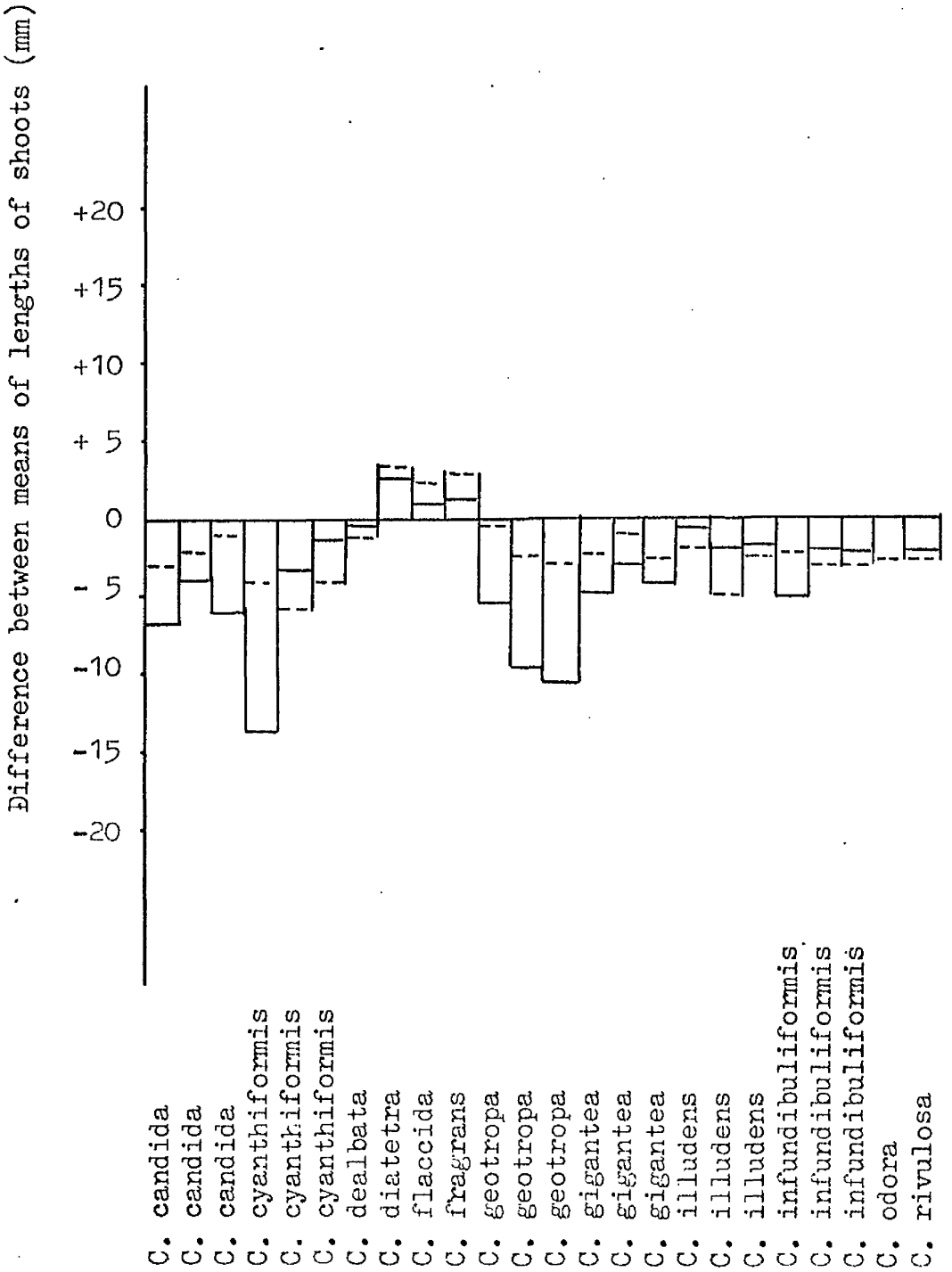
Figure (xxii)(a). Differences in % germination of lettuce seeds in assemblies containing lettuce paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.



— = observed difference between means in each experiment.

-- = least significant difference between means in each experiment.

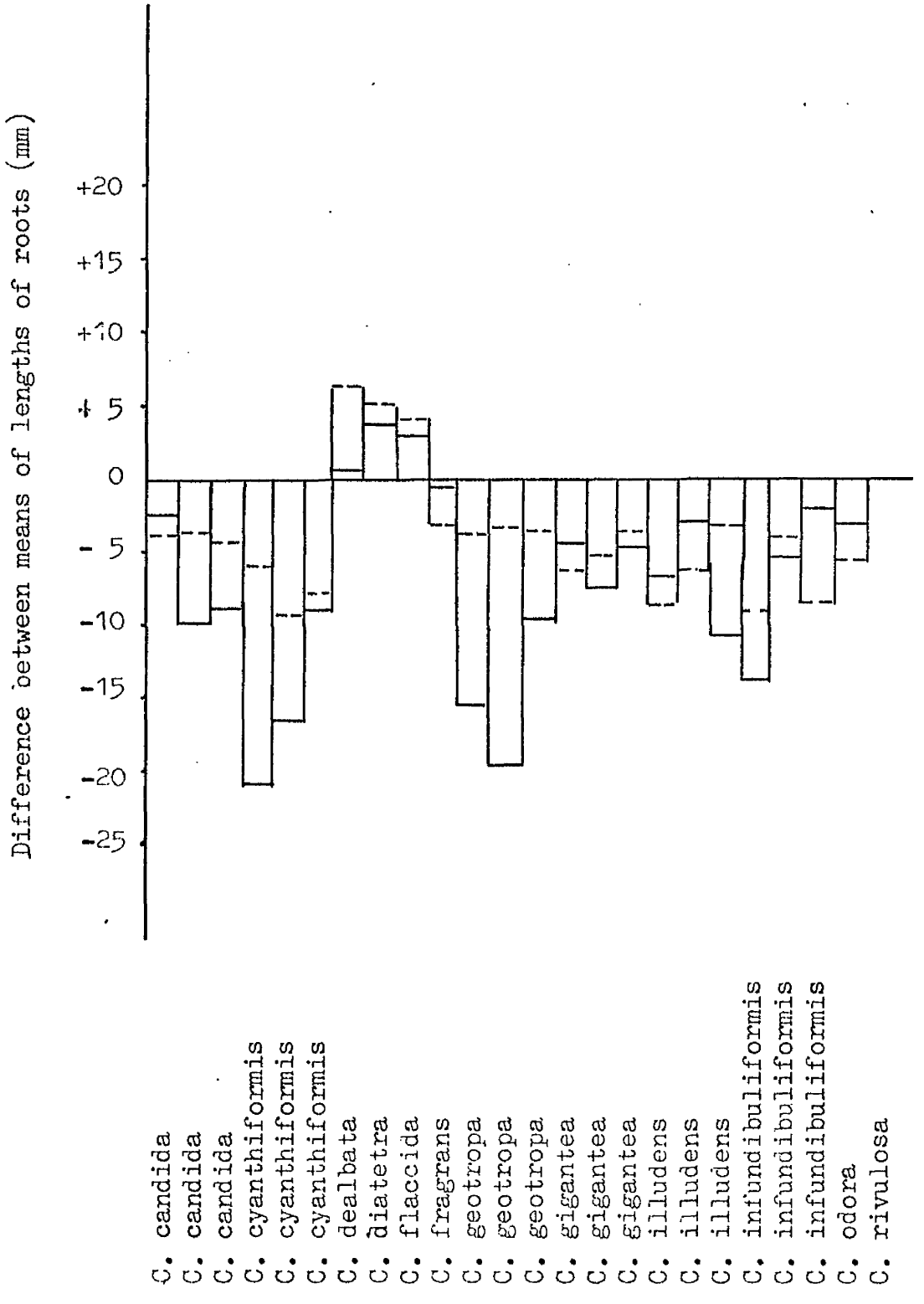
Figure (xxii)(b). Differences in mean lengths of shoots of germinated seedlings in assemblies containing lettuce paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.



—— = observed difference between means in each experiment

- - = least significant difference between means in each experiment.

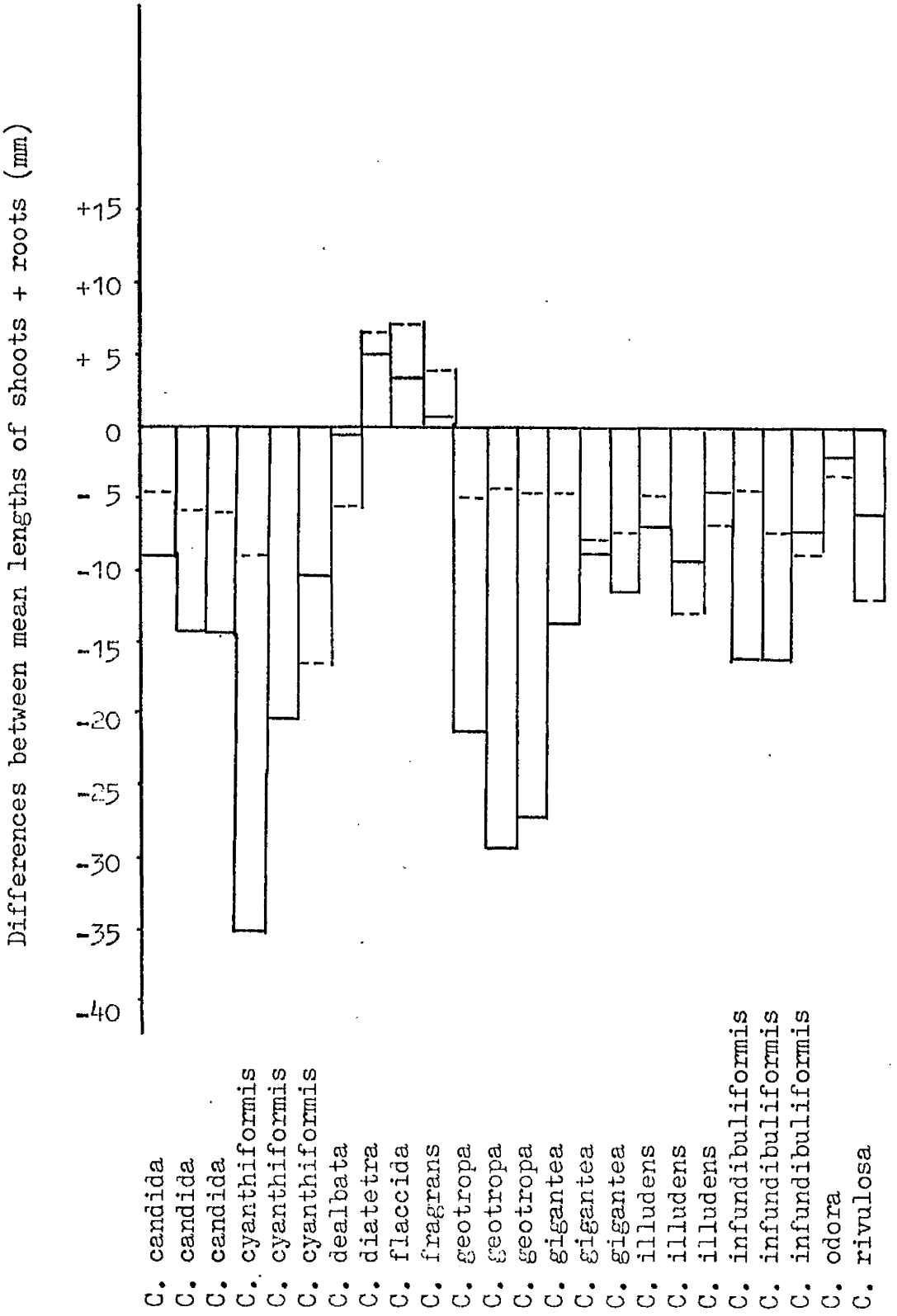
Figure (xxii)(c). Differences between mean lengths of roots of germinated seedlings in assemblies containing lettuce paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.



— = observed difference between means in each experiment

-- = least significant difference between means in each experiment.

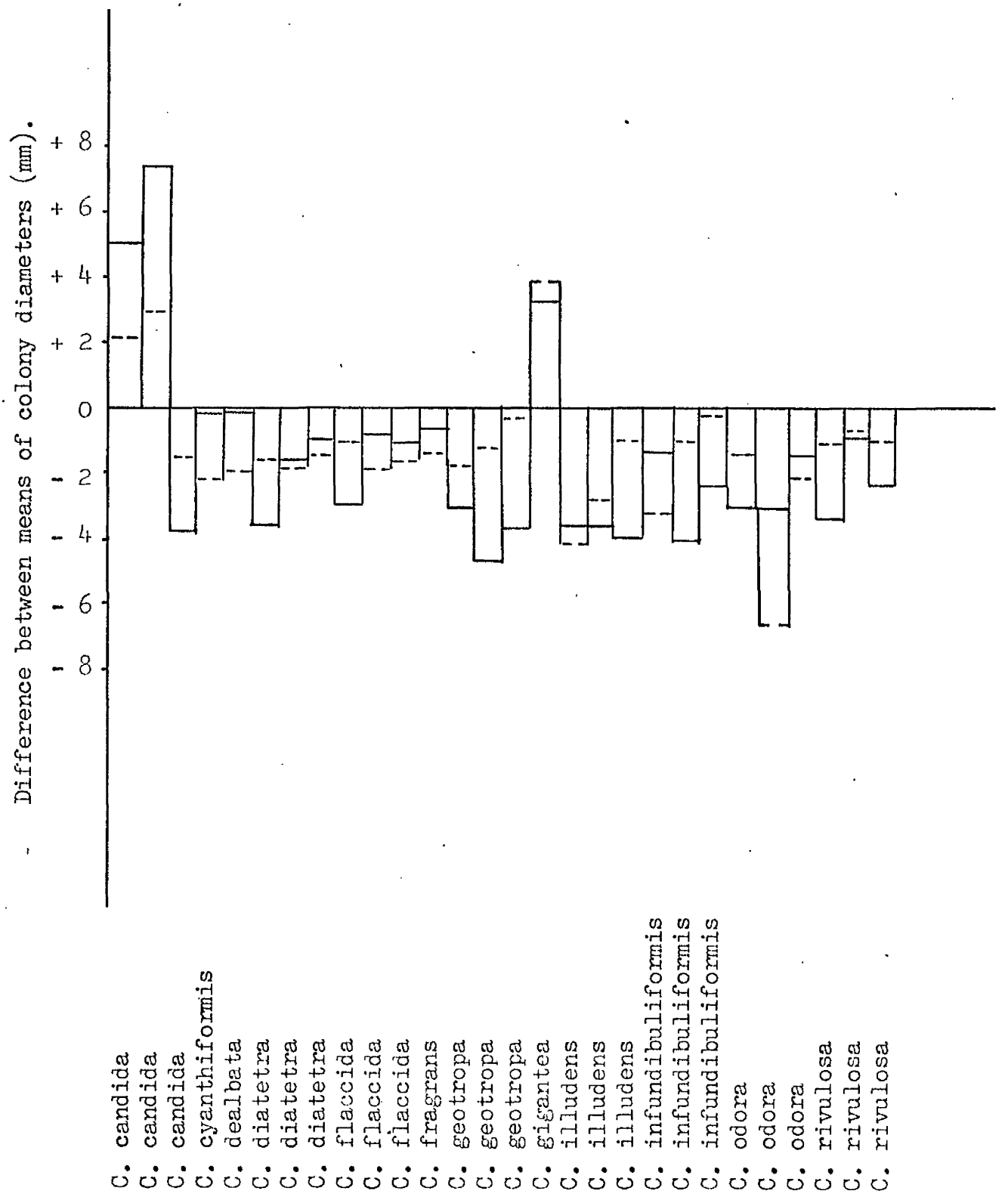
Figure (xxii)(d). Differences in mean lengths of shoots + roots of germinated seedlings in assemblies containing lettuce paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.



— = observed difference between means in each experiment.

- - = least significant difference between means in each experiment.

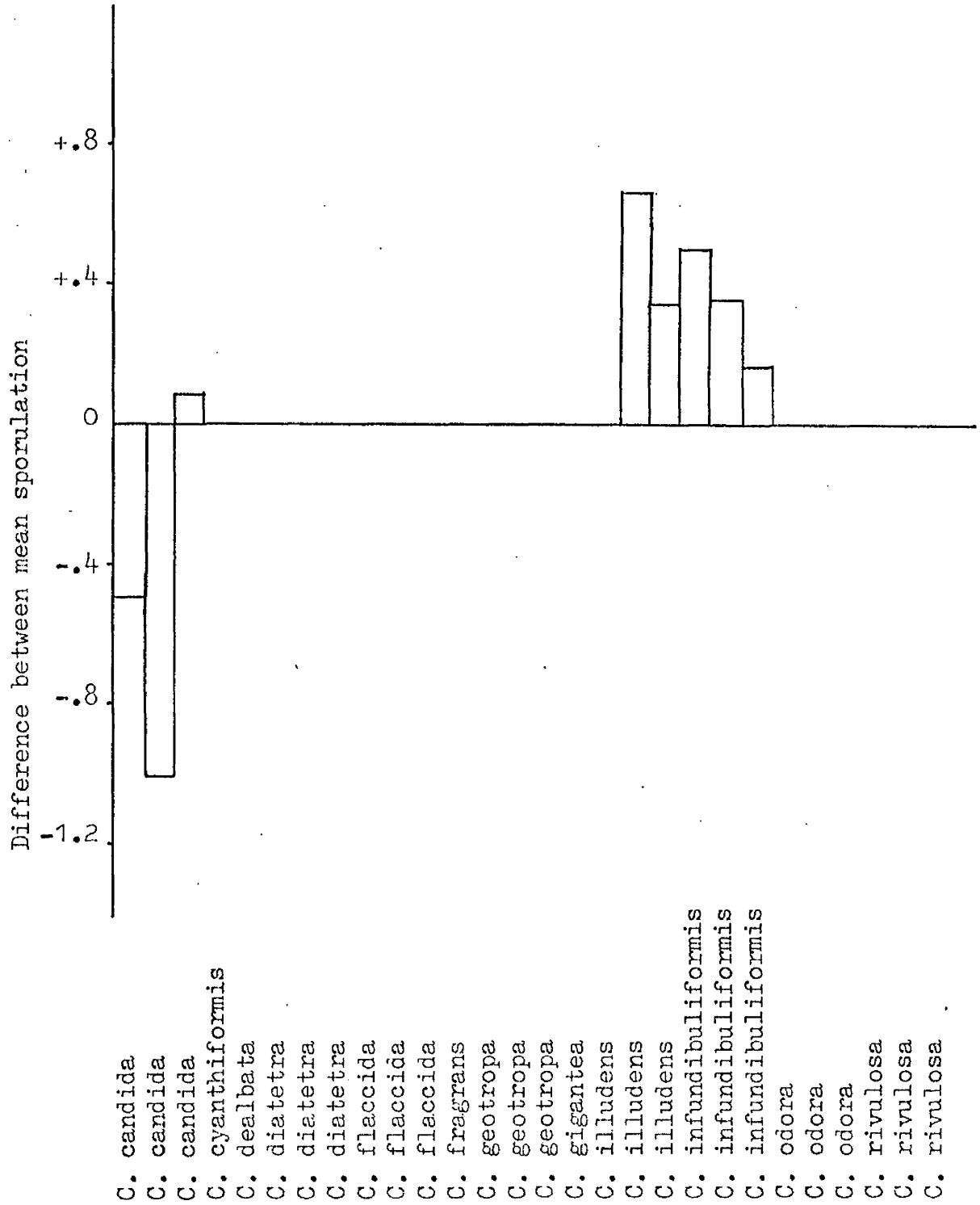
Figure (xxiii)(a). Differences between means of diameters of A. niger colonies in assemblies containing A. niger paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.



— = observed difference between means in each experiment.

– – = least significant difference between means in each experiment.

Figure (xxiii)(b). Differences between mean values for sporulation of A. niger in assemblies containing A. niger paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.



— = observed difference between means in each experiment.

Table 18. Species producing biological activity, in survey.

Assay organism	Lettuce				A. niger		Bacteria
Facet of growth affected / Test species	% germination	Length of shoots	Length of roots	Length of shoots + roots	Colony diameter	Sporulation	Growth
C. candida		- - -	- -	- - -	+ + -	- - +	
C. cyan.		- - -	- - -	- - -			
C. flaccida					-	- - -	
C. geotropa	- - -	- - -	- - -	- - -	- - -	+ + +	- - -
C. gigantea		- - -	- - -	- - -			
C. illudens		- - -	-	-	- - -	+	
C. inf.		- - -	- - -	- - -	- -	+ + +	
C. odora					- - -	+ +	
C. rivulosa					- - -		

\- = inhibitory effect.
\+ = stimulatory effect.

C. cyan. = C. cyanthiformis
C. inf. = C. infundibuliformis

2. Gas chromatographic survey.

There were only two patterns observed which were produced by more than one species:-

Group I.

Illustration and description as for Fomes Group I (see text Figure (iii)).

Cultures following this pattern: Uninoculated 5% malt agar, C. geotropa, C. infundibuliformis and C. rivulosa.

Group II.

Illustration: Text Figure (xxiv).

Description: Injection peak followed by one peak which appeared only

Figure (xxiv). Group II.

Typical G.L.C. trace produced by a 6 week old culture of C. cyanthiformis.

Column: Carbowax 20M

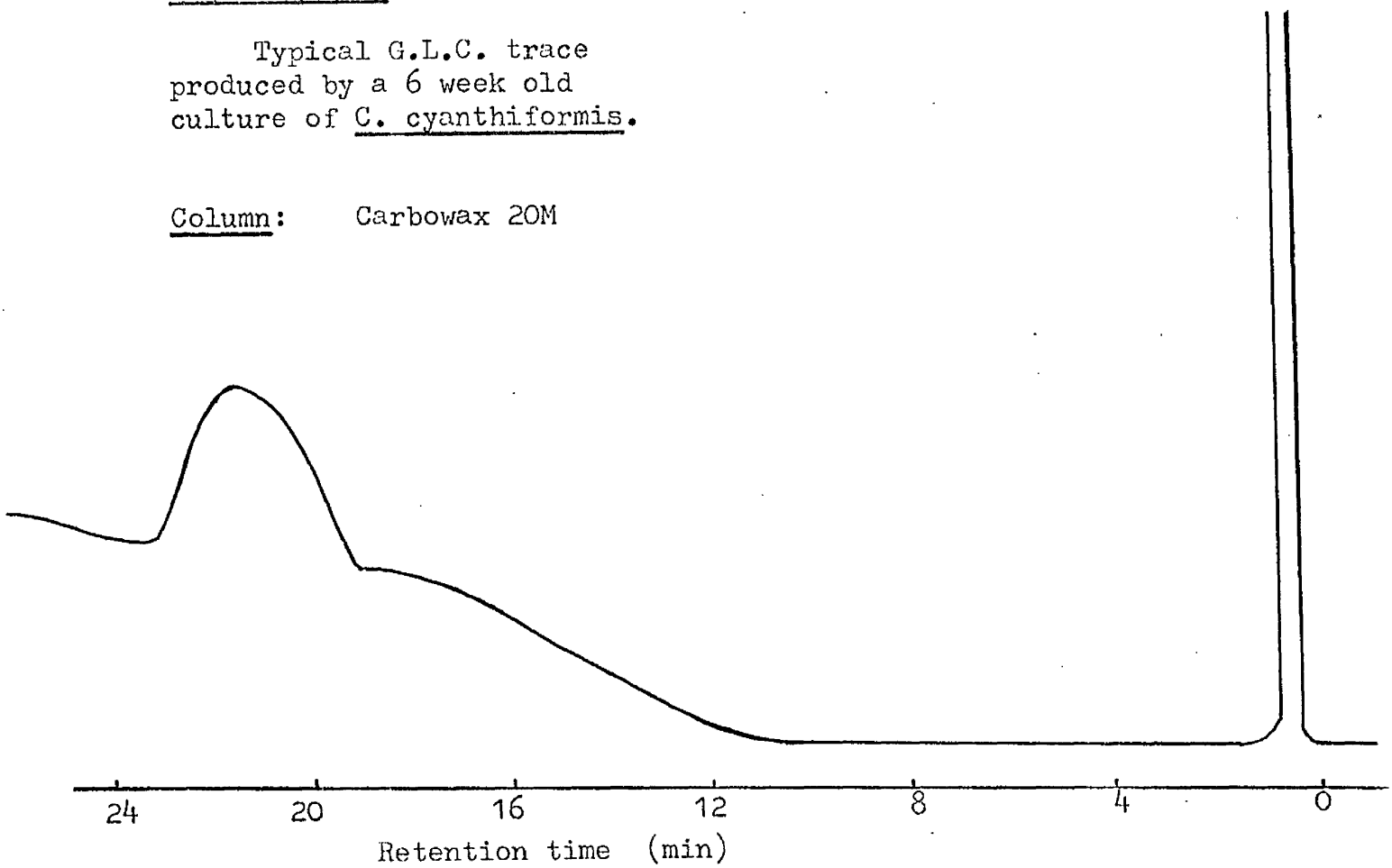


Figure (xxv).

Typical G.L.C. traces produced by 6 week old cultures of C. fragrans.

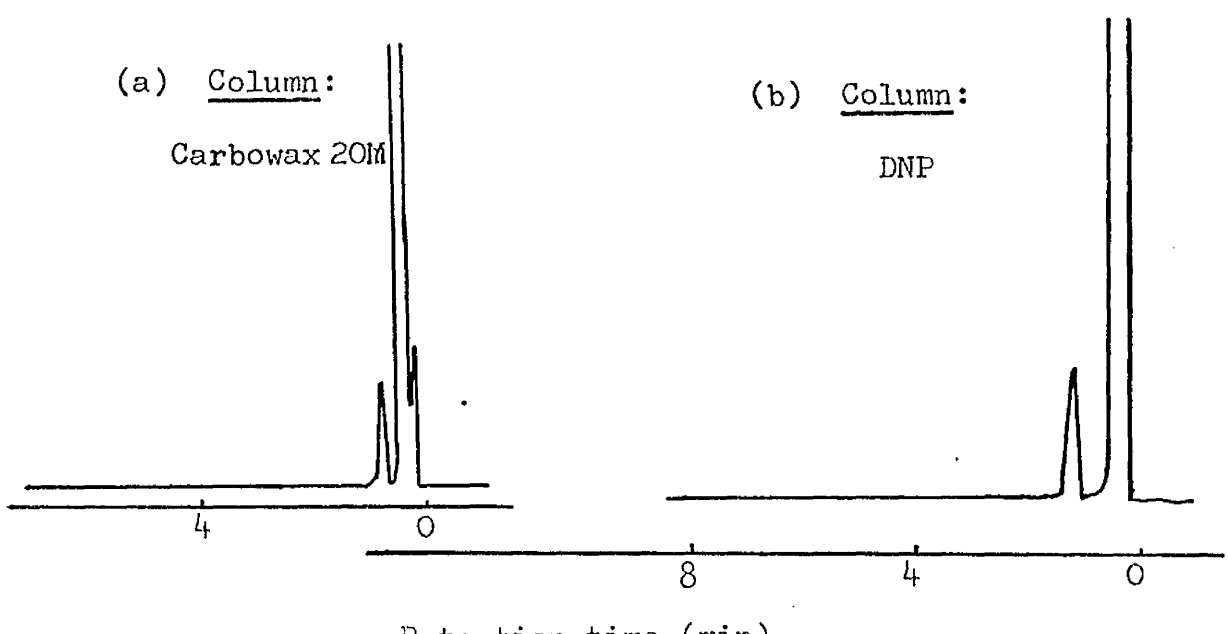
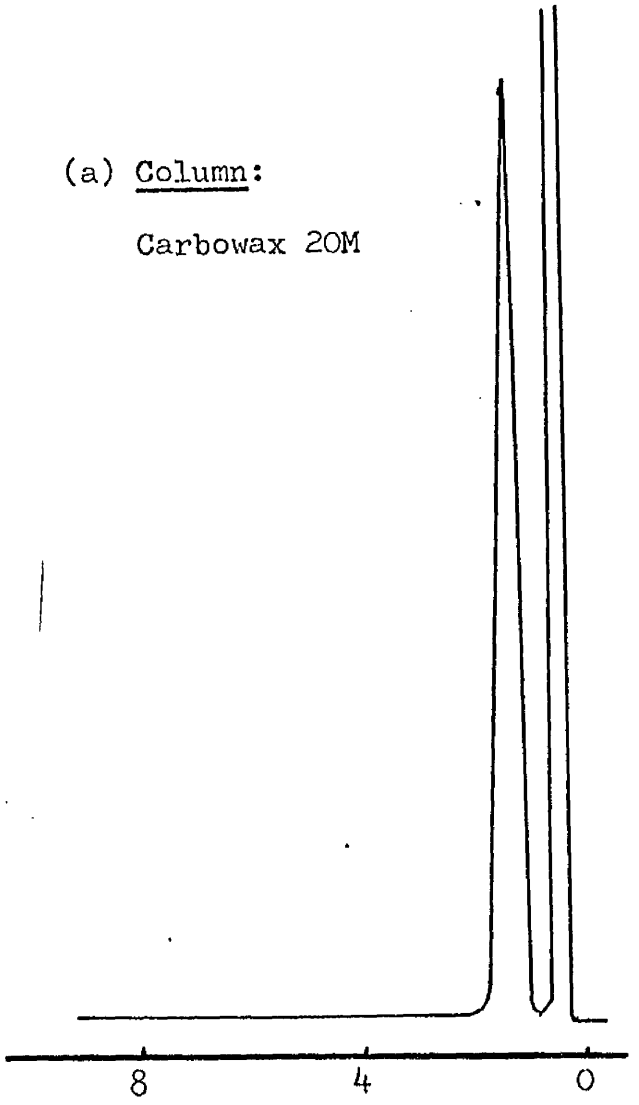


Figure (xxvi).

Typical G.L.C. traces from 5 week old cultures of C. illudens.

(a) Column:

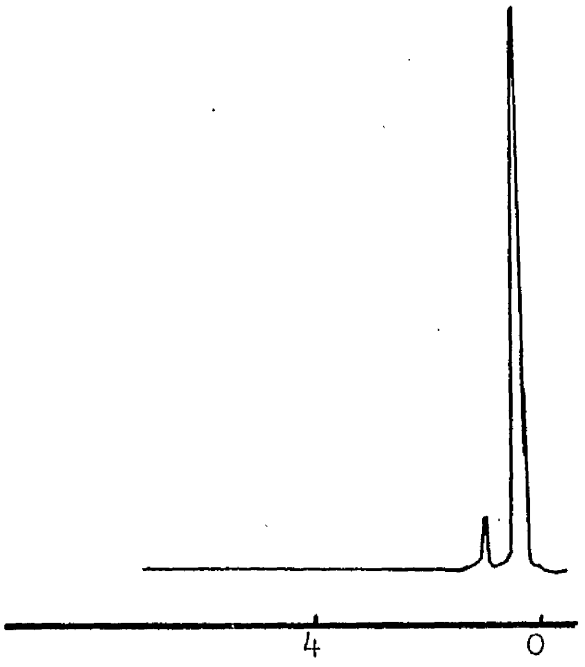
Carbowax 20M



Retention time (min)

(b) Column:

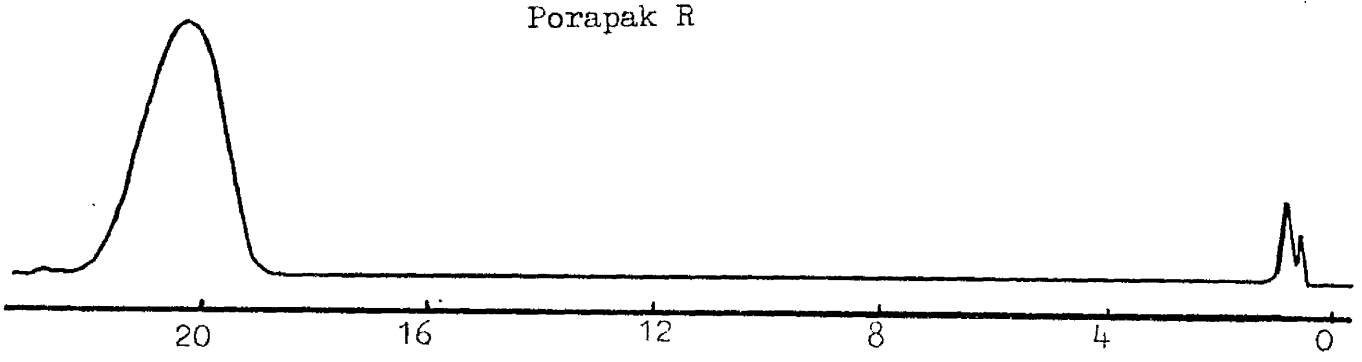
DNP



Retention time (min)

(c) Column:

Porapak R



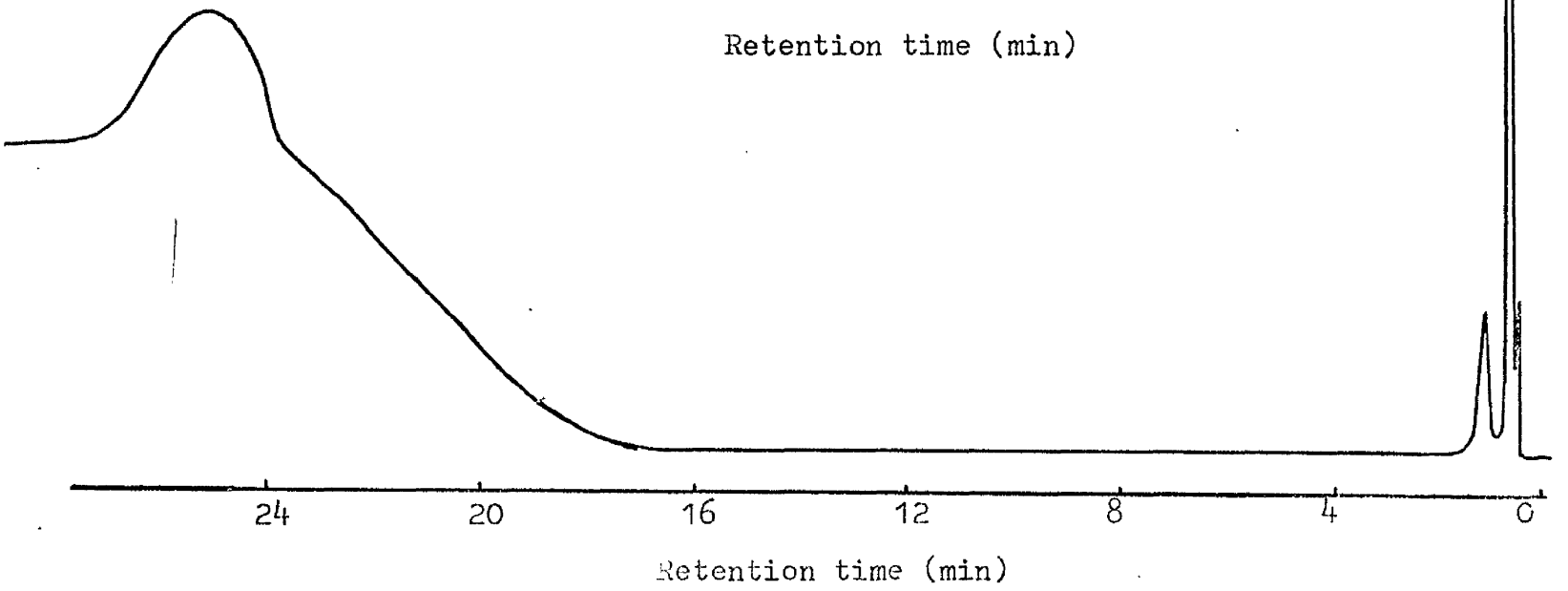
Retention time (min)

Figure (xxvii).

Typical G.L.C. traces from 7 week old cultures of C. flaccida.

(a) Column:

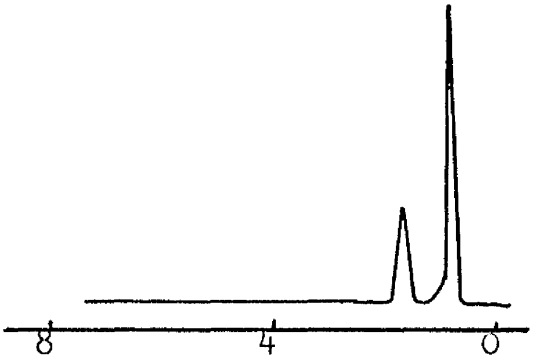
Carbowax 20M



Retention time (min)

(b) Column:

DNP



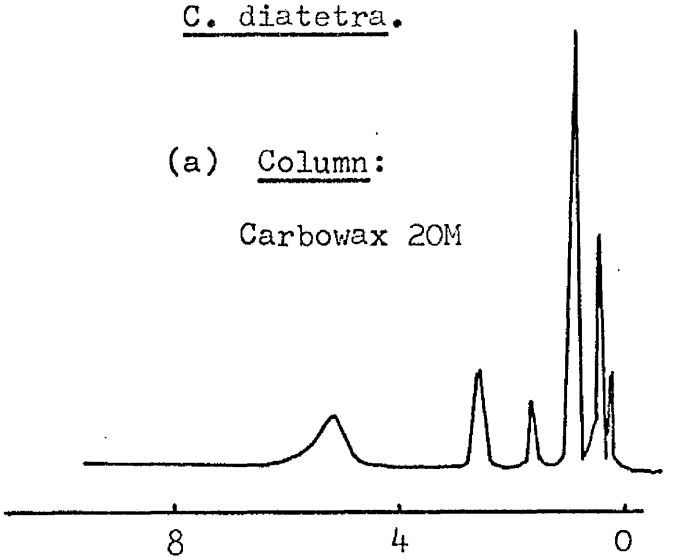
Retention time (min)

Figure (xxviii).

Typical G.L.C. traces from 7 week old cultures of C. diatetra.

(a) Column:

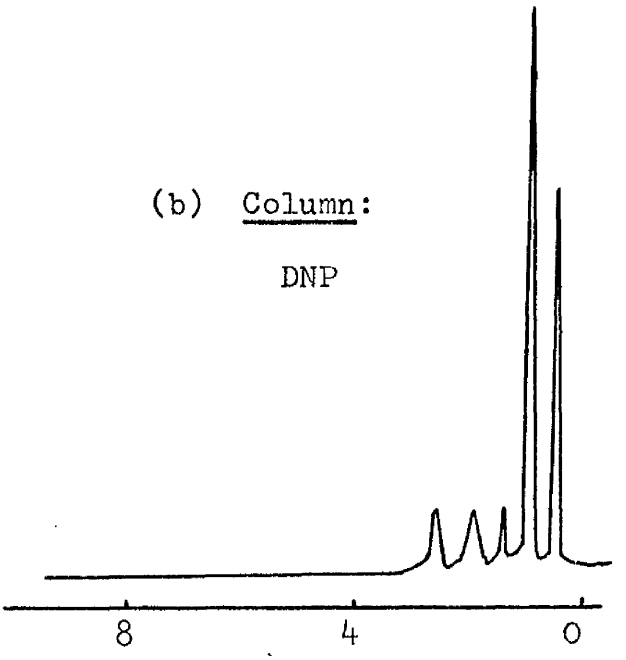
Carbowax 20M



Retention time (min)

(b) Column:

DNP

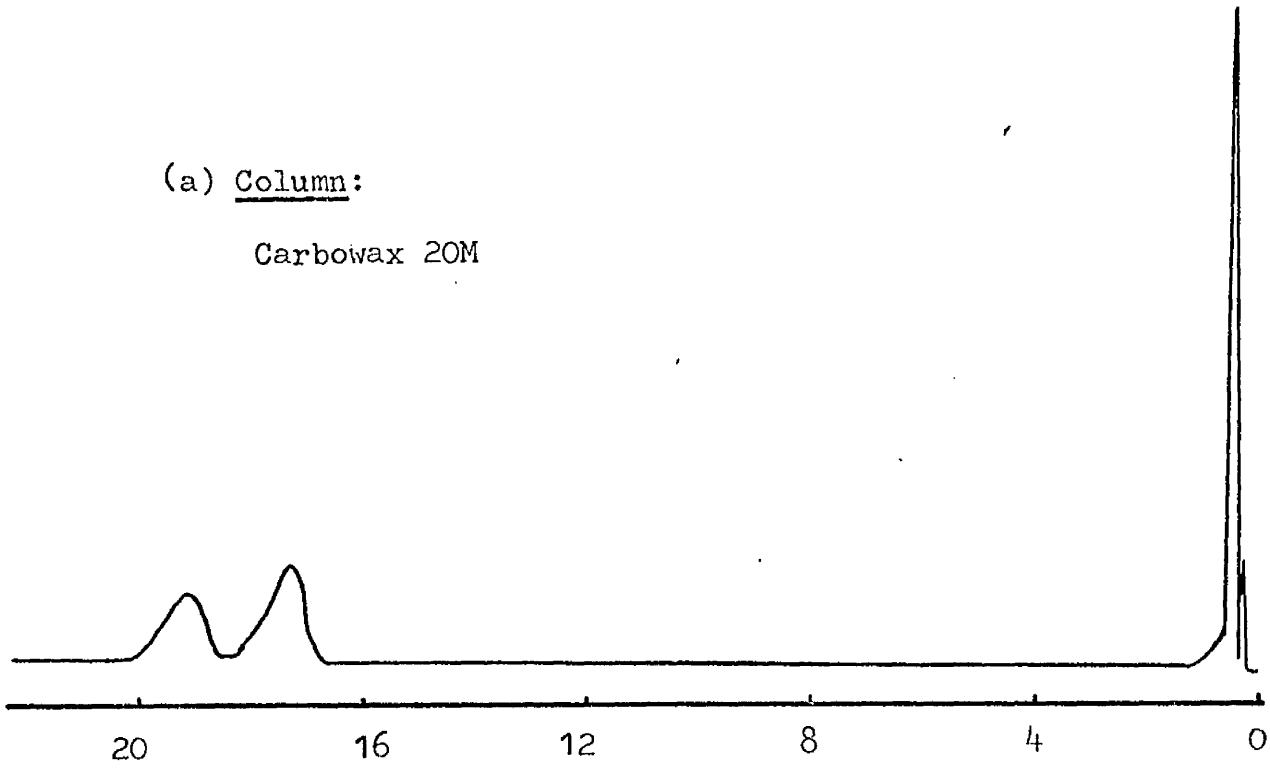


Retention time (min)

Figure (xxix). Typical G.L.C. traces from 7 week old cultures of C. dealbata.

(a) Column:

Carbowax 20M



(b) Column:

DNP

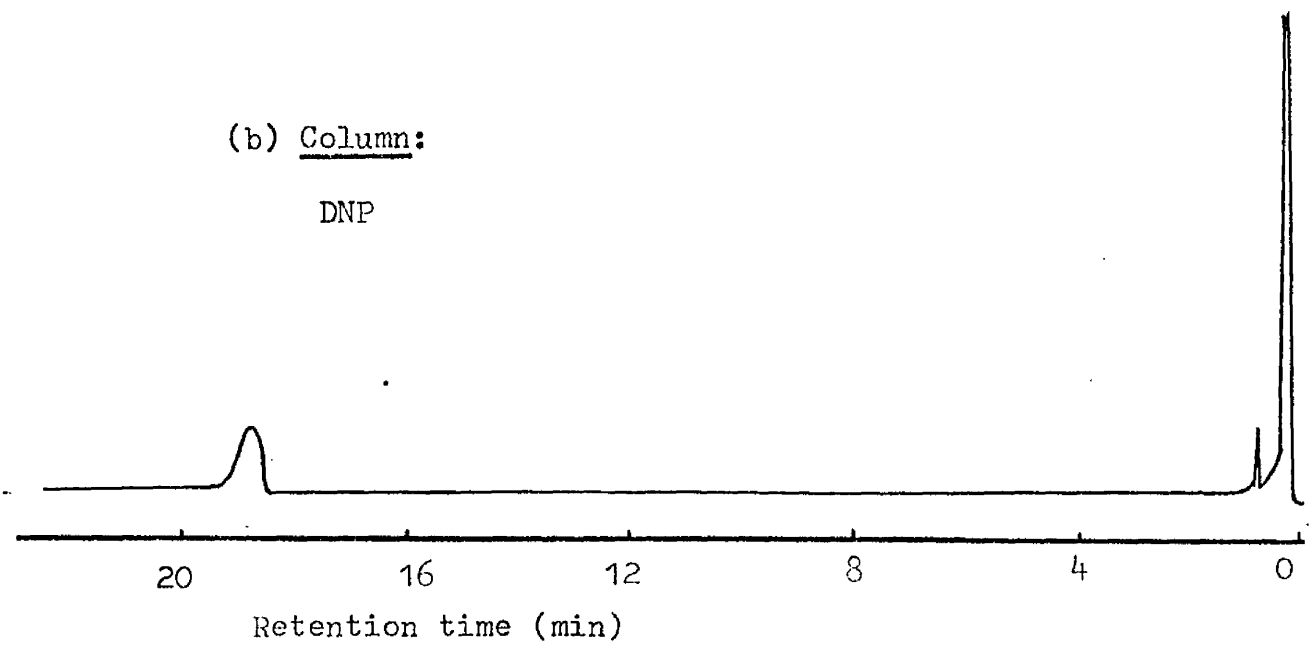


Figure (xxx). Typical G.L.C. traces from 6 week old cultures of C. gigantea.

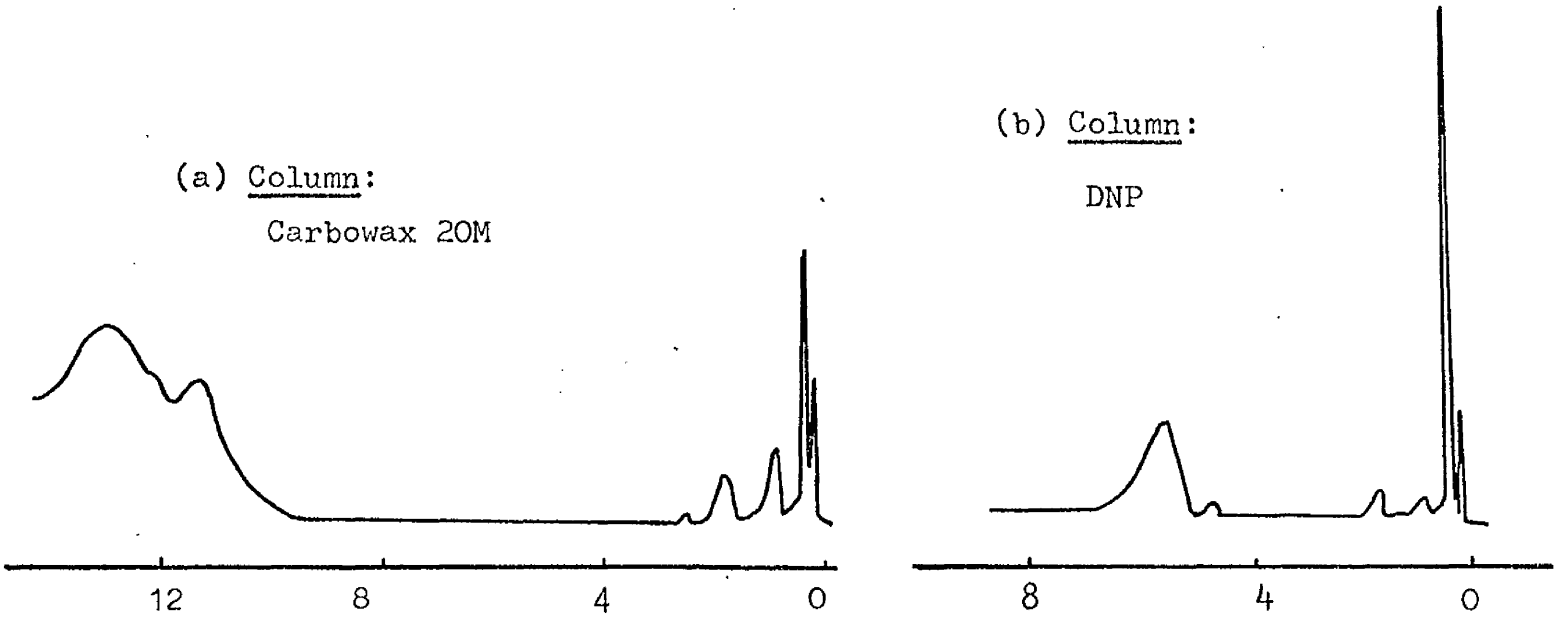
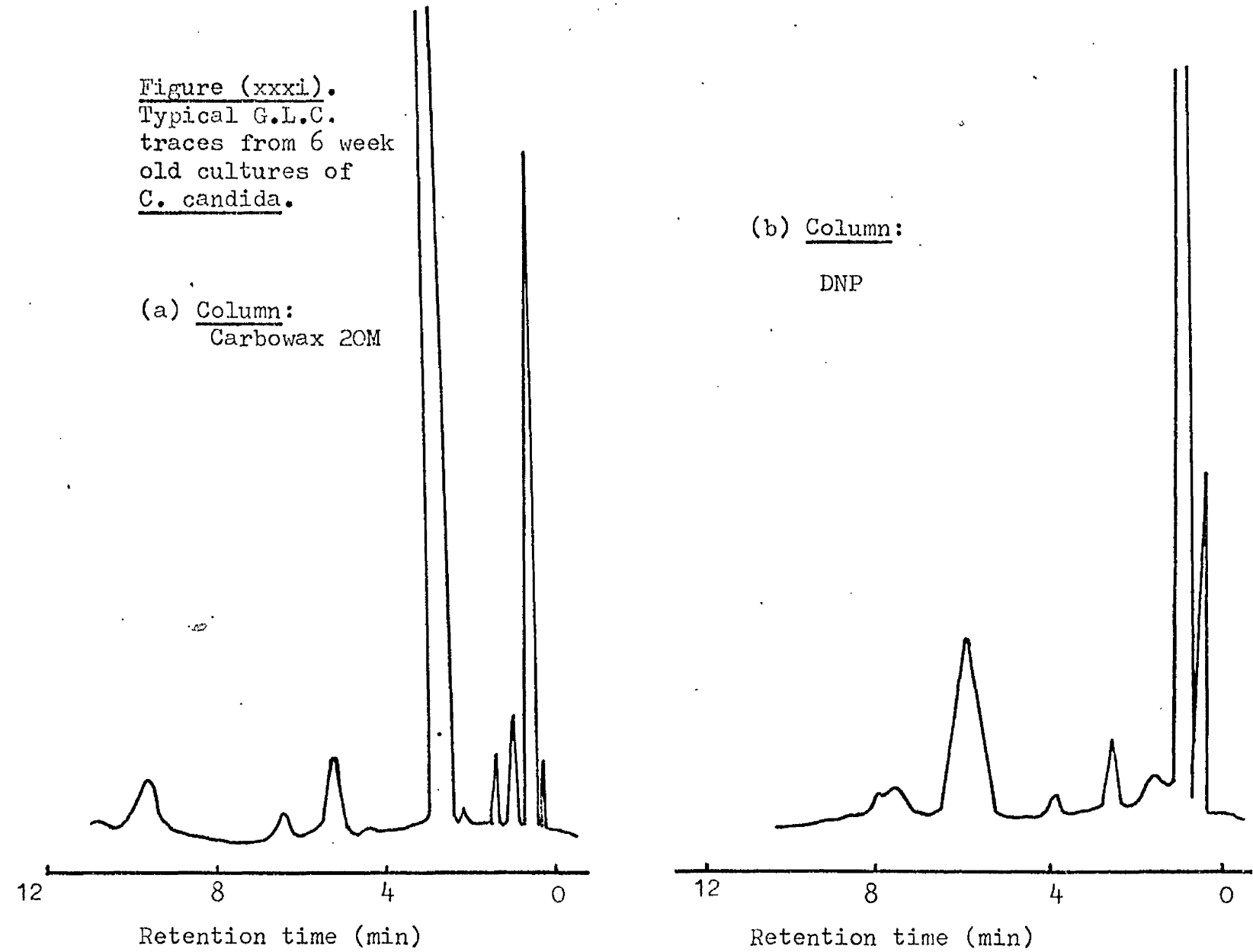


Figure (xxxi). Typical G.L.C. traces from 6 week old cultures of C. candida.



on the Carbowax 20M column when the column temperature had been raised to 100°C. Cultures following this pattern: C. cyanthiformis and C. odora.

No general pattern was seen which applied to more than one species of the remainder.

The patterns are illustrated in text Figures (xxv) to (xxxi). Numbers of peaks produced in these traces varied from one prominent peak, in traces from C. illudens (Figure (xxvi)) and C. fragrans (Figure (xxv)) to seven small poorly defined peaks in traces from C. candida (Figure (xxxi). There are indications that some peaks shown on the above Figures may be common to several species, e.g. that shown in Figure (xxv) from C. fragrans has a similar retention time to the peak in traces from C. flaccida on both Carbowax 20M and DNP (Figure (xxvii)) and the first peak on traces from C. diatetra (Figure (xxviii)) and C. gigantea (Figure (xxx)). However, no accurate comparisons of retention times of these and of other peaks have been made.

3. Examination for the production of hydrogen cyanide.

Hydrogen cyanide was detected in the culture gases of C. candida, C. cyanthiformis, C. geotropa and C. infundibuliformis. C. geotropa caused a more rapid change of colour in test papers than did the other species suggesting that higher concentrations of hydrogen cyanide were present in this species (see Section IB of this thesis).

A further examination of the production of hydrogen cyanide and effects of the culture gases of these species is described in Section IIB.

Discussion.

1. Biological assay.

The inhibitory effects of the culture gases of the six species shown to inhibit consistently the root and shoot growth of lettuce (i.e. C. candida, C. cyanthiformis, C. geotropa, C. gigantea and C. infundibuliformis and, to a lesser extent C. illudens) closely resemble those produced by the gases of F. scutellatus described in Section IA results paragraph 1, and subsequently demonstrated in the presence of concentrations of gaseous HCN equivalent to those detected in the culture gases of this species (Section IB). The possibility that these inhibitory effects could be due to the production of this gas is discussed below.

In tests with A. niger, three of the species shown to inhibit the growth of lettuce, C. candida, C. geotropa and C. infundibuliformis, were also shown to inhibit the colony growth of A. niger significantly in a few trials. They were also shown to stimulate sporulation in the same trials and in one additional trial in the case of C. candida. Again these results compare closely with those observed with F. scutellatus (Section IA results paragraph 1) and are discussed below in relation to metabolite production.

The culture gases of C. flaccida, C. illudens, C. odora and C. rivulosa also inhibited the colony growth of A. niger, the culture gases of C. illudens and C. odora also causing stimulation of sporulation

in some trials. The tendency for correlation between inhibition of colony growth and stimulation of sporulation and the converse in two trials with C. candida compares with the observations made in the Fomes survey. It again differs from the correlation between inhibition of both facets of growth reported by Martin (1963); the only instance where this correlation was reported being in one trial with C. flaccida. Stimulations of colony growth were, however, again rare, occurring only in two trials with C. candida.

C. geotropa, the one species shown to affect the growth of bacteria, also inhibits the other assay organisms. The possible relationship of this trend of consistency in biological effects is compared below with trends in metabolite production.

2. G.L.C. survey.

Group I.

This is similar to Group I discussed in the Fomes survey (Section IA). Again, further examination of these species would not seem profitable from these results alone. However, from experience with F. scutellatus, the possibility of active metabolites being present was not dismissed.

Group II and other species.

All these patterns look worthy of further investigation. At present, insufficient information is available to compare individual peaks in the different patterns and hence to comment further on the frequency of occurrence of the metabolites detected by G.L.C. analysis in this group. Of special interest too is the single prominent peak

given by the culture gases of C. illudens since this peak behaves in a manner closely similar to that identified in the F. pomaceus group as methyl chloride. The correlation of these results with the biological work discussed below would obviously affect the priorities in choice for further investigation.

3. HCN production.

The production of HCN by C. candida, C. cyanthiformis, C. geotropa, and C. infundibuliformis has been reported by other authors (see text Table 16 in the introduction to this Section), but only by the fruiting bodies of these species. From the results reported here, it would appear that, in these species, HCN production is not limited to the sporophore, but can also be produced by the mycelium, contrary to the report by Mirande (1932) who could not detect HCN production by the mycelium of C. geotropa. In this survey, however, no HCN could be detected in the culture gases of C. fragrans, although the sporophores of this species have been reported to produce HCN (Mirande, 1932; Muller, 1944; Singer, 1962); this suggests that HCN production may be confined to the sporophores of this species, or, of course, that the conditions of these tests were unsuitable for the production and/or the detection of HCN production by this species. Neither could HCN be detected in the culture gases of C. gigantea whose sporophores are also reported to produce this compound (Locquin, 1944 and 1947). However, the taxonomy of C. gigantea is rather confused and Locquin's report refers to the

species C. gigantea var. candida which Heim (1947) considers to be possibly the same species as C. candida and physiologically different from C. gigantea. These results certainly support the hypothesis that C. gigantea and C. candida are physiologically different.

The differences in the amounts of HCN detected in the four species producing this metabolite are discussed below in relation to the biological results.

Correlation of results of biological and chemical surveys.

The most striking correlation of biological activity with volatile metabolite production is that of the hydrogen cyanide producing species, all of which inhibited lettuce growth, some affected colony growth and sporulation of A. niger and one of which inhibited bacterial growth. This trend in biological activity seems to be related to the amount of HCN produced in these species. Further examination of HCN production in these species and the biological effects of the culture gases in more controlled environments is described in Section IIB of this thesis. The possibility that HCN in these culture gases could cause these effects on A. niger has not been further investigated (for further discussion of the effect of HCN on A. niger, see Section IB). No experiments have been carried out to investigate the possibility that HCN was the factor causing inhibition of bacterial growth in tests with C. geotropa. The observations of Locquin (1947) who showed that the inhibition of growth

of the bacteria Staphylococcus aureus, Micrococcus tuberculosis, Brucella abortus, Bacterium coli, and Bacillus pyocyaneus could be accounted for by the HCN in the culture gases of C. gigantea var. candida, are consistent with this hypothesis.

Neither of the other two species shown to inhibit the growth of lettuce in this survey produced HCN in detectable quantities. Both, however, produced interesting G.L.C. traces quite different from one another. The possibility that the activity of C. illudens could be due solely to the action of the antibiotic previously reported from this species i.e. muscarine seems unlikely since other species, examined here, also reported to produce this compound, C. infundibuliformis and C. rivulosa, show different patterns of activity to that of C. illudens.

The two species in Group II, C. cyanthiformis and C. odora, showed no consistent correlation of biological activity in this survey although obviously in C. cyanthiformis and other cyanide producing species the presence of HCN may affect the possible biological interactions between other metabolites produced and the assay organisms.

General conclusions.

The results of this survey open up many interesting possibilities for further investigation. Of these, only one has been examined briefly and is reported in the following section.

SECTION IIB.

INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF THE VOLATILE METABOLITES FROM CULTURES OF CLITOCYBE CANDIDA, C. CYANTHIFORMIS, C. GEOTROPA AND C. INFUNDIBULIFORMIS.

SECTION IIB. INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES FROM CULTURES OF CLITOCYBE CANDIDA, C. CYANTHIFORMIS, C. GEOTROPA AND C. INFUNDIBULIFORMIS.

Introduction.

The results in Section IIA, paragraph 1 show that, in the conditions examined, gases from C. candida, C. cyanthiformis, C. geotropa and C. infundibuliformis showed consistent biological effects on the growth of lettuce and in some cases on the germination of lettuce, on the growth and/or sporulation of A. niger and on the growth of bacteria. In addition, HCN was detected in the culture gases of these species (Section IIA, paragraph 2). The following report is restricted to an investigation of the hypothesis that the concentrations of HCN present could account for the inhibition of the growth of lettuce. It is based on a comparison of tests carried out as part of the investigation described in Section IB on F. scutellatus.

A. Analysis of culture gases.

1) Carbon dioxide and oxygen.

Methods.

The methods used are described in the General Methods, paragraph 5.

To avoid the effects of pressure changes, etc. involved in sampling, any variations in the amounts were not determined during tests.

Results.

Text Table 19 gives the amounts of these metabolites found at the end of representative experiments with paired bottle assemblies and with paired petri dish assemblies.

Table 19. Carbon dioxide and oxygen concentrations in assemblies testing the effects of gases from Clitocybe species on lettuce.

Treatment (lettuce in presence of:-)		Range of carbon dioxide (% volume/volume of gas space)	Range of oxygen concentration (% volume/volume of gas space)
Paired bottle assemblies	C. candida	0.110 - 0.232	17.02 - 23.75
	C. cyanthiformis	0.092 - 0.220	18.32 - 24.51
	C. geotropa	0.094 - 0.136	19.32 - 24.00
	C. infundibuliformis	0.075 - 0.153	18.63 - 19.52
	Control	0.039 - 0.109	20.40 - 22.38
Paired petri dish assemblies	C. candida	0.032 - 0.044	20.00 - 22.17
	C. cyanthiformis	0.024 - 0.037	18.68 - 20.97
	C. geotropa	0.025 - 0.041	19.41 - 20.04
	C. infundibuliformis	0.031 - 0.039	19.88 - 21.52
	Control	0.028 - 0.042	19.90 - 21.60

No greater concentration of carbon dioxide or lower concentration of oxygen was seen in any assembly in measurements made in many other similar experiments after shorter and longer incubation periods.

Discussion.

The range of carbon dioxide and oxygen in paired petri dish assemblies differed little from that of the normal laboratory air. The carbon dioxide concentrations detected in paired bottle assemblies were within the range

shown in Section IB, paragraph 2(a) to have no effect on the growth of lettuce. The changes in oxygen concentration were not more than 22.0% in assemblies with Clitocybe cultures compared with 7.0% in assemblies with uninoculated 5% malt agar. These changes were considered to be trivial and the possibility of their having a significant effect was not investigated.

Conclusion.

Levels of carbon dioxide and oxygen present are unlikely to contribute significantly to the effects under these conditions.

2) Hydrogen cyanide.

Methods.

Hydrogen cyanide levels were measured in paired bottle assemblies containing lettuce and Clitocybe cultures using the techniques described in Section IB, paragraph 2(c). Sampling procedures used and the conditions under which these experiments were carried out here were similar to those described in that Section. No parallel tests with authentic KCN were carried out and interpretation of results was based on comparison with data obtained in the above-mentioned Section with parallel tests with Clitocybe cultures.

Results.

a) Analysis of HCN in assemblies used in biological tests.

Text Figure (xxxiii) gives the daily changes in the concentration of HCN in paired bottle assemblies containing lettuce paired with cultures of Clitocybe.

The detailed results of these experiments are given in appendix Table LVII.

b) Biological tests with Clitocybe cultures under similar conditions to those with authentic HCN in Section IB.

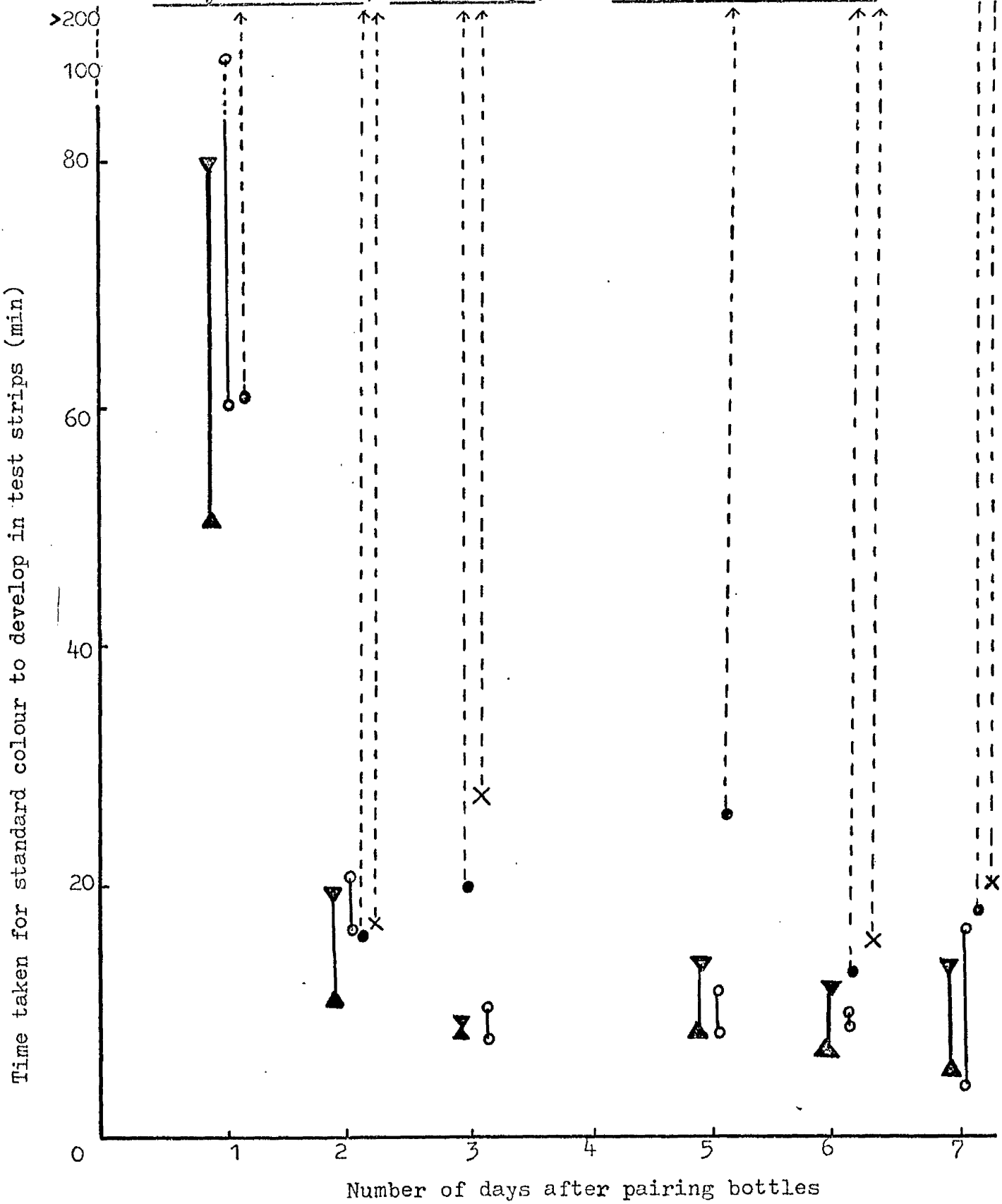
Text Figure (xxxiv) gives the germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with cultures of each species of Clitocybe, and in controls, paired with uninoculated 5% malt agar.

In these tests, the growth of lettuce seedlings was significantly reduced in the presence of the culture gases of each species.

The detailed results of these and two replicate experiments, including the terminal HCN concentrations measured in all three experiments, are given in appendix Tables LVIII, LIX, LX and LXI.

The results of all three experiments with each species are summarized in text Table 20. In these tests, the results of replicate experiments with C. geotropa and C. infundibuliformis were closely similar to those in Figure (xxxiv) (experiment 1 in each case). However, inhibition of seedling growth was more variable in tests with C. candida and C. cyanthiformis, and was not significant in one test with each species.

Figure (xxxiii). Changes in HCN concentrations in paired Roux bottle assemblies containing lettuce and gases from cultures of C. candida, C. cyanthiformis, C. geotropa, and C. infundibuliformis.



Each line shows the range of the readings from the 3 assemblies sampled each day (all for day 7)

●—● = C. candida gases, ×—× = C. cyanthiformis gases,
O—O = C. geotropa gases, ▶—◀ = C. infundibuliformis gases.

For detailed results see appendix Table LVII.

Table 20. Summary of results of experiments examining the germination of lettuce seeds and growth of germinated seedlings in the presence of gases from cultures of Clitocybe species and in controls with uninoculated 5% malt agar.

Treatment	Expt. no.	% reduction in mean of total seedling lengths compared with control treatments with uninoculated 5% malt agar	
		Observed	Least significant (P = 0.05)
C. candida	I	39.6	27.9
	II	49.0	50.9
	III	53.1	12.6
C. cyanthiformis	I	30.4	22.6
	II	25.8	27.1
	III	36.8	11.7
C. geotropa	I	73.4	23.9
	II	54.5	21.3
	III	50.0	25.7
C. infundibuliformis	I	84.3	24.7
	II	67.4	32.7
	III	27.1	18.1

Discussion.

Both the inhibition of lettuce seedling growth and concentrations of HCN detected in assemblies containing lettuce paired with cultures of C. geotropa and C. infundibuliformis (see Figure (xxxiii)) were slightly lower than those detected in comparable tests with F. scutellatus and authentic solutions of 0.001N KCN (see Section IB Figure (xii)). However, the concentrations of HCN detected in the tests with C. geotropa and C. infundibuliformis were considered sufficient to account for the inhibition observed.

The inhibition of lettuce seedling growth and the concentrations of hydrogen cyanide detected in assemblies with lettuce and cultures of C. candida and C. cyanthiformis (see Figure (xxxiii) were considerably lower than those detected in assemblies with F. scutellatus and 0.001N KCN solutions. In many assemblies containing C. candida and C. cyanthiformis HCN was not detectable in the time period conveniently measured with the analytical technique used.

The extent of inhibition of lettuce seedling growth was correlated consistently, however, with the level of HCN detected in paired bottle assemblies and where inhibition was seen, the level of HCN was sufficiently high to account for the inhibition.

SECTION III

INVESTIGATION OF THE PRODUCTION OF ZYGOSPORES AT LOW TEMPERATURES BY RHIZOPUS SEXUALIS (SMITH) CALLEN.

SECTION III. INVESTIGATION OF THE PRODUCTION OF ZYGOSPORES AT LOW TEMPERATURES BY RHIZOPUS SEXUALIS (SMITH) CALLEN.

Introduction.

Hawker, Hepden and Perkins (1957) reported that cultures of this strain produced a few gametangial initials when grown at temperatures between 5° and 10°C. These are, however, produced erratically and do not mature. Cultures grown at 20°C produced normal mature zygospores, also cultures grown at 20°C until gametangial initials had formed (after about 2 days) produced normal mature zygospores on transfer to 10°C. Those grown at 5° to 10°C rapidly develop normal mature zygospores after transfer to, and subsequent incubation at, 15° to 20°C.

Hepden and Hawker (1961) subsequently found that normal zygospores were formed in cultures incubated at 5° to 10°C in the presence of gases from cultures growing at 20°C. They found that no comparable stimulation could be produced with extracts of mycelium grown at 20°C, solutions of known growth substances or various natural extracts, apart from extracts of carrot although not by individual components of this extract. Similarly no comparable stimulation was reported using carbon dioxide, ammonia or ethanol. They found that the proportion of DNA to RNA rose considerably during zygospore production suggesting that inhibition of zygospore initiation could be due to the lack of methyl donors preventing conversion of RNA to DNA (Hepden & Folkes, 1960). However, methylamine and several

other methyl donors did not produce comparable stimulation in cultures grown at 10°C.

The most recent publication on this topic concerns an investigation of the intensity of respiration of R. sexualis cultures during sporulation (Hawker and Hepden, 1962). A period of intense respiration was consistently observed prior to sporulation; the occurrence of this intense peak of respiration was essential to zygospore initiation, no such peak occurring in cultures grown at 9°C. However, they found that zygospore production did not inevitably follow a peak in respiration e.g. with non-compatible strains of fungus, or on addition of thiamine to the growth medium. These investigations have led to the hypothesis that some qualitative change in the environment gives rise to a quantitative change in respiration, which, in turn, causes a morphological change in the growth of the fungus.

The investigation carried out as part of this thesis was commenced to analyse the effects of the culture gases more rigorously. Great variation in the initial results led to the expansion of this study to an examination of other effects in the interaction.

Methods.

The methods described in this section were used in many of the following examinations. Methods used in particular experiments are described in the relevant places below.

1. Strain of fungus used.

Rhizopus sexualis (Smith) Callen, obtained from Prof. Hawker was used throughout.

2. Medium.

The cultures of R. sexualis were grown on 1% malt agar since Hawker et al. (1957) found that this medium allowed moderate formation of zygospores at 20°C, but insufficient growth of aerial mycelium to make counting of zygospores difficult.

3. Assay assemblies.

Most tests were carried out using either paired petri dish assemblies or paired bottle assemblies, described in the general methods section.

4. Inoculation technique.

Petri dishes containing 20 ml aliquots of 1% malt agar were inoculated centrally with mycelium and incubated for 1 to 2 days at 20°C. The surface of colonies was then examined using a x100 magnification on a Watson binocular microscope and 4.0 mm discs containing no recognizable gametangial initials were cut from the edge of the colonies using a cork borer. One each of these discs was placed centrally on to the surface of 20 ml aliquots of 1% malt agar in petri dishes or boiling tubes, 70 ml aliquots in 12 oz. bottles, or 120 ml aliquots in

Roux bottles. By this procedure, colonies were purely vegetative at the time of inoculation, and colonies formed were of regular shape.

5. Control of environment.

Cultures were grown in incubators at chosen temperatures. Changes in temperatures in incubators were recorded on thermographs throughout each assay period.

6. Number of zygospores.

In assay colonies grown in petri dishes, two 20 mm wide transects on previously marked diameters at right angles to each other were examined under x100 magnification. The average of the numbers of zygospores on the two transects was recorded.

For assay colonies grown in bottles, the number of zygospores on a 1 cm wide transect removed from the centre of the colony was recorded.

For assay colonies grown in boiling tubes, the total number of zygospores produced was recorded.

7. Gas chromatographic analysis.

This was carried out on samples from Roux bottle cultures using the medium and inoculation methods described in the General Methods, and also from paired assemblies. Gas samples were taken from vessels incubated as described in subsequent sections. G.L.C. traces from these were compared with traces produced by similar samples from bottles containing

uninoculated culture medium.

Carbon dioxide and oxygen were examined as described in the General Methods.

Compounds other than these were analysed on the Carbowax 20M column and the DNP column described in Section IA text Table 2 under the conditions given there.

Experimental.

1. Re-examination of Hawker's findings.

Introduction.

A preliminary examination of Hawker and Hepden's findings was carried out by:-

a) Comparing the number of zygospores produced after 8 days incubation at 10°C with the number produced at 20°C.

b) Examining the effects of exposing cultures grown at 10°C to gases produced by cultures incubated at 20°C.

Methods.

Tests were carried out with petri dish cultures using the methods described above; details are given on the relevant tables below.

Results.

a) Appendix Table LXII gives the numbers of zygospores produced in

petri dish colonies incubated at 10°C and 20°C for 8 days. The results are summarized in text Table 21.

Table 21. Number of zygospores in petri dish cultures grown at 10°C and 20°C.

Expt. no.	Mean number of zygospores per transect[+] 10°C	20°C	Least[*] significant difference between means
I	14.1	135.7	19.4
II	17.5	147.0	26.0
III	13.7	159.3	21.4

[+]Six replicate petri dishes cultures were set up for each treatment in each experiment, as described in the methods.

[*]P = 0.05

b) Appendix Table LXIII gives the number of zygospores produced in assay colonies of R. sexualis grown at 10°C in paired petri dish assemblies containing 2 or 5 day old cultures grown at 10°C paired with cultures of the same age grown at 20°C and in controls, paired with uninoculated 1% malt agar. Measurements given were made after 10°C colonies had been incubated for a total of 12 days at 10°C. These results are summarized in text Table 22.

Table 22. Mean numbers of zygospores produced in paired petri dish assemblies by assay colonies of R. sexualis paired with 2 and 5 day old mature colonies of the same species and in controls, paired with uninoculated 1% malt agar.

Expt. no.	Treatment	Mean of average number of zygospores per transect+ Test fungus present	Test fungus absent	*Least significant difference between means
I	Paired with 2 day culture	12.7	21.9	14.4
	Paired with 5 day culture	43.1	16.9	
II	Paired with 2 day culture	2.6	4.5	7.5
	Paired with 5 day culture	11.7	0.0	
III	Paired with 2 day culture	0.0	0.0	0
	Paired with 5 day culture	0.0	0.0	

+Six replicate pairs of petri dish assemblies were set up for each treatment in each experiment, as described in the methods.

* P = 0.05

The presence of "pseudophores", first described by Callen (1940) was confirmed in colonies grown at 10°C in tests in sections (a) and (b) above.

Discussion.

The effects of low temperature in relation to zygospore production

and pseudophore formation were generally similar to those observed by Hepden & Hawker (1961). There was some quantitative difference, in that here some mature zygospores were produced at 10°C though the total number was very greatly less than that produced at the higher temperature.

The stimulation effect described by them could be demonstated with 5 day old cultures in the first two of three trials, both of which produced zygospores in test and control treatments, but in the third trial, no zygospores were produced in either treatment.

This lack of consistent behaviour i.e. a change in the stimulation effect, correlated with a change in zygospore production at 10°C, was the most significant point observed in these tests. It was therefore decided to investigate the factors underlying this change in behaviour. The remainder of this report deals with this. The factors possibly contributing to this were examined as follows:-

a) Investigation of possible changes in the ability of the fungus to form zygospores at particular temperatures.

b) Investigation of possible changes in ability to produce the stimulant.

c) Investigation of possible changes in response to this stimulant.

2. Investigation of the factors underlying the change in behaviour observed in initial tests.

a) Investigation of possible changes in the ability of the fungus to form zygospores at particular temperatures.

Introduction.

It is well known that changes in behaviour of fungal cultures can occur during subculturing. In the first experiments carried out in October and November, 1967 with this strain, zygospores were produced consistently at 10°C, but by December, 1967 they were produced only erratically in assay colonies at this temperature although production at 20°C was still abundant. The following tests were therefore carried out to investigate this possibility, that the behaviour of the fungus had changed during these investigations.

Methods.

This was examined by measuring the amount of zygospore production in standard conditions by three culture strains, each derived from Hawker's strain and comparing the results with known history of these strains. Culture strains were as follows:-

Strain A. Last subcultured November, 1967. Recultured and measured, June to July, 1968.

Strain B. " " March, 1968 " " " "

Strain C. " " June, 1968 " " " "

Results.

Appendix Table LXIV gives the number of zygospores produced in petri dish cultures at 10°C, 11°C, 12°C, 13°C, 14°C and 20°C. These results are summarized in text Table 23.

Table 23. Results of statistical analyses on number of zygospores produced by colonies of R. sexualis at standard temperatures in three replicate experiments.

Strain	Temperature treatment	Expt. I Mean	Expt. I L.S.M.D.	Expt. II Mean	Expt. II L.S.M.D.	Expt. III Mean	Expt. III L.S.M.D.
A	10°C	0.0	23.2	0.0	21.3	0.0	29.7
	11°C	0.0		0.0		0.0	
	12°C	10.0		11.3		9.3	
	13°C	16.5		19.2		46.3	
	14°C	74.0		89.3		44.5	
	20°C	155.3		131.2		139.8	
B	10°C	0.0	17.6	0.0	14.5	0.0	13.2
	11°C	0.8		0.0		0.0	
	12°C	8.7		5.0		2.7	
	13°C	7.8		31.7		4.3	
	14°C	57.5		31.3		32.8	
	20°C	156.3		168.3		153.8	
C	10°C	0.0	22.2	0.0	19.1	0.0	17.9
	11°C	0.8		0.0		0.0	
	12°C	2.7		1.5		2.7	
	13°C	3.5		30.3		22.3	
	14°C	22.2		57.3		41.7	
	20°C	155.8		170.3		164.7	

L.S.M.D. = Least significant difference between the mean of the average number of zygospores produced at each temperature in each experiment. (P = 0.05).

Discussion.

A change in behaviour had taken place; none of these culture strains showed the behaviour observed in the initial tests i.e. none produced zygospores at 10°C. The minimum temperature supporting zygospore production in these tests was 11-12°C, similar to that observed by Hepden & Hawker (1961). Culture strain C had changed most i.e. that strain which had been subcultured most.

An additional point of interest arose in this investigation, that differences of 1°C in the critical range may have a very big effect on zygospore production. There were large differences between the numbers of zygospores recorded at any one temperature in different experiments. Analysis of between experiment variance and between plate variance within each experiment (appendix Table LXV) shows that these are substantially less than between treatment variance in all critical cases. The differences therefore appear to be due either to variation in the behaviour of the fungus, or to sensitivity to other variables, or to sensitivity to smaller temperature changes than those recorded in the apparatus used. These possibilities are examined further in the general discussion at the end of this Section.

The possibility that the failure of the fungus to produce zygospores at 10°C could alone be responsible for the failure to demonstrate stimulation at this temperature was investigated. This was done running tests with mature colonies in paired petri dishes similar to those described in paragraph 1, but at an incubation temperature of 13°C, a temperature shown to permit zygospore production.

Results.

Zygospore production was not stimulated in the presence of mature colonies in any of three replicate experiments each consisting of six test and six control assemblies (see appendix Table LXVI).

Discussion.

This suggests that change of the minimum temperature supporting zygospore production alone is not responsible for the observed correlation in failure to demonstrate the stimulation effect and hence that other factors are likely to be responsible.

b) Investigation of possible changes in ability to produce the stimulant.

Introduction.

Little actual analysis of the culture gases had been carried out in the past. Hepden & Hawker (1961) found no correlation between stimulation of zygospore production and levels of carbon dioxide and ethanol. It seemed appropriate therefore to reinvestigate Hepden & Hawker's findings by G.L.C.

Methods.

Since it was appreciated that the paired petri dishes gave little control of gas exchange with the environment, gases from the following assemblies were also examined in addition to the assemblies used in the initial tests described in paragraph 1 of this Section.

a) Paired petri dishes sealed together with sellotape.

b) Paired bottle assemblies (both Roux and 12oz. bottle pairs).

c) Sealed assemblies at 10°C aerated by a constant flow of gases from cultures grown at 20°C.

d) Assemblies in which condensates of culture gases were added. The condensates were prepared by passing gases from a culture producing zygospores through a trap cooled in solid carbon dioxide.

Results.

Carbon dioxide and oxygen.

There were no consistent changes in the amounts of these metabolites produced in successive experiments and no correlation between the levels detected and zygospore production.

Other metabolites.

The only metabolites identified consistently were acetaldehyde and ethanol. In most tests, no zygospores were produced. Where they were produced, there was no correlation with the amounts of metabolites detected. This applied even where levels of these metabolites were as high as x10 those detected in the paired petri dish assemblies in the initial tests described in paragraph 1 of this Section, where stimulation was demonstrated.

Three replicate experiments, each with a range of concentrations of authentic acetaldehyde or ethanol showed no correlation between zygospore production and concentrations of these metabolites. Because

no stimulation could be demonstrated here and because of Hepden & Hawker's observations, this line was not carried further.

c) Investigation of possible variation in response to the stimulant.

By this stage, it was apparent that stimulation by the culture gases no longer could be demonstrated consistently in any conditions. It was also well appreciated that the factor might not be a chemical one, but a physical one of some sort e.g. slight changes in temperature or humidity in the different treatments. Hence, the reason for the failure to produce zygospores in any test could not be specifically attributed either to changes in the reaction of the fungus to the stimulant or to differences in the level of stimulant present. It was thought, therefore, that another useful line of attack would be to examine the possibility of inducing an increase in the numbers of zygospores produced in a condition in which zygospores would be formed anyway. It was appreciated that negative results would not advance the investigation, but that positive ones would give useful information for analysis.

i) Examination for the occurrence of the stimulation effect at 20°C.

Introduction.

Hepden & Hawker (1961) and Martin (1963) reported the occurrence of the stimulation effect in assay colonies of R. sexualis grown at 20°C in the presence of gases produced by similar colonies grown at 20°C, compared with controls, in the presence of gases from uninoculated agar. They

make no distinction between the factors considered to be producing stimulation at 10°C and those acting at 20°C. Obviously these cannot be assumed to be the same. However, with these reservations in mind, the following tests were carried out.

Methods.

Paired petri dish assemblies were used as described before. Assemblies were paired immediately after inoculation of cultures and were incubated for 12 days at 20°C. Tests were carried out with assemblies bound with rubber bands and also in assemblies sealed with sellotape, for reasons discussed above.

Results.

Appendix Table LXVII gives the results of these tests. The differences in the numbers of zygospores produced in test and control treatments were not significant in any case.

Discussion.

These results indicate that there may have been a change in response of the fungus to the stimulant effective previously at 20°C. The possibility that the stimulant was no longer being produced cannot, however, be eliminated.

ii) Examination for the presence of the stimulation effect with gaseous metabolites from mature cultures of three species of Zygomycete.

Introduction.

Another line of approach was to see if the failure to react occured

only with R. sexualis, but could still occur with other species.

Hepden & Hawker (1961) reported the occurrence of stimulation at 7°C and 20°C in the presence of sporulating cultures of Phycomyces blakesleeanus and at 20°C with other sporing species of the Mucorales. Martin (1963) demonstrated stimulation at 20°C with several species of Zygomycete including Mucor plumbens, Absidia spinosa and Rhizopus stolonifer. These last three species were examined here. The same reservations concerning the nature of the stimulant are made here as in the last experiment.

Methods.

Test species were grown on 20 ml aliquots of potato dextrose agar. Tests were carried out at 10°C and 13°C as described before.

Results.

No consistent production of zygospores was observed in any treatment at 10°C. Only one assembly in any of three replicate experiments produced a few zygospores.

Appendix Table LXVIII gives the results of tests at 13°C. The results of the three replicate experiments are summarized in text Table 24.

Table 24. Numbers of zygospores produced at 13°C by assay colonies of *R. sexualis* in paired petri dish assemblies with mature colonies of *M. plumbens*, *A. spinosa* and *R. stolonifer* and in controls with uninoculated potato dextrose agar.

Expt. no.	Mean of number of zygospores per transect+				Least significant difference between means		
	Test fungus present			Test fungus absent			
	M.plumbens	*A.spinosa*	*R.stolonifer*		*M.plumbens*	*A.spinosa*	*R.stolonifer*
I	94.5	29.9	34.7	17.8	15.5	23.9	19.9
II	94.0	84.0	120.9	59.0	12.8	15.8	8.8
III	92.0	78.1	84.0	54.7	23.2	23.2	16.1

+Six assemblies were set up for each treatment in experiments I and II and at least four per treatment in experiment III.

Examination of the metabolites present at the end of biological tests showed ethanol and acetaldehyde in concentrations about twice those observed in initial tests with *R. sexualis*. A few other small peaks were observed, but not identified.

Discussion.

These results suggest that *R. sexualis* can still respond to a stimulant at temperatures permitting zygospore production, but not at 10°C, a temperature at which no zygospores were produced in these tests. This may indicate that it is the production of a stimulant which has changed in *R. sexualis*. However, the stimulatory principle active in these tests may differ qualitatively or quantitatively from the factor producing

stimulation in initial tests. Hence the possibility that assay colonies of R. sexualis may have changed in response to the stimulant produced by R. sexualis cannot be eliminated. These tests could, however, provide a tool for analysis of the stimulant to which R. sexualis is still sensitive.

General conclusions.

The stimulant has not been identified by this investigation.

It has added only two significant points to previous knowledge i.e. that the zygospore production may be affected by very slight changes in temperature and that the minimal temperature for zygospore production may change during subculturing. Both these factors could affect the variability of the results seen here although there is evidence that the second is not alone responsible in tests with R. sexualis, but may affect stimulation in tests with other Zygomycetes. However, both these factors should be considered in the interpretation of older records e.g. the possibility of slight temperature changes being introduced by any continuous aeration system seems particularly relevant.

SECTION IV

THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES IN THE SOIL.

SECTION IV. THE BIOLOGICAL ACTIVITY OF VOLATILE METABOLITES IN THE SOIL.

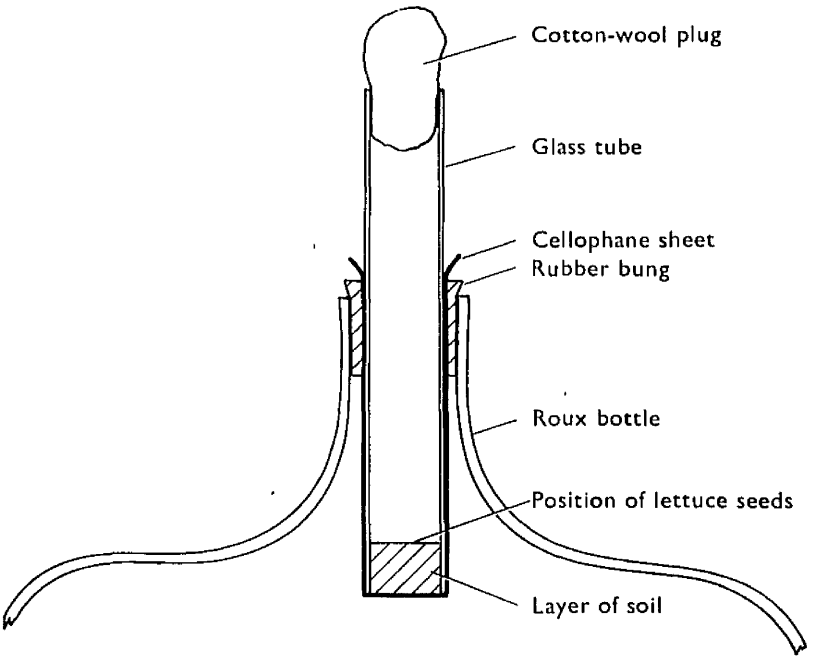
Introduction.

The results reported in Sections IB and IC of this thesis showed that under certain conditions the culture gases of *F. scutellatus* and *F. noxius* could affect the growth of other organisms. It is obviously possible to suggest many situations in nature where such products may have an ecological effect. Investigations on these effects are very limited (see review of literature). This report contains the results of a brief study of the effects of the culture gases of *F. scutellatus* and *F. noxius* on the growth of lettuce in three soils.

Methods.

Text Figure (xxxv) shows the assay assemblies used throughout. The assay tube consists of a six inch length of glass tubing (diameter 10 mm) fitted at one end with a cotton wool plug and with a cellophane disc covering the other. The cellophane disc is held in position with a rubber band. One of three standard soil types, peat, peralite or sand was poured into assay tubes to a depth of 6-7 mm, 4-5 mm, 4-5 mm for the three types respectively. Assay tubes were autoclaved at 120°C for 20 minutes.

Figure (xxxv). Apparatus used to assay the effects of culture gases on lettuce growing on soil.



Six surface sterilized lettuce seeds were inoculated on to the moistened surface of each soil type and the tubes were fitted into the necks of Roux bottles containing test fungus or uninoculated growth medium. Text Table 25 gives the germination of seeds in soil assay tubes of each type, paired with bottles containing uninoculated 5% malt agar and incubated at 19°C ± 2° under warm-white fluorescent light for seven days.

Table 25. Germination of lettuce seeds in a typical experiment with soil tube assemblies.

Soil type	Assembly no.	Number of seeds germinated out of 6 planted
Peat	1	5
	2	6
	3	1
Peralite	1	6
	2	3
	3	5
Sand	1	2
	2	0
	3	0

In this test and in many replicate trials, germination was poor. It was thought that desiccation might be a factor involved. To examine this further, capillary tubes containing water were inserted into the soil assay tubes and rested on the soil surface. However, this did not overcome the problem of desiccation during the first night of incubation. Rather than investigate this further at the time, the difficulty was avoided by using pregerminated seeds in these tests as follows:-

Surface sterilized seeds were inoculated on to the surface of sterile moist filter papers in petri dishes until the radicle was 1 to 3 mm long. Four germinated seeds were then placed on to the moistened surface of the soil in assay tubes. Soil assay tubes were paired with bottles and incubated as described above.

Carbon dioxide was measured as described in the General Methods paragraph 2(c).

Experiments were replicated suitably throughout the investigations.

Particular methods used for F. scutellatus or F. noxius are described in the appropriate places below.

1. F. scutellatus.

Methods.

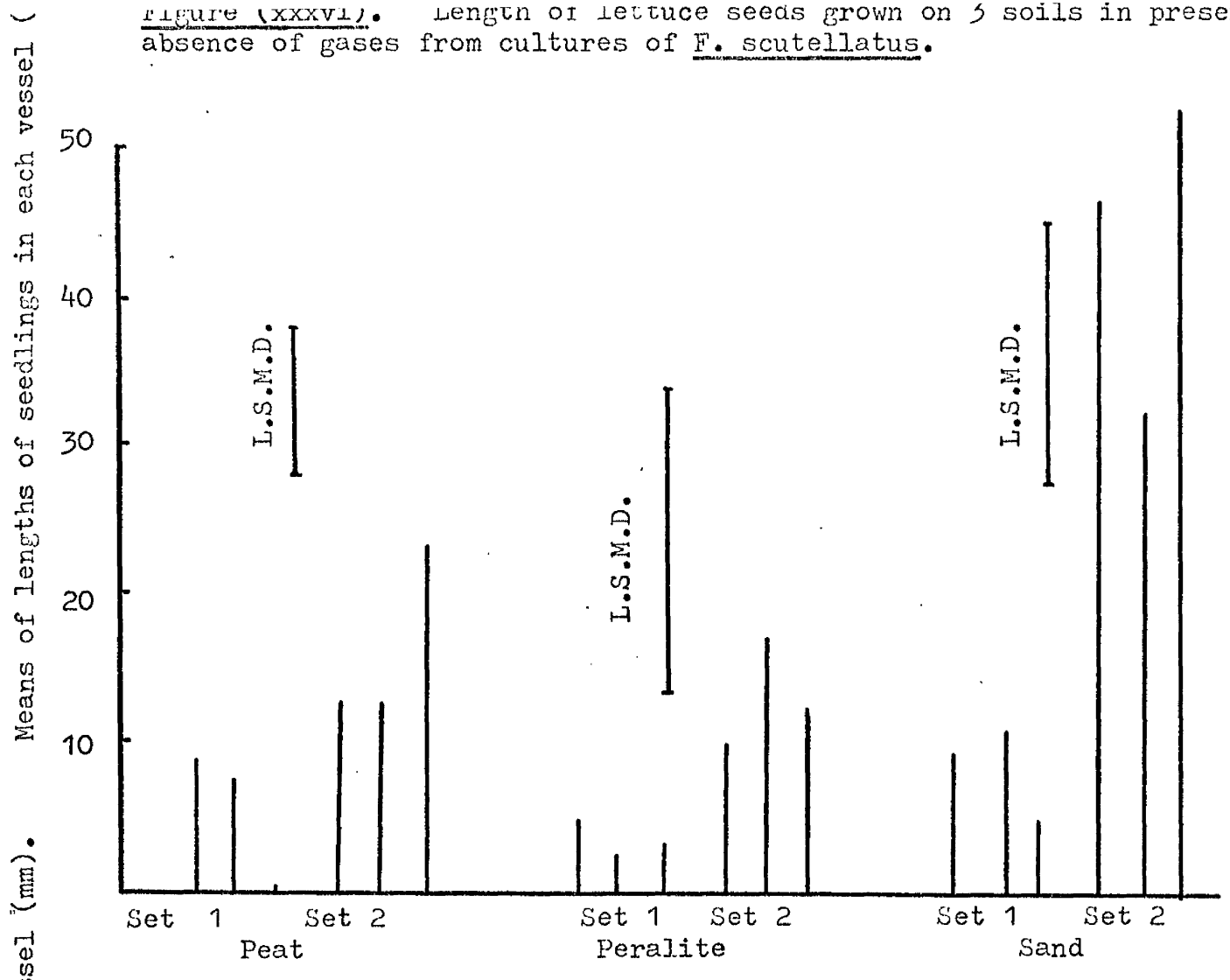
As above except that examination for HCN in the gases above the soil in the soil tubes was made using the test described in Section IB.

Results.

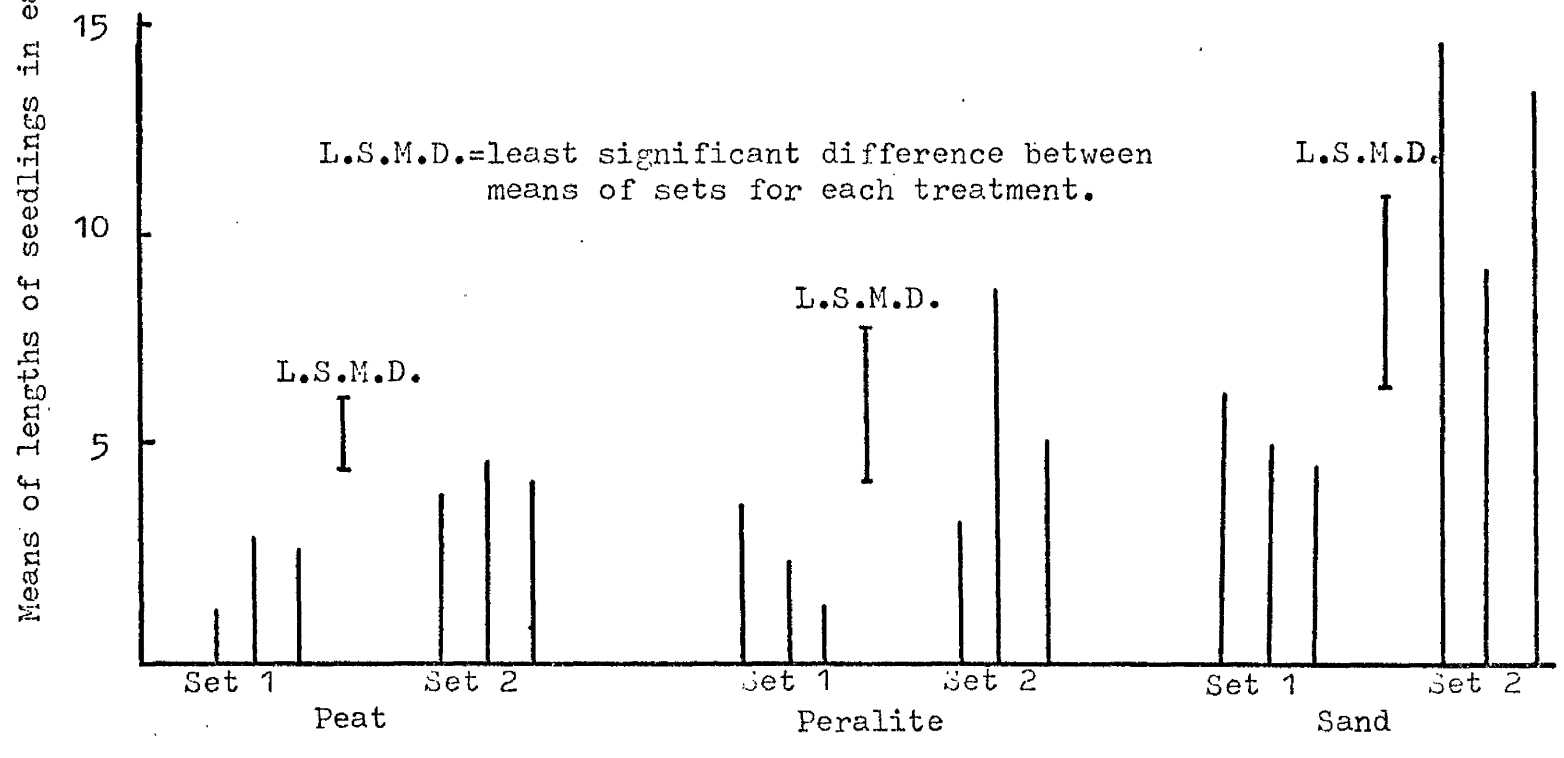
Text Figure (xxxvi) gives the growth of lettuce seedlings in assemblies containing lettuce paired with F. scutellatus cultures and in controls, paired with uninoculated 5% malt agar in a typical experiment.

In this test, the growth of the lettuce seedlings was significantly reduced in the presence of F. scutellatus culture gases compared with that in controls. The results of this and two replicate experiments are given in appendix Table LXIX and are summarized in text Table 26. They agree closely with those in Figure (xxxvi), apart from in one test on peralite where inhibition was not significant. This deviation was not investigated more closely at this stage.

Figure (xxxvi). Length of lettuce seeds grown on 3 soils in presence or absence of gases from cultures of F. scutellatus.



Figure(xxxvii). Lengths of lettuce seedlings grown on 3 soils in assemblies containing lettuce paired with F. noxius cultures and in controls paired with uninoculated 5% malt agar.



4 lettuce seeds were planted in each vessel for each soil type, set 1 were paired with F. noxius cultures, set 2 were paired with uninoculated 5% malt agar. Similarly with F. scutellatus in Figure (xxxvi).

Table 26. Differences in growth of lettuce seedlings in three soil environments in assemblies with F. scutellatus cultures expressed as a percentage of the growth in control assemblies with uninoculated 5% malt agar.

Soil type	Expt. no.	Mean shoot lengths: Observed difference (%)	Mean shoot lengths: *LSMD (%)	Mean root lengths: Observed difference (%)	Mean root lengths: LSMD (%)	Mean total lengths: Observed difference (%)	Mean total lengths: LSMD (%)
Peat	I	67.2	62.9	46.9	36.8	67.5	47.3
	II	60.0	58.8	89.6	13.5	72.7	67.4
	III	87.9	44.0	31.3	41.7	61.5	36.3
Peralite	I	41.4	24.7	40.5	59.6	62.7	50.8
	II	94.6	64.0	95.9	70.6	94.5	64.0
	III	37.8	13.7	42.5	62.3	35.8	75.5
Sand	I	78.2	56.4	84.6	35.3	42.3	41.8
	II	75.9	42.2	87.4	72.1	81.7	39.9
	III	76.4	74.4	96.1	86.0	85.2	84.8

*LSMD = Least significant difference between means, (P=0.05).

The carbon dioxide concentrations measured in the many assemblies examined containing F. scutellatus or 5% malt agar were within the range of 0.02% to 0.04% volume/volume. The range in oxygen concentrations was no greater than 18.6% to 23.9% volume/volume in any assemblies.

Hydrogen cyanide was detected in the atmosphere above the soil in assemblies containing F. scutellatus cultures.

Discussion.

The carbon dioxide concentrations detected were well within the range shown to have no inhibitory effect on the growth of lettuce

(cf. Section IB, paragraph 2). Changes in oxygen concentration were again considered trivial and their possible effects were not investigated.

The shoot and root growth were inhibited to a similar extent in these assemblies despite the greater proximity of the roots to the culture gases, (cf. text Table 26). Also, the inhibition in these tests compares closely with that in tests in paired bottle assemblies, reported in Section IB, suggesting that the soil barrier had little effect in reducing the activity of the culture gases.

Thus, these brief investigations showed that, under certain conditions, the culture gases of this species could affect the growth of lettuce separated from the culture by an air space and a layer of moist soil. Although, in these tests, no quantitative work on the build up of concentrations of HCN has yet been done, it does seem probable from the results reported in Section IB that the inhibitory effect is due to the activity of HCN. These observations may have some relevance in connection with fairy ring disease and snow mould of alfalfa mentioned in the discussion of Section IB. However, the significance of the role of gaseous HCN as opposed to HCN diffusing in solution must remain a matter for speculation until further experimental information is available.

2. *F. noxius.*

Methods.

As above except that the gases in assemblies were examined by G.L.C. analysis using a Carbowax 1500 column under the conditions described in Section IA, methods paragraph 5.

Results.

a) Examination of growth of lettuce seedlings.

The results are recorded in appendix Table LXX, summarized in Table 27 and illustrated in text Figure (xxxvii).

Table 27. Differences in growth of lettuce seedlings in three soil environments in assemblies with *F. noxius* cultures expressed as a percentage of the growth in control assemblies with uninoculated 5% malt agar.

Soil type	Expt. no.	Mean shoot lengths		Mean root lengths	
		Observed difference (%)	*LSMD (%)	Observed difference (%)	LSMD (%)
Peat	I	20.7	79.0	24.3	39.0
	II	12.8	71.5	52.2	23.9
	III	54.4	28.6	94.0	43.3
Peralite	I	4.3	53.0	60.7	64.3
	II	13.3	50.9	52.7	48.7
	III	15.5	91.0	75.5	10.2
Sand	I	91.9	70.4	57.4	39.3
	II	25.0	63.0	64.8	35.9
	III	32.0	79.0	79.8	35.7

*LSMD = Least significant difference between means, (P = 0.05).

In the test illustrated in Figure (xxxvii) the root lengths were significantly reduced in the assemblies with F. noxius cultures. The results of the two replicate experiments were closely similar to this except in one test with peat (experiment III) where root length was not significantly reduced in the F. noxius treatment. This deviation was not investigated further at this stage. In all tests, the roots of seedlings showed the characteristic symptoms of F. noxius inhibition described in Section IA results paragraph 1(a) and Section IC paragraph 1.

b) Analysis of carbon dioxide and oxygen.

The carbon dioxide concentrations measured in the many assemblies examined containing F. noxius or 5% malt agar were within the range 0.018% to 0.032% volume/volume. The range in oxygen concentration was from 19.0% to 22.8% volume/volume in assemblies with F. noxius cultures and in control assemblies.

c) Analysis of other metabolites.

Traces of acetaldehyde, ethanol, isobutanol and pentanol were detected in the atmosphere above the lettuce seedlings in assemblies containing F. noxius cultures.

Discussion.

The carbon dioxide concentrations differed little from atmospheric and were well within the range shown to have no inhibitory effect on the

growth of lettuce (cf. Section IB). Changes in oxygen concentration were again considered trivial and their possible effects were not further investigated. As in tests with F. scutellatus, the presence of a layer of soil had little effect on the extent or nature of the inhibitory effect (cf. text Figure (i) (b) and (c) and text Table 27).

It is thought that the above observations on the growth of lettuce under these conditions may have some relevance in connection with the pathogenicity of F. noxius mentioned in the discussion in Section IC.

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A P P E N D I X

Table I.

Fomes species examined for the production of volatile metabolites:

Fungus.	Origin.
F. Cleland Lloyd.	+C.B.S.
F. connatus (Weinm) Gill.	C.B.S.
F. conchatus (Pers. ex Fries) Gill.	*F.P.R. 142.
F. dependens (Murr.) Sacc. et Trott.	C.B.S.
F. fastuosus Lév.	F.P.R. 132.
F. ferruginosus (Schrad) Massee Nobles.	C.B.S.
F. fomentarius (L. ex Fries) Kickx. Noble.	C.B.S.
F. fraxineus (Bull. ex Fries) Cooke.	F.P.R. 17B.
F. hartigii All. Hubert.	C.B.S.
F. hemitephrus Berk.	C.B.S.
F. ignarius (L.) Gillet Lohwag.	F.P.R. 97B.
F. juniperinus v. Schrenk.	F.P.R. 158.
F. lignosus Klotzsch. Müller.	F.P.R. 202A.
F. melanoporus Mont.	F.P.R. 283.
F. nigricans (Fr.) Lloyd.	C.B.S.
F. nigrolimitatus (Rommell) Egeland.	C.B.S.
F. noxius Corner.	F.P.R. 224A.
F. noxius Corner.	C.B.S. 170.32.
F. occidentalis Overh.	C.B.S.
F. officinalis Lloyd.	F.P.R. 81B.
F. pinicola (Swartz. ex Fries) Cooke.	F.P.R. 98A.
F. pinicola (Swartz. ex Fries) Cooke.	F.P.R. 98B.
F. pomaceus (Pers.) Lloyd.	F.P.R. 33A.
F. pseudo-senex (Murr.) Sacc. et Trott	F.P.R. 190.
F. ribis (Schum. ex Fries) Gill.	F.P.R. 42.
F. rimosus (Berk.) Cooke.	F.P.R. 180.
F. robustus Karst..	F.P.R. 139A.
F. scutellatus (Schw.) Cooke.	F.P.R. 181.
F. sub-roseus (Weir) Overh..	F.P.R. 301.
F. torulosus (Per.) Lloyd.	F.P.R. 222.
F. ulmarius Lloyd.	F.P.R. 241A.
F. ulmarius Lloyd.	F.P.R. 241B.
F. vinosus Berk.	F.P.R. 143.

+ = Centraalbureau voor Schimmelcultures, Baarne.

* = Forest Products Research Laboratories.

Germination of lettuce seeds and growth of germinated seedlings in assemblies containing lettuce paired with *Fomes* cultures and in controls paired with uninoculated 5% malt agar.

Treatment	% germination — Assembly no. 1	2	3	4	5	6	Mean	Mean lengths of shoots of germinated seedlings (mm) — Assembly no. 1	2	3	4	5	6	Mean	Mean lengths of roots of germinated seedlings (mm) — Assembly no. 1	2	3	4	5	6	Mean
F. Clelandi	100	100	100	90	90	100	96.7	16.9	14.7	12.1	13.9	14.3	12.3	14.0	11.0	18.6	18.0	14.9	19.7	14.9	16.2
Control	100	100	100	100	100		100	13.2	12.0	10.8	14.1	13.4		12.7	18.4	14.1	14.3	16.8	15.3		15.8
F. conchatus	100	90	100	90	100		96.0	12.4	10.0	17.6	15.8	11.7		13.5	25.7	23.0	21.8	27.0	32.7		26.0
Control	100	90	100	100	100	100	98.3	12.4	8.0	12.4	10.0	11.2	9.6	10.6	24.7	16.0	25.3	23.9	22.7	18.0	21.8
F. conchatus	100	90	100	100	100	100	98.3	16.0	16.1	19.4	14.7	19.2	14.6	16.7	27.7	22.9	24.9	22.3	25.2	26.7	24.9
Control	90	100	100	100	90	100	96.7	16.2	16.0	13.8	13.2	13.1	10.2	13.7	26.9	26.8	21.9	24.4	24.9	27.4	25.4
F. conchatus	80	100	90	100			92.5	19.6	22.7	16.1	18.5			19.2	26.5	35.7	22.2	31.9			29.1
Control	100	100	100	100			100	13.8	17.3	18.4	20.6			17.5	24.5	27.9	23.7	33.1			27.3
F. connatus	100	100	90	100	100	100	98.3	14.9	13.9	13.6	15.4	15.4	13.0	14.4	15.7	22.1	23.8	22.8	21.5	23.6	21.6
Control	100	100	90	100	100	100	98.3	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. dependens	100	90	90	100	100	100	96.7	8.1	9.6	10.0	13.6	11.8	8.0	10.2	17.4	15.6	14.5	12.3	20.2	18.2	16.4
Control	90	100	100	100	100		98.0	13.2	12.0	10.8	14.1	13.4		12.7	18.4	14.1	14.3	16.8	15.3		15.8
F. fastuosus	100	100	100	100	100	100	100	12.6	12.5	12.6	11.4	18.0	18.5	14.3	21.0	17.7	24.0	18.4	27.4	29.9	23.1
Control	100	100	100	100	90		98.0	11.3	11.6	12.6	12.1	16.8		12.9	27.6	15.9	21.5	24.6	37.2		25.4
F. fraxineus	90	90	100	100	100	90	95.0	12.8	16.0	12.6	20.4	12.9	16.0	15.1	23.0	30.1	26.7	32.6	23.6	30.4	27.7
Control	90	100	100	100			97.5	20.8	14.1	19.6	19.8			18.6	31.7	24.7	28.2	35.1			29.9
F. fomentarius	80	90	100	90			90.0	17.9	24.0	17.1	23.2			20.5	22.4	22.8	23.3	26.2			23.7
Control	100	100	100	90	100	90	96.7	17.7	20.2	21.4	14.1	17.0	14.1	17.4	19.1	25.8	27.5	25.0	19.8	28.0	24.2
F. hartigii	100	100	100	100	100	100	100	13.4	17.7	12.5	13.8	11.9	16.9	14.4	15.1	19.0	18.9	16.6	18.2	22.8	18.4
Control	100	100	100	100	100	100	100	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. hemitephrus	100	100	100	100	100	90	98.3	12.5	17.6	25.3	13.8	14.1	17.4	16.8	18.2	28.2	30.5	17.5	15.3	27.4	22.9
Control	100	100	100	100	100		100	16.2	16.1	18.0	14.6	20.6	13.2	16.4	18.5	24.0	16.2	24.4	25.8	13.6	20.4
F. ignarius	100	90	100	90	100	100	96.7	10.3	18.6	14.3	13.2	12.2	14.5	13.9	12.3	18.6	16.1	12.3	12.6	12.7	14.1
Control	90	100	100	100	100		98.0	13.2	12.0	10.8	14.1	13.4		12.7	18.4	14.1	14.3	16.8	15.3		15.8
F. juniperinus	100	90	100	100	100	90	96.7	14.1	17.1	14.7	16.9	18.9	16.1	16.3	27.9	31.0	24.0	23.3	30.6	29.2	27.7
Control	100	100	100	100	100	90	98.0	15.3	21.0	15.3	15.3	16.9	16.7	16.8	27.3	22.1	23.0	25.0	25.1	26.4	24.8
F. lignosus	100	100	100	100	100	100	100	14.5	17.1	16.2	16.6	15.3	13.9	15.6	17.0	19.1	18.3	19.7	21.6	16.2	18.6
Control	100	100	100	100	90	100	98.3	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. melanoporus	100	100	100	100	90	100	98.3	13.7	15.0	18.4	15.8	13.3	16.3	15.4	15.7	14.9	17.3	22.8	20.0	19.1	18.3
Control	100	100	100	90	100	100	98.3	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. nigrolimitatus	100	100	100	100	100	90	98.3	14.9	12.6	11.2	15.3	13.6	11.3	13.2	27.5	21.7	19.9	22.5	21.8	20.4	22.3
Control	100	100	100	90	100	90	96.7	9.1	11.1	14.8	12.1	10.4	11.2	11.4	23.6	23.0	26.8	19.9	22.5	24.6	23.4
F. noxius 224A	100	90	80	50	90	70	80.0	16.1	12.8	17.2	9.5	8.9	9.6	12.3	7.9	7.6	10.6	6.8	8.0	12.0	8.8
Control	100	100	100	90	100	100	98.3	16.6	16.6	17.0	18.1	13.2	12.9	15.7	29.7	19.9	29.0	29.3	25.0	22.2	25.9
F. noxius 224A	100	100	100	100	90	100	98.3	23.0	25.4	23.6	22.1	26.1	23.3	23.9	18.0	14.7	16.0	19.1	14.8	19.1	16.9
Control	90	100	90	100	100	100	96.7	25.4	24.9	19.8	14.1	18.2	20.3	20.4	23.7	23.9	25.6	25.5	20.7	26.7	24.3
F. noxius 224A	100	100	100	100	90	100	98.3	17.2	11.7	11.2	14.8	13.7	22.3	15.2	4.6	2.7	2.9	3.7	3.2	7.2	4.1
Control	100	90	100	100	100	100	98.3	14.0	8.4	13.7	10.1	9.8	15.0	11.8	23.1	19.2	19.8	19.9	17.0	17.0	19.3
F. noxius 170.32	80	100	100	100	100	100	96.7	7.9	11.1	7.5	13.4	10.6	9.7	10.0	11.5	16.2	13.5	19.0	21.0	12.2	15.6
Control	80	100	100	90	100	100	95.0	10.8	9.9	10.0	7.6	9.9	9.3	9.6	20.5	23.7	24.5	18.8	20.6	20.1	21.3
F. noxius 170.32	90	100	90	100			95.0	13.0	13.0	18.7	14.4			14.8	10.4	13.0	22.0	19.1			16.1
Control	80	100	100	100	100		96.0	15.2	15.6	15.6	12.8	16.3		15.1	15.9	21.4	23.6	22.3	21.3		20.9
F. noxius 170.32	100	100	90	100	100	90	96.7	8.3	7.7	8.9	8.3	7.8	11.4	8.7	20.5	18.7	26.3	18.6	29.6	19.2	22.1
Control	90	90	100	100			95.0	7.6	8.4	7.7	7.0			7.7	18.0	24.3	26.8	18.8			21.9
F. officinalis	100	100	100	100	100	100	100	21.3	12.8	13.8	11.3	16.3	14.3	15.0	31.3	18.3	23.5	23.9	22.7	22.8	23.7
Control	90	100	100	100	100	100	98.3	17.0	17.4	21.6	17.8	16.2	16.8	17.8	18.4	20.7	17.1	20.6	14.0	18.0	18.1
F. officinalis	80	80	100	100			90.0	14.4	8.6	7.3	10.0			10.1	17.8	15.5	12.7	15.3			15.3
Control	60	90	90	100			85.0	11.2	12.7	14.8	11.6			12.6	13.5	20.6	23.3	15.9			18.8
F. officinalis	100	100	100	100	90	90	96.7	21.4	16.5	18.6	14.6	14.7	18.0	17.3	20.4	21.8	21.2	21.5	15.7	29.8	21.7
Control	100	100	100	90	100		98.0	17.7	18.8	15.6	17.1	17.4		17.3	25.5	31.6	24.2	26.1	23.0		26.1
F. pinicola 98A	100	90	100	90	100	100	96.7	28.5	23.7	28.3	24.3	27.8	25.1	26.3	24.3	37.1	29.3	24.6	25.9	28.2	28.2
Control	100	100	90	90			95.0	20.6	18.7	21.3	21.6			20.5	50.7	37.8	49.3	49.6			46.9
F. pinicola 98A	90	100	100	100	100		98.0	17.0	20.0	15.7	12.7	13.3		15.7	21.9	30.0	22.2	19.3	18.4		22.4
Control	100	100	100	100			100	12.2	14.9	12.5	12.6			13.1	20.4	23.8	20.1	23.8			22.0
F. pinicola 98A	100	100	100	100	90	90	96.7	13.8	12.8	16.0	15.0	16.6	9.7	14.0	18.9	18.7	18.9	21.1	16.0	14.0	17.9
Control	100	100	100	90	100	100	98.3	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. pinicola 98B	100	100	90	100	100	100	98.3	14.2	22.8	16.0	12.5	15.0	11.2	15.3	15.2	19.8	15.6	15.2	19.0	15.1	16.7
Control	100	80	100	90	100	100	95.0	16.2	17.3	17.6	13.0	16.3	22.2	17.1	13.7	22.8	16.6	21.0	16.5	26.3	19.5
F. pinicola 98B	100	100	90	100	100	80	95.0	19 7	23.1	21.7	22.7	26.0	22.8	22.7	20.5	22.5	18.2	19.1	24.3	21.9	21.1
Control	90	100	90	100	100		96.0	25.4	24.9	19.8	18.2	20.3		21.7	23.7	23.9	25.6	20.7	26.7		24.1
F. pinicola 98B	100	100	100	100	100	100	100	14.3	12.8	14.7	16.7	11.7	16.6	14.5	16.9	21.1	22.7	19.0	24.4	17.6	20.3
Control	100	100	100	90	100	100	98.3	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. possecus	100	100	100	100	100	100	100	9.8	13.8	14.3	13.1	15.5	13.8	13.9	20.2	24.5	21.6	22.8	22.7	21.5	22.2
Control	100	100	100	100	100	100	100	13.5	13.2	12.8	14.4	13.2	17.5	14.1	25.7	26.0	20.1	22.2	22.1	24.5	23.4
F. pseudo-senex	100	90	90	100	100	90	95.0	17.8	17.3	21.0	14.9	15.6	17.1	17.3	21.5	22.7	23.3	26.8	20.7	23.6	23.1
Control	100	90	100	100	100		98.0	14.6	13.5	15.0	14.9	12.7	14.6	14.2	23.9	21.0	20.8	18.3	16.2	23.8	20.7
F. pseudo-senex	100	100	100	100	90		98.0	21.9	20.7	15.5	22.1	14.2		18.9	22.9	25.7	23.2	24.8	21.8		23.7
Control	100	100	100	100			100	20.4	20.3	13.4	15.2			17.3	24.0	20.9	17.7	21.7			21.1
F. pseudo-senex	100	100	100	100			100	11.1	14.5	17.9	14.1			14.4	14.9	18.7	29.4	23.7			21.7
Control	100	100	90	100			97.5	12.9	16.0	12.0	12.7			13.4	22.3	31.5	18.4	22.5			23.7
F. ribis	90	100	100	90	100	100	96.7	14.1	13.7	16.8	19.0	11.4	10.8	14.4	19.6	23.3	26.6	22.0	20.7	20.2	22.1
Control	100	100	100	100	90	90	96.7	14.9	11.4	10.4	12.4	15.7	11.8	12.8	18.4	24.1	14.2	20.8	17.2	12.2	17.8
F. ribis	100	90	90	100	100	100	96.7	10.2	11.0	11.0	14.2	12.5	10.7	11.6	23.2	19.8	16.1	25.2	22.8	18.7	21.0
Control	100	100	100	100	100	90	98.3	13.5	13.2	12.8	14.4	13.2	17.5	14.4	25.7	26.0	20.1	22.2	22.1	24.5	23.4
F. ribis	90	100	100	100			97.5	13.4	18.1	13.0	14.2			14.7	16.8	13.3	12.6	14.9			14.4
Control	100	100	90	100	100	100	98.3	17.9	20.0	15.6	13.5	18.7	13.1	16.5	21.6	21.1	19.8	22.6	17.3	21.1	20.6
F. rimosus	100	100	100	100	100	100	100	14.8	19.3	13.4	16.0	16.2	13.3	15.5	19.9	26.3	22.4	19.3	18.6	18.6	20.9
Control	100	100	100	100			100	12.1	11.2	13.6	14.7			12.9	16.8	20.9	20.1	18.2			19.0
F. robustus	100	100	100	100	100	100	100	13.7	15.0	16.0	20.2	16.7	16.4	16.7	21.1	17.1	17.3	21.0	20.0	21.8	19.7
Control	90	90	100	100	100	100	96.7	14.0	12.7	13.1	13.2	14.4	18.9	14.4	26.8	24.7	28.7	28.6	24.2	25.1	26.3
F. robustus	100	90	100	90	100	90	95.0	18.5	20.0	21.3	18.3	17.5	22.7	19.7	18.4	22.7	29.5	23.6	25.4	17.2	22.8
Control	100	80	100	90	100	100	95.0	16.2	17.3	17.6	13.0	16.3	22.2	17.1	13.7	22.8	16.6	21.0	16.5	26.3	19.5
F. robustus	100	100	90	90	100	90	95.0	22.7	21.6	20.4	22.1	23.1	20.0	21.7	23.4	29.5	23.6	15.7	18.8	26.1	22.9
Control	100	100	90	90	100		96.0	21.6	20.3	21.8	18.9	19.6		20.5	25.2	25.9	23.3	21.8	24.6		24.2
F. scutellatus	40	90	100	100			82.5	1.5	10.6	5.6	5.8			5.7	1.0	5.3	2.4	1.3			2.8
Control	90	100	100	100			97.5	12.5	13.4	14.5	13.2			13.9	25.1	27.0	22.5	28.2			25.7
F. scutellatus	40	100	90	40			67.5	1.2	4.7	5.6	3.5			3.8	0.3	3.5	2.8	7.5			3.5
Control	90	90	90	100			92.5	14.0	7.2	8.0	9.9			9.8	26.2	21.3	14.4	24.8			20.8
F. scutellatus	0	0	60	0	0	40	16.7	0	0	0	0	0	0	0			6.7			2.9	4.8
Control	90	100	100	90			95.0	10.9	10.2	10.6	10.3			10.5	28.8	25.1	23.6	28.1			25.9
F. sub-roseus	100	100	90	90	100	100	96.7	15.9	11.0	17.7	17.1	12.1	12.3	14.3	23.3	19.9	27.3	19.1	23.1	22.2	22.5
Control	100	100	100	100	90		98.0	14.3	12.6	12.0	16.3	15.1		14.1	28.6	26.4	22.7	25.4	21.8		26.0
F. torulosus	90	100	100	100	100	100	98.3	17.9	20.7	18.2	19.5	17.3	16.1	18.3	24.4	23.7	19.8	20.3	21.6	22.0	21.9
Control	100	100	100	100	90	100	98.3	15.1	14.9	17.9	11.4	16.8	13.4	14.9	17.2	19.8	21.9	17.9	25.7	25.5	21.4
F. torulosus	100	90	100	90	100		96.0	21.4	13.3	16.8	14.2	19.7	19.3	17.5	25.5	23.6	25.3	21.1	23.7	21.8	23.5
Control	100	80	100	90	100	100	95.0	16.2	17.3	17.6	13.0	16.3	22.2	17.1	13.7	22.8	16.6	21.0	16.5	26.3	19.5
F. torulosus	100	90	100	100	100	80	95.0	22.7	22.8	23.9	21.8	26.9	26.4	24.1	22.7	17.4	16.6	19.5	24.2	23.1	20.6
Control	90	100	90	100	100		96.0	25.4	24.9	19.8	18.2	20.3		21.7	23.7	23.9	25.6	20.7	26.7		24.1
F. ulmarius 241A	100	100	100	100	100	100	100	16.4	18.2	20.0	17.1	14.0	16.3	17.1	23.8	38.6	27.8	27.2	22.1	26.8	27.7
Control	90	100	100	100			97.5	20.8	14.3	14.0	19.2			17.1	31.7	22.9	29.0	29.2			28.2
F. ulmarius 241B	100	100	100	100	100		100	19.5	14.0	17.8	15.1	20.9		17.4	16.3	16.4	29.7	17.8	23.5		20.7
Control	100	100	100	100	100		100	16.3	18.0	14.6	17.9	16.2		16.6	22.7	21.3	17.8	25.8	19.6		21.4
F. vinosus	100	100	100	100	100	90	98.2	18.3	17.6	16.0	14.4	14.0	15.4	15.9	24.1	20.2	22.6	30.6	34.3	24.4	26.3
Control	100	100	100	100			100	16.2	17.8	16.6	13.1			15.9	27.5	25.3	25.5	27.7			26.5

Diameters and sporulation of Aspergillus niger colonies in assemblies containing A. niger paired with Fomes cultures and in controls paired with uninoculated 5% malt agar.

Treatment	Assembly no.	Colony diameter after 7 days (mm) 1	2	3	4	5	6	Mean	Sporulation after 7 days
F. Clelandi		54	53	60	55	53		55.0	3.0+
Control		60	57	53	57	56		56.6	3.0+
F. conchatus		49	48	49	50	49		49.0	2.5+
Control		50	48	45	50	50	49	48.7	2.5+
F. connatus		52	52	54	51	50		51.8	2.5+
Control		58	54	52	53	54		54.2	2.6+
F. connatus		53	50	50	53	51	50	51.2	3.0+
Control		53	51	49	53	52	55	52.2	3.0+
F. connatus		51	50	50	50	50		50.2	2.0+
Control		53	50	50	51	51	52	51.2	2.0+
F. dependens		45	49	49	49	50		48.4	2.0+
Control		54	47	52	47	51	53	50.7	2.0+
F. fastuosus		53	72	51	74	54		60.8	2.5+
Control		55	54	53	54			54.0	2.5+
F. fastuosus		55	55	54	57	59	55	55.8	2.5+
Control		59	57	58	60	60		58.8	2.5+
F. fastuosus		54	55	53	53	56		54.2	2.5+
Control		54	56	56	55	54		55.0	2.5+
F. fraxineus		51	51	53	53	51		51.8	2.0+
Control		52	53	53	52	51		52.2	2.3+
F. fraxineus		48	48	48	48			48.0	3.0+
Control		51	51	51	51	51	54	51.5	3.0+
F. fraxineus		49	49	45	45	47		47.0	2.0+
Control		46	47	49	48	49		47.8	2.0+
F. fomentarius		70	66	61	62			64.7	2.0+
Control		65	65	67	63	67	61	64.7	2.0+
F. hartigii		50	53	49	52			50.8	2.0+
Control		49	52	53	53			51.7	2.0+
F. hemitephrus		50	51	51	51	50		50.6	2.5+
Control		56	55	52	52	54	55	54.0	2.5+
F. hemitephrus		56	54	54	53	57	57	55.2	2.5+
Control		60	59	61	60			60.0	2.5+
F. hemitephrus		53	51	50	53	50		51.4	3.0+
Control		53	51	49	53	52	55	52.2	3.0+
F. igniarius		51	51	50	50	50	50	50.3	2.0+
Control		54	54	52	51	52	52	52.5	2.0+
F. juniperinus		63	63	65	65	65		64.2	2.0+
Control		63	62	61	63	63		62.4	2.0+
F. lignosus		51	45	46	50	49	49	48.3	2.0+
Control		53	52	53	52	52	51	52.2	2.0+
F. lignosus		51	50	49	51	50		50.2	2.0+
Control		50	52	51	53	50	50	51.0	2.0+
F. lignosus		50	51	50	49	50		50.0	2.0+
Control		53	50	50	51	51	52	51.1	2.0+
F. melanoporus		49	49	46	46	48	50	48.0	2.75+
Control		52	55	50	51	55	52	52.5	2.5+
F. nigrolimitatus		46	47	46	46	46	46	46.2	3.0+
Control		46	47	46	48	49		47.2	3.0+
F. noxius 224A		53	56	48	50			51.7	3.2+
Control		60	57	61	61			59.8	2.8+
F. noxius 224A		48	50	48	48			48.5	3.0+
Control		51	51	51	51	54	51	51.5	2.8+
F. noxius 224A		45	45	42	43	41		43.2	3.2+
Control		45	45	45	43	45		44.6	2.8+
F. noxius 170.32		50	51	51	52	50	51	50.8	2.8+
Control		53	51	49	53	52	55	52.2	2.8+
F. officinalis		62	65	46	61	57	52	57.2	2.0+
Control		52	61	64	61	62		60.0	2.0+
F. pinicola 98A		62	66	58	64	57	60	61.2	2.0+
Control		64	65	63	64	63	59	63.0	2.0+
F. pinicola 98B		47	50	54	52	49	51	50.5	2.0+
Control		54	53	55	46	49	50	51.2	2.0+
F. pomaceus		58	66	70	70	63	63	65.0	2.0+
Control		62	74	67	67	70	64	67.3	2.0+
F. pseudo-senex		59	59	57	58	60	60	58.8	2.5+
Control		68	59	62	58			61.7	2.5+
F. ribis		52	50	48	50	49		49.8	2.0+
Control		51	55	55	57	58	57	55.5	2.0+
F. rimosus		50	47	46	53	50		49.2	2.5+
Control		48	48	54	50	49		49.8	2.5+
F. robustus		56	52	54	55			54.2	2.5+
Control		50	60	60				56.7	2.5+
F. sub-roseus		55	58	58	55	56	54	56.0	2.0+
Control		60	60	58	60	59	60	59.5	2.0+
F. scutellatus		49	50	52	51	50	50	50.3	3.0+
Control		52	52	51	51	51	54	51.8	3.0+
F. scutellatus		41	40	40	39	41	42	40.5	3.0+
Control		45	43	42	43	43	43	43.2	3.0+
F. scutellatus		47	45	43	47	46	44	45.3	3.0+
Control		47	47	48	47	47	48	47.3	3.0+
F. torulosus		49	46	57	56	75	76	59.8	2.0+
Control		65	61	47	56	70		59.8	2.0+
F. ulmarius 241A		54	53	54	53	52		53.2	2.0+
Control		56	53	54	56	53	54	54.3	2.0+
F. ulmarius 241B		48	47	53	47	48		48.6	2.5+
Control		50	48	49	48	50	49	49.0	2.5+
F. vinosus		62	58	58	55	55	57	57.5	2.0+
Control		59	61	59	55	59		58.6	2.0+

Table IV.

Growth of bacteria in assemblies containing bacteria paired with Fomes cultures and in controls, paired with uninoculated 5% malt agar.

Species having no apparent effect on bacteria.

F. Clelandi	F. noxius 170.32
F. conchatus	F. occidentalis
F. connatus	F. officinalis
F. dependens	F. pinicola 98A
F. fastuosus	F. pinicola 98B
F. ferruginosus	F. pomaceus
F. fomentarius	F. pseudo-senex
F. fraxineus	F. ribis
F. hartigii	F. rimosus
F. ignarius	F. robustus
F. juniperinus	F. scutellatus
F. lignosus	F. sub-roseus
F. melanoporus	F. torulosus
F. nigricans	F. ulmarius 241A
F. nigrolimitatus	F. ulmarius 241B
F. noxius 224A	F. vinosus

Growth in assemblies with F. hemitephrus cultures and in controls.

Treatment	Bacterial growth							
	M.ph.	M.r.	C.x.	St.a.Ox.	B.s.	Ps.a.	K.a.	Rh.2001
	Assembly no.							
	123	123	123	123	123	123	123	123
F. hemitephrus	+++	+++	+++	+++	+++	+++	+++	+++
Control	+++	+++	+++	+++	+++	+++	+++	+++
F. hemitephrus	+++	+++	+++	+++	+++	+++	+++	+++
Control	+++	+++	+++	+++	+++	+++	+++	+++
F. hemitephrus	+++	+++	+++	+++	+++	+++	+++	+++
Control	+++	+++	+++	+++	+++	+++	+++	+++

Table V. Differences in mean % germination of lettuce seeds and mean growth of germinated seedlings, mean diameters and sporulation of Aspergillus niger colonies and growth of bacteria in assemblies containing assay species paired with Fomes cultures and in controls, paired with uninoculated 5% malt agar.

Test species	Assay species: Lettuce						Total
	% Germination		Shoot length (mm)		Root length (mm)		
	Observed mean difference	L.S.M.D.	Observed mean difference	L.S.M.D.	Observed mean difference	L.S.M.D.	Observe mean differe
F. Clelandi	- 3.3	4.7	+ 1.3	2.2	+ 0.4	3.7	+ 1.
F. conchatus	- 2.3	6.2	+ 2.9	3.3	+ 4.2	7.7	+ 7.
F. conchatus	+ 1.6	4.7	+ 3.0	2.9	- 0.5	4.1	+ 2.
F. conchatus	- 7.5	10.4	+ 1.7	4.5	+ 1.8	8.3	+ 3.
F. connatus	0.0	2.7	- 2.1	2.7	+ 1.0	9.2	- 1.
F. connatus							
F. connatus							
F. dependens	- 1.3	6.7	- 2.5	2.6	- 0.6	2.6	- 3.
F. fastuosus	+ 2.0	2.7	+ 1.4	3.4	- 2.3	2.9	+ 0.
F. fastuosus							
F. fastuosus							
F. fraxineus	- 2.5	6.6	- 3.5	4.4	- 2.2	6.5	- 5.
F. fraxineus							
F. fraxineus							
F. fomentarius	- 6.7	8.6	+ 3.1	4.8	-0.5	7.1	+ 2.
F. hartigii	+ 1.7	2.1	- 2.1	3.4	-1.8	2.7	- 3.
F. hemitephrus	- 1.7	2.1	+ 0.4	4.9	+2.5	8.4	+ 2.
F. hemitephrus							
F. hemitephrus							
F. ignarius	- 1.3	6.7	+ 1.2	3.1	-1.7	3.2	- 0.
F. juniperinus	- 1.3	9.4	- 0.5	8.1	+2.9	3.5	+ 2.
F. lignosus	+ 1.7	1.9	- 0.9	2.8	-2.0	2.5	- 2.
F. lignosus							
F. lignosus							
F. melanoporus	0.0	2.7	- 1.1	3.1	-2.3	3.1	- 3.
F. nigrolimitatus	+ 1.6	2.6	+ 1.8	2.6	-1.0	3.2	+ 0.
F. noxius 224A	-18.3	16.3	- 3.4	3.8	-7.1	4.2	-20.
F. noxius 224A	+ 1.6	5.1	+ 3.5	4.1	-7.4	7.4	- 4.
F. noxius 224A	0.0	2.7	+ 3.4	4.5	-5.2	2.5	-12.
F. noxius 170.32	+ 1.7	10.5	- 0.4	2.2	-1.7	4.0	- 5.
F. noxius 170.32	+ 1.0	12.3	- 0.3	3.1	-1.8	6.6	- 5.
F. noxius 170.32	+ 1.7	8.1	+ 1.0	1.7	+1.2	7.0	+ 1.
F. officinalis	- 1.7	1.9	- 2.8	3.8	+.6	4.8	+ 2.
F. officinalis	- 5.0	25.5	- 2.5	4.3	- .5	5.3	- 6.
F. officinalis	- 3.0	6.7	0.0	2.9	- .4	5.8	- 4.
F. pinicola 98A	+ 1.7	8.1	+ 5.8	3.7	-1.7	10.0	-12.
F. pinicola 98A	- 2.0	4.1	+ 2.6	5.7	- .4	5.3	+ 3.
F. pinicola 98A	- 1.6	4.7	- 2.5	3.2	- .7	2.8	- 5.
F. pinicola 98B	+ 3.3	7.4	- 1.8	4.6	- .8	4.7	- 4.
F. pinicola 98B	- 1.0	9.0	+ 1.0	3.6	- .0	3.1	- 2.
F. pinicola 98B	+ 1.7	2.1	- 2.0	3.2	- .3	3.2	- 2.
F. pomaceus	0.0	13.2	- 0.2	2.0	- .2	2.5	- 1.
F. pseudo-senex	- 3.0	7.0	+ 3.1	2.1	+ .4	3.1	+ 5.
F. pseudo-senex	- 2.0	5.4	+ 1.6	5.9	+ .6	3.5	
F. pseudo-senex	+ 2.5	6.4	+ 1.0	4.3	- .0	4.0	- 1.
F. ribis	0.0	5.6	+ 1.6	1.7	+ .3	4.6	+ 5.
F. ribis	- 1.6	4.6	- 2.8	3.4	+ .1	3.7	- 5.
F. ribis	- 0.8	5.2	- 1.8	3.0	- .2	2.8	- 8.
F. rimosus	0.0	0.0	+ 2.4	3.0	+ .3	3.5	+ 4.
F. robustus	+ 3.3	4.5	+ 2.3	2.4	- .5	3.1	- 4.
F. robustus	0.0	9.1	+ 2.6	3.3	+ .4	5.9	+ 5.
F. robustus	- 1.0	7.0	+ 1.2	2.1	- .4	5.3	- 0.
F. scutellatus	-68.3	35.9	-10.5	4.8	-21.	3.3	-31.
F. scutellatus	-25.0	48.7	- 6.0	4.4	-17.	9.0	-23.
F. scutellatus	-60.0	54.6	- 8.2	6.6	-22.	3.5	-31.
F. sub-roseus	- 1.0	8.1	+ 0.2	3.5	- 3.	3.7	- 3.
F. torulosus	- 1.0	2.7	+ 3.4	2.6	+ 0.	3.6	+ 3.
F. torulosus	+ 1.0	2.5	+ 0.4	4.0	+ 4.	4.9	+ 4.
F. torulosus	0.0	7.5	+ 2.4	3.7	- 3.	3.3	- 1.
F. ulmarius 241A	+ 2.0	4.7	0.0	4.0	- 0.	7.7	- 0.
F. ulmarius 241B	0.0	0.0	+ 0.8	3.4	- 0.	7.0	+ 0.
F. vinosus	- 1.8	2.2	0.0	3.3	- 0.	4.2	- 0.

Table VI.

Growth of young colonies of F. scutellatus in paired petri dish assemblies containing young colonies paired with mature colonies of the same species.

Expt. no.	Treatment	Diameters of young colonies of F. scutellatus in each assembly (mm) Weeks after time of pairing					
		0	1	2	3	4	5
I	F. scutellatus	5 5 5	6 6 6	10 15 14	20 20 19*	30 33 31	50 48 47
	Control	5 5 5	7 6 7	13 14 15	26 25 28	30 41 44	45 50 48
II	F. scutellatus	5 5 5	6 6 6	8 9 7	18 21 20	28 27 30	35 37 42
	Control	5 5 5	7 8 6	8 17 12	20 30 25	22 34 32	29 44 45
III	F. scutellatus	5 5 5	6 6 6	12 13 12	22 18 18	28 27 26	35 38 35
	Control	5 5 5	6 6 6	13 11 12	19 16 18	27 26 29	33 39 38

*Difference between means for F. scutellatus and control significantly different at 0.05 P. level (observed mean difference = 6.6 mm ; least significant mean difference = 3.5 mm).

For illustration of experiment I, see text Figure (x).

Table VII.

Germination of lettuce seeds, growth of lettuce seedlings and concentrations of carbon dioxide in sealed bottles in tests with authentic carbon dioxide.

Expt. no.	Assembly no.	Concentrations of carbon dioxide (% volume/volume of gas space) Initial	Final	Mean	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)
I	1	0.026	0.036	0.031	90	8.1	31.2	39.3
	2	0.030	0.030	0.030	90	7.4	26.3	33.7
	3	0.036	0.040	0.038	90	8.1	34.1	42.2
	1	0.069	0.053	0.061	100	6.8	21.6	28.4
	2	0.109	0.066	0.087	90	6.4	19.7	26.1
	3	0.125	0.076	0.101	100	9.2	34.2	43.4
	1	0.175	0.185	0.180	90	6.3	17.1	23.4
	2	0.188	0.168	0.178	100	6.8	33.2	40.0
	3	0.178	0.228	0.203	80	6.8	23.7	30.5
II	1	0.027	0.025	0.026	100	8.0	37.3	45.3
	2	0.030	0.032	0.031	90	8.1	22.3	31.4
	3	0.040	0.025	0.032	90	8.7	37.6	46.3
	1	0.096	0.088	0.092	100	11.6	35.9	47.5
	2	0.076	0.120	0.098	100	11.5	45.1	56.6
	3	0.089	0.102	0.096	100	12.7	51.7	64.4
	1	0.168	0.169	0.169	100	11.6	54.3	65.9
	2	0.178	0.162	0.170	100	7.7	30.8	38.5
	3	0.155	0.166	0.160	100	9.0	34.4	43.4
	1	0.311	0.411	0.360	100	6.6	26.7	33.3
	2	0.326	0.240	0.283	100	8.4	15.0	23.4
	3	0.396	0.240	0.318	100	10.2	31.7	41.9
III	1	0.050	0.020	0.035	100	11.2	51.1	62.3
	2	0.050	0.060	0.055	90	10.3	28.3	38.6
	3	0.030	0.068	0.049	100	11.6	48.7	59.3
	1	0.050	0.036	0.043	100	9.3	50.5	59.8
	2	0.071	0.065	0.068	90	10.3	34.9	45.2
	3	0.070	0.051	0.060	100	11.0	45.5	56.5
	1	0.240	0.330	0.275	100	16.7	45.2	61.9
	2	0.205	0.294	0.250	90	8.8	51.8	60.6
	3	0.254	0.264	0.260	90	10.2	31.7	41.9

The differences between the means for % germination and lengths of shoots + roots of seedlings in different treatments are not significant in any experiment. (P=0.05)

For illustration of experiment I, see text Figure (xi).

Table VIII.

Time taken for three standard colours to develop in cupric ethylacetoacetate plus titrabase on filter paper in tests with standard KCN solutions.

Concentration of KCN solution (xN)	Time for pallid violet blue to develop (min)				Time for light violet blue to develop (min)				Time for smalt blue to develop (min)			
	Expt. no. I	II	III	Mean	Expt. no. I	II	III	Mean	Expt. no. I	II	III	Mean
0.1	0.30	0.10	0.15	0.18	1.3	1.5	0.9	1.3	10.0	12.0	9.0	10.5
0.09	0.40	0.15	0.25	0.27	1.6	1.5	1.1	1.4	13.0	13.0	10.0	12.0
0.08	0.40	0.15	0.30	0.28	1.8	1.1	1.4	1.4	14.0	13.0	10.0	12.5
0.07	0.40	0.20	0.35	0.32	1.7	1.2	1.4	1.4	14.0	14.0	13.0	14.0
0.06	0.40	0.20	0.40	0.33	2.0	1.3	1.5	1.6	15.0	18.0	13.0	15.5
0.05	0.60	0.30	0.60	0.50	2.7	1.4	2.3	2.1	15.0	20.0	15.0	17.0
0.04	0.60	0.35	0.60	0.52	3.1	1.6	2.0	2.2	15.0	20.0	15.0	17.0
0.03	0.70	0.40	0.80	0.63	3.1	1.9	2.6	2.5	16.0	18.0	19.0	18.0
0.02	0.70	0.55	1.0	0.75	5.2	2.4	3.1	3.6	20	18.0	25	21
0.01	0.90	0.55	1.1	0.85	5.4	2.6	3.1	3.7	27	18.0	25	23
0.009	1.2	0.90	2.3	1.5	6.0	3.5	5.3	3.9	28	18.0	22	23
0.008	1.2	0.80	3.0	1.7	6.0	6.0	5.3	5.8	28	20	22	26
0.007	1.2	0.85	2.6	1.6	6.0	8.5	5.3	6.6	28	28	23	37
0.006	1.3	0.80	3.6	1.9	8.0	8.7	6.5	7.7	40	17.0	24	43
0.005	1.3	0.90	3.8	2.0	8.0	12.0	6.6	8.9	70	22	28	44
0.004	1.5	1.1	3.8	2.1	8.0	13.0	6.6	9.2	80	24	28	47
0.003	1.5	1.2	4.7	2.5	9.0	14.0	7.4	10.0	100	25	35	53
0.002	2.0	2.5	4.8	3.1	9.0	18.0	10.0	12.5	>*24 hrs	45	45	
0.001	2.7	3.0	5.1	3.7	9.5	20	10.0	13.0	>24 hrs	55	50	

The least significant difference between the mean of readings for 0.001N and 0.01N = 2.1 min for pallid violet blue and 9.8 min for light violet blue and between the mean of readings for 0.01N and 0.1N = 0.41min for pallid violet blue and 2.5 min for light violet blue.

In these and all subsequent experiments the time taken to attain standard colour was measured to the following accuracy:-

0 to 1 min	to the nearest	0.05	min.
1 "10 "	" " "	0.1	min.
10 "20 "	" " "	0.5	min.
20 "60 "	" " "	1	min.
Over 60 "	" " "	10	min.

*>24 hrs = no development of colour by 24 hours after insertion of test paper.

Pallid violet blue = Ridgway No. 53-BF.
Light violet blue = Ridgway No. 53-VBb.
Smalt blue = Ridgway No. 53-v-Bi.

Table IX.

Times taken (min) for pallid violet blue colour to develop in paired bottle assemblies containing lettuce paired with F. scutellatus cultures, or standard KCN solutions.

a) F. scutellatus

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	36	28	30						
1				20	17.0	24			
2							5.1	10.5	15.0
3	5.5	7.3	7.6						
5				6.5	7.0	5.5			
6							6.0	7.0	5.5
7	5.0	6.0	5.5	5.0	5.5	5.0	7.0	5.0	5.0

b) 0.001N KCN

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	15.5	18.5	12.0						
1				6.8	4.1	4.0			
2							7.0	4.0	3.8
3	4.8	3.4	8.6						
5				8.0	3.1	4.2			
6							7.4	4.2	3.8
7	6.0	5.6	3.6	4.5	4.5	11.0	11.0	3.0	6.0

c) 0.01N KCN

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	1.1	1.1	3.0						
1				0.40	1.0	0.50			
2							1.1	0.60	0.80
3	0.80	0.60	0.90						
5				0.90	0.40	0.40			
6							0.90	0.50	0.30
7	1.0	0.50	0.30	1.1	0.40	0.60	0.70	0.50	0.30

d) 0.1N KCN

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	0.40	0.10	0.05						
1				0.10	0.10	0.15			
2							0.05	0.10	0.10
3	0.10	0.05	0.10						
5				0.10	0.15	0.10			
6							0.10	0.15	0.10
7	0.10	0.10	0.05	0.10	0.15	0.10	0.10	0.15	0.10

e) F. scutellatus (replicate experiment)

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	45	20	360						
1				38	24	15.0			
2							14.0	10.0	17.0
3	14.0	5.5	25.0						
5				11.0	6.4	9.0			
6							6.1	5.5	10.0
7	5.4	7.3	8.2	10.0	8.5	11.0	6.0	6.7	9.6

For illustration of (a), (b), (c) and (d), see text Figure (xii).

Table X.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *F. scutellatus* cultures, 10 ml standard 0.001N KCN solutions and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Expt. I.

Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
A	1	80	6.5	11.5	18.0	9.0
F. scutellatus	2	90	8.1	22.4	22.4	15.0
	3	60	2.8	3.3	6.1	24.0
B	1	100	13.6	48.8	62.4	
Uninoculated	2	100	15.8	41.5	57.3	
5% malt agar	3	100	12.8	49.2	62.0	
C	1	90	7.9	11.4	19.3	120
0.001N KCN	2	100	9.6	24.8	34.4	160
	3	90	8.6	7.2	15.8	20.0
D	1	100	17.8	44.5	62.3	
H_2O	2	100	11.1	35.2	46.3	
	3	100	15.1	55.1	70.2	
E	1	90	7.6	16.8	17.8	12.0
0.001N KCN	2	100	10.8	36.2	31.6	20.0
	3	100	10.5	16.7	22.8	17.0
F	1	100	12.9	52.3	78.0	
H_2O	2	100	10.8	42.4	63.5	
	3	100	10.3	38.3	69.5	

For illustration, see text Figure (xiii).

Table XI.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *F. scutellatus* cultures, 10 ml standard 0.001N KCN solutions and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Expt. II.

Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
A	1	90	4.6	8.4	13.0	5.5
F. scutellatus	2	100	6.2	10.1	16.3	10.0
	3	70	3.7	3.1	6.8	6.0
B	1	90	9.9	34.4	44.3	
Uninoculated	2	100	12.3	57.9	70.2	
5% malt agar	3	100	11.2	45.4	56.6	
C	1	100	6.2	7.0	13.2	50.0
0.001N KCN	2	90	4.9	3.6	8.5	15.0
	3	100	6.4	6.3	12.7	25.0
D	1	100	10.7	53.9	64.6	
H_2O	2	100	11.8	49.0	50.8	
	3	100	11.2	45.4	56.6	
E	1	80	9.6	15.2	24.8	45.0
0.001N KCN	2	90	13.3	31.1	44.4	54.0
	3	100	9.0	10.8	19.8	14.0
F	1	100	12.1	39.1	51.2	
H_2O	2	100	12.3	55.2	67.2	
	3	100	13.9	48.3	62.2	

Table XII.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *F. scutellatus* cultures, 10 ml standard 0.001N KCN solutions and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Espt. III.

Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
A	1	100	5.4	8.8	14.2	7.5
F. scutellatus	2	90	6.5	13.8	20.3	5.5
	3	100	6.2	13.4	19.6	7.5
B	1	100	10.6	48.5	59.1	
Uninoculated 5% malt agar	2	100	8.1	38.4	46.5	
	3	100	8.9	49.7	58.6	
C	1	100	5.2	7.6	12.8	8.5
0.001N KCN	2	80	7.2	7.4	14.6	6.5
	3	90	7.2	9.8	17.0	9.0
D	1	100	9.8	49.6	59.4	
H_2O	2	100	9.2	56.0	65.2	
	3	100	9.4	62.7	72.1	
E	1	100	7.6	19.7	27.6	19.5
0.001N KCN	2	90	7.3	23.6	30.9	21.0
	3	100	4.6	7.0	11.6	6.0
F	1	100	10.1	42.0	52.1	
H_2O	2	100	9.8	30.3	40.1	
	3	100	11.5	62.3	73.8	

Table XIII.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with 10 ml standard 0.1N and 0.01N KCN solutions and in controls, paired with 10 ml sterile deionized water.

Expt. no.	Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
I	0.1N KCN	1	0	0.0	0.0	0.0	0.1
		2	0	0.0	0.0	0.0	0.1
		3	0	0.0	0.0	0.0	0.2
	0.01N KCN	1	0	0.0	0.0	0.0	1.0
		2	10	0.0	1.0	1.0	0.6
		3	10	0.0	1.0	1.0	0.6
	H_2O	1	70	8.0	19.0	27.0	
		2	100	9.0	29.4	38.4	
		3	100	9.1	20.2	29.3	
II	0.1N KCN	1	0	0.0	0.0	0.0	0.2
		2	0	0.0	0.0	0.0	0.15
		3	0	0.0	0.0	0.0	0.15
	0.01N KCN	1	0	0.0	0.0	0.0	0.9
		2	0	0.0	0.0	0.0	0.6
		3	0	0.0	0.0	0.0	1.0
	H_2O	1	80	5.2	6.4	11.6	
		2	80	6.2	12.5	18.7	
		3	80	8.5	11.6	20.1	
III	0.1N KCN	1	0	0.0	0.0	0.0	0.05
		2	0	0.0	0.0	0.0	0.10
		3	0	0.0	0.0	0.0	0.10
	0.01N KCN	1	0	0.0	0.0	0.0	1.2
		2	0	0.0	0.0	0.0	0.4
		3	0	0.0	0.0	0.0	0.8
	H_2O	1	90	7.1	15.0	22.1	
		2	100	7.4	16.2	23.6	
		3	100	6.0	15.5	21.5	

Table XIV.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *F. scutellatus* cultures, 10 ml standard 0.002N KCN solutions, and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
A	1	60	3.3	2.7	6.0	1.0
F. scutellatus	2	80	3.4	4.0	7.4	3.7
	3	100	4.6	10.8	15.4	8.6
B	1	100	12.0	30.3	43.3	
Uninoculated	2	100	11.5	35.5	47.0	
5% malt agar	3	100	9.1	34.4	43.5	
C	1	30	1.0	1.7	2.7	2.0
0.002N	2	20	1.5	1.0	2.5	2.0
KCN	3	40	1.5	1.5	3.0	7.0
D	1	100	10.6	33.7	44.3	
H_2O	2	100	8.3	24.3	32.6	
	3	90	12.1	44.7	56.8	
E	1	70	4.7	3.7	8.4	10.2
0.002N	2	60	1.8	2.0	3.8	2.0
KCN	3	90	2.1	3.7	5.8	3.2
F	1	100	11.4	48.2	59.6	
H_2O	2	100	9.6	46.4	56.0	
	3	90	12.8	45.4	58.2	

Assemblies in treatments A, B, C and D were paired immediately after planting of lettuce seeds. Assemblies in treatments E and F were paired 24 hrs after planting.

Table XV.

Times taken (min) for pallid violet blue colour to develop in paired petri dish assemblies containing lettuce seedlings paired with F. scutellatus cultures, or 10 ml standard KCN solutions.

a) F. scutellatus

Assembly no. / Day no.	1	2	3	4
0	>24 hrs	45	*>24 hrs	40
1	2.0	360	3.0	>24 hrs
2	20.0	21	20.0	20.0
3	10.0	100	70	>24 hrs
5	2.0	70	5.0	25
6	30	17.0	20.0	10.0
7	>24 hrs	10.0	40	12.0

b) 0.1N KCN solution

Assembly no. / Day no.	1	2	3	4
0	0.05	0.05	0.05	0.05
1	"	"	"	"
2	"	"	"	"
3	"	0.1	0.1	0.1
5	0.10	0.10	0.20	0.10
6	0.40	0.05	0.20	0.30
7	0.10	0.10	0.10	0.10

c) 0.01N KCN solution

Assembly no. / Day no.	1	2	3	4
0	0.20	0.20	0.20	0.20
1	0.20	0.30	0.20	0.20
2	0.10	0.20	0.20	0.20
3	0.40	0.50	0.40	0.70
5	1.8	1.7	1.9	2.4
6	19.0	18.0	1.3	5.2
7	2.1	2.3	>24 hrs	>24 hrs

d) 0.001N KCN solution

Assembly no. / Day no.	1	2	3	4
0	0.70	0.60	0.80	0.70
1	0.30	0.40	0.40	0.40
2	2.0	2.1	1.0	0.90
3	1.0	1.0	1.1	1.2
5	25.0	35.0	40.0	75.0
6	35.0	>24 hrs	70.0	>24 hrs
7	40	50	40	"

* >24 hrs = no development of colour by 24 hours after insertion of test paper.

Table XVI.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. scutellatus cultures, 10 ml standard KCN solutions, and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Treatment	Assembly no.	Expt. I					Expt. II					Expt. III				
		% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Mean lengths of shoots + roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
F. scutellatus	1	100	14.5	11.3	25.8	20	100	12.4	12.9	25.3	24 hrs	40	1.5	1.0	2.5	8.0
	2	100	9.3	6.6	15.9	20	100	6.7	2.5	9.2	15	100	10.6	5.3	15.9	18.0
	3	80	4.2	1.8	6.0	5.0	100	10.2	6.5	16.7	30	100	5.6	2.4	8.0	5.0
	4	100	6.7	3.4	10.1	5.0	100	10.3	2.9	13.2	20	100	5.8	1.3	7.1	5.0
Uninoculated 5% malt agar	1	90	16.8	26.2	43.0		100	15.1	26.7	41.8		80	12.6	25.1	37.7	
	2	90	15.3	21.8	37.1		100	15.2	27.3	42.5		100	15.4	27.0	42.4	
	3	100	13.6	28.7	42.3		90	12.0	21.1	33.1		100	14.5	22.5	37.0	
	4	100	19.3	26.3	45.6		100	13.6	21.6	35.2		100	13.2	28.2	41.4	
0.001N KCN	1	70	11.0	17.9	28.9	>24 hrs*	90	8.2	15.8	24.0	24 hrs	90	14.7	18.7	33.4	>24 hrs
	2	90	10.0	15.4	25.4	>24 hrs	100	6.4	14.8	21.2	24 hrs	100	7.8	9.8	17.6	>24 hrs
	3	100	11.0	18.7	29.7	>24 hrs	90	3.3	5.0	8.3	24 hrs	100	8.4	17.2	25.6	>24 hrs
	4	90	9.8	18.2	28.0	>24 hrs	100	6.4	17.8	24.2	24 hrs	100	12.9	27.5	40.4	>24 hrs
0.01N KCN	1	50	1.6	2.2	3.8	>24 hrs.	10	2.0	1.0	3.0	24	20	0.0	1.0	1.0	4.0
	2	0.0	0.0	0.0	0.0	7.0	90	5.7	6.7	12.4	22	100	14.4	22.7	37.1	>24 hrs
	3	0.0	0.0	0.0	0.0	13.0	90	1.9	2.0	3.9	200	0	0.0	0.0	0.0	10.0
	4	60	1.7	1.1	2.8	13.0	100	4.5	8.4	12.9	360	90	10.1	12.2	22.3	360
0.1N KCN	1	0.0	0.0	0.0	0.0	3.0	0	0.0	0.0	0.0	0.05	0	0.0	0.0	0.0	0.05
	2	0.0	0.0	0.0	0.0	2.0	0	0.0	0.0	0.0	0.05	0	0.0	0.0	0.0	0.50
	3	0.0	0.0	0.0	0.0	4.0	0	0.0	0.0	0.0	0.20	0	0.0	0.0	0.0	0.10
	4	0.0	0.0	0.0	0.0	2.0	0	0.0	0.0	0.0	1.0	0	0.0	0.0	0.0	0.10
H_2O	1	100	18.7	31.6	50.3		90	13.6	18.8	32.4		100	12.8	21.4	34.2	
	2	90	16.6	22.8	39.4		100	12.6	20.7	33.3		100	20.5	29.4	49.9	
	3	100	13.4	21.3	35.7		100	14.9	23.9	38.8		100	17.3	21.3	38.6	
	4	100	16.1	28.9	45.0		90	12.6	25.7	38.3		100	12.7	20.2	32.9	

*>24 hrs = no development of colour by 24 hours after insertion of test paper.

Table XVII.

Summary of results of tests in paired petri dish assemblies containing lettuce paired with *F. scutellatus* cultures, standard KCN solutions, uninoculated 5% malt agar or sterile deionized water.

		% reduction compared with control treatment			
		% germination		Total seedling length (mm)	
Expt. no.	Treatment	Observed	*Least significant	Observed	Least significant
I	*F. scutellatus*	0	14	65.7	27.1
	0.001N KCN	16	46	9.6	19.2
	0.01N KCN	72	41	96.2	19.5
	0.1N KCN	100	6	100	18.3
II	*F. scutellatus*	5+	6	69.8	24.9
	0.001N KCN	0	10	45.6	28.2
	0.01N KCN	24	56	77.3	12.3
	0.1N KCN	100	8	100	11.4
III	*F. scutellatus*	11	20	90.8	37.1
	0.001N KCN	3	6	24.9	40.1
	0.01N KCN	48	6	61.2	77.1
	0.1N KCN	100	0	100	25.5

+ Stimulation * P = 0.05

Table XVIII. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures for 7 or 14 days and in controls, paired with uninoculated 5% malt agar.

Expt. I

Treatment	% germination*	After incubation for 7 days				After incubation for 14 days			
		Mean lengths of shoots (mm)		Mean lengths of roots (mm)		Mean lengths of shoots (mm)		Mean lengths of roots (mm)	
	Value/assembly	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment
A F. noxius days 1-7 5% M.A. days 7-14	90 100 100	15.8 11.8 11.2	12.9	4.8 2.7 2.9	3.5	19.9 11.6 14.3	15.3	13.8 8.3 11.5	11.2
B F. noxius days 1-14	100 90 100	14.8 13.3 22.8	17.0	3.7 3.2 7.2	4.7	16.7 14.6 21.3	17.2	3.6 2.7 5.1	3.8
C 5% M.A. days 1-14 (changed on day 7)	100 90 100	14.0 8.4 13.7	12.0	23.1 19.6 19.8	20.8	24.9 15.9 26.7	22.5	31.0 32.2 25.7	29.6
D 5% M.A. unchanged	100 100 100	10.1 9.8 15.0	11.6	19.9 17.0 17.0	18.0	20.0 18.1 22.5	20.2	24.7 26.6 30.0	27.1

* % germination unchanged on day 14.

Differences between means of numbers of germinated seeds in each treatment were not significant ($P = 0.05$).

For further statistical treatment see text Figure (xlv).

Table XIX. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures for 7 or 14 days and in controls, paired with uninoculated 5% malt agar.

Expt. II

Treatment	* % germination	After incubation for 7 days				After incubation for 14 days			
		Mean lengths of shoots (mm)		Mean lengths of roots (mm)		Mean lengths of shoots (mm)		Mean lengths of roots (mm)	
	Value/assembly	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment
A									
F. noxius									
days 1-7	90	8.8		2.3		11.7		12.6	
5% M.A.	90	8.0	9.2	2.5	2.4	17.5	13.6	12.6	11.3
days 7-14	100	10.9		2.5		11.6		8.8	
B	90	9.0		1.0		9.0		1.0	
F. noxius	90	7.1	8.6	4.1	3.0	9.1	10.4	4.2	4.3
days 1-14	90	9.7		3.9		13.2		7.8	
C									
5% M.A.	90	16.0		22.0		21.2		30.5	
days 1-14	90	9.9	11.8	18.7	18.9	18.1	17.3	30.1	28.5
(changed	100	9.6		16.1		11.6		25.0	
on day 7)									
D									
5% M.A.	80	12.4		23.5		27.9		41.6	
unchanged	80	10.9	11.6	20.8	22.3	12.9	18.1	33.0	33.7
days 1-14	100	11.5		22.6		13.4		26.4	

Treatments	Differences between mean shoot extension between days 7 & 14 (mm)		Differences between mean root extension between days 7 & 14 (mm)	
	Observed	L.S.M.D.	Observed	L.S.M.D.
A and B	2.4	8.0	6.9	7.1
A and C	1.1	8.9	1.3	5.1
B and D	4.7	14.2	10.0	12.4
C and D	1.4	15.8	1.8	11.2

L.S.M.D. = least significant difference between mean extension in each treatment (P = 0.05).

* % germination unchanged on day 14.

Differences between means of numbers of germinated seeds in each treatment were not significant (P = 0.05).

Table XX. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures for 7 or 14 days and in controls, paired with uninoculated 5% malt agar.

Expt. III

Treatment	* % germination	After incubation for 7 days				After incubation for 14 days			
		Mean lengths of shoots (mm)		Mean lengths of roots (mm)		Mean lengths of shoots (mm)		Mean lengths of roots (mm)	
	Value/assembly	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment	Value/assembly	Mean/treatment
A F. noxius days 1-7 5% M.A. days 7-14	100 100 90	20.0 19.8 25.3	21.7	6.7 3.6 11.0	7.1	21.0 18.8 21.1	20.3	13.9 8.3 16.1	12.7
B F. noxius days 1-14	90 100 90	16.9 11.5 24.0	17.5	3.9 4.9 6.4	5.1	14.3 12.3 21.3	16.0	2.9 2.9 4.9	3.2
C 5% M.A. days 1-14 (changed on day 7)	100 90 100	18.7 16.2 15.7	16.9	20.6 21.8 22.8	21.7	22.2 22.1 23.1	22.5	28.5 26.1 32.0	28.9
D 5% M.A. unchanged days 1-14	100 100 100	14.9 16.2 16.9	16.0	20.3 20.6 19.4	20.1	21.1 21.6 22.2	21.6	25.9 28.4 29.2	27.8

Treatments	Differences between mean shoot extension between days 7 & 14 (mm)		Differences between mean root extension between days 7 & 14 (mm)	
	Observed	L.S.M.D.	Observed	L.S.M.D.
A and B	0.8	7.8	7.1	3.3
A and C	6.3	7.5	1.4	6.4
B and D	7.1	5.7	9.2	4.9
C and D	0.0	5.3	0.7	7.4

L.S.M.D. = least significant difference between mean extension in each treatment (P = 0.05).

* % germination unchanged on day 14.

Differences between means of numbers of germinated seeds in each treatment were not significant (P = 0.05).

Table XXI. Germination of cress seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls, paired with uninoculated 5% malt agar.

Expt. No.	Treatment	% germination							Mean lengths of shoots (mm)							Mean lengths of roots (mm)						
		Assembly No.						Mean	Assembly No.						Mean	Assembly No.						Mea
		1	2	3	4	5	6		1	2	3	4	5	6		1	2	3	4	5	6	
I	*F. noxius*	90	100	100	100	90	100	97	16.2	21.1	12.3	12.6	17.4	16.0	15.9	8.6	8.6	5.3	5.7	16.1	12.4	9.
	Control	100	100	90	100	90	100	97	17.7	18.8	20.1	15.6	17.1	17.4	17.0	25.5	31.6	23.1	24.2	26.1	23.0	27.
II	*F. noxius*	100	90	90	90	90	100	93	33.0	30.4	27.2	23.2	27.3	25.7	27.8	23.9	20.0	10.7	8.2	12.7	13.6	14.
	Control	90	80	90	100	70	80	88	31.8	37.0	26.1	29.3	26.1	29.3	29.1	39.1	37.6	35.6	35.3	38.3	36.4	37.
III	*F. noxius*	90	80	70	90	80	80	82	27.3	18.1	24.6	24.8	17.0	29.1	23.5	65.7	25.0	35.0	20.4	31.4	24.7	35.
	Control	90	100	100	90	90	80	92	24.4	29.3	27.1	25.6	30.1	34.2	28.6	64.9	61.3	60.1	63.7	52.1	75.1	66.

Least significant differences between mean lengths of shoots for different treatments = 3.2 mm, 4.0 mm, 5.4 mm and between mean lengths of roots for different treatments = 5.1 mm, 5.6 mm, 5.2 mm in Experiments I, II and III respectively.

Differences in numbers of germinated seeds were not significant.

Table XXII. Germination of lettuce seeds, growth of germinated seedlings and carbon dioxide and oxygen concentrations in paired bottle assemblies containing lettuce paired with F. noxius cultures and in controls, paired with uninoculated 5% malt agar.

Expt. No.	Treatment	Assembly No.	% germination	Mean	Mean shoot lengths (mm)	Mean	Mean root lengths (mm)	Mean	CO_2 concentration as % volume/volume of air space	O_2 concentration as % volume/volume of air space
I	F. noxius	1	70		4.1		6.1		0.858	20.15
		2	50	47	2.4	2.5	5.8	4.5	0.990	19.61
		3	20		1.0		1.5		0.607	18.31
	Control	1	100		14.4		35.5		0.025	22.21
		2	100	100	13.9	14.4	40.5	37.8	0.028	17.60
		3	100		15.0		37.3		0.041	23.28
II	F. noxius	1	10		2.0		1.0		0.467	18.56
		2	40	30	4.0	3.5	1.0	2.3	0.416	19.40
		3	40		4.5		5.0		0.272	18.81
	Control	1	100		12.1		30.3		0.042	20.23
		2	90	93	13.7	12.4	41.1	34.7	0.031	20.10
		3	90		11.3		32.8		0.038	20.23
III	F. noxius	1	60		3.0		7.3		0.87	20.20
		2	60	63	5.8	5.6	15.2	12.2	0.86	20.90
		3	70		8.1		14.2		0.64	20.90
	Control	1	100		11.3		28.5		0.027	20.15
		2	100	100	10.6	11.7	51.3	40.4	0.028	18.09
		3	100		13.1		41.4		0.033	19.60

Least significant differences between mean numbers of germinated seedlings for each treatment are 40.4%, 29.3% and 9.9% in experiments I, II and III respectively.

Least significant differences between means of lengths of roots for each treatment are 5.0 mm, 9.9 mm and 19.6 m respectively, and for shoots of germinated seedlings = 2.8 mm, 2.9 mm and 7.3 mm in experiments I, II and III respectively (P = 0.05).

Table XXIII. Germination of lettuce seeds, growth of lettuce seedlings and concentrations of carbon dioxide in sealed bottles in tests with authentic carbon dioxide/air mixtures.

Expt. No.	Concentrations of carbon dioxide (% vol./vol. of gas space)			% germination		Lengths of shoots (mm) in each assembly	Lengths of roots (mm) in each assembly	Lengths of shoots and roots (mm)	
	Initial	Final	Mean	Value/ assembly	Mean/ treatment			Value/ assembly	Mean/ treatment
I	0.032	0.015	0.024	100		9.3	38.9	48.2	
	0.030	0.014	0.022	100	100	8.8	48.0	56.8	53.3
	0.032	0.015	0.024	100		8.6	46.3	54.9	
	0.588	0.362	0.420	100		5.8	32.8	38.6	
	0.782	0.327	0.560	100	100	4.3	10.3	14.6	28.9
	0.668	0.240	0.450	100		5.6	27.8	33.4	
	1.145	0.608	0.876	0		0.0	0.0	0.0	
	1.081	0.689	0.835	90	57	1.8	1.0	2.8	2.3
	1.094	0.599	0.847	80		3.0	1.0	4.0	
	1.272	0.933	1.102	0		0.0	0.0	0.0	
	1.437	0.642	1.039	30	10	1.3	0.3	1.6	0.5
	1.590	0.959	1.274	0		0.0	0.0	0.0	
II	0.030	0.018	0.024	90		8.8	37.2	46.0	
	0.040	0.021	0.035	100	97	10.2	34.0	44.0	44.4
	0.025	0.023	0.024	100		9.7	33.4	43.1	
	0.608	0.573	0.591	90		7.0	22.4	29.4	
	0.631	0.518	0.405	100	90	8.8	44.4	53.2	29.3
	0.581	0.579	0.580	80		3.4	1.8	5.2	
	1.162	0.462	0.812	20		1.0	1.0	2.0	
	1.142	0.876	1.009	50	57	2.4	1.0	3.4	3.1
	1.310	0.702	1.006	100		2.6	1.4	4.0	
	1.540	1.404	1.497	0		0.0	0.0	0.0	
	1.550	1.344	1.447	0	0	0.0	0.0	0.0	0.0
	1.633	1.080	1.337	0		0.0	0.0	0.0	
III	0.029	0.027	0.028	90		7.3	40.8	48.1	
	0.028	0.027	0.028	100	97	7.8	43.1	50.9	48.1
	0.028	0.028	0.028	100		8.1	37.3	45.4	
	0.745	0.227	0.486	100		5.3	33.2	38.5	
	0.588	0.312	0.450	90	90	5.8	37.3	43.1	33.5
	0.755	0.360	0.557	80		5.6	13.3	18.9	
	1.220	0.906	1.063	70		1.9	1.0	2.9	
	1.238	0.486	0.862	70	70	4.3	4.1	8.4	5.3
	1.164	1.840	1.002	70		2.6	1.9	4.5	
	1.577	1.242	1.409	0		0.0	0.0	0.0	
	1.577	1.338	1.457	0	0	0.0	0.0	0.0	0.0
	1.466	1.500	1.483	0		0.0	0.0	0.0	

Least significant differences (P= 0.05) between means of lengths of shoots and roots in each 3 assemblies in the ranges tested = 20.7 mm, 22.8 mm and 12.0 mm for experiments I, II and III respectively.

Least significant differences (P = 0.05) between means of numbers of germinated seeds in each 3 assemblies in the ranges tested = 55.6%, 49.1% and 14.65% for experiments I, II and III respectively.

Table XXIV. Concentrations of acetaldehyde, ethanol, isobutanol and pentanol in paired petri dish assemblies with lettuce paired with F. noxius cultures and in controls, paired with uninoculated 5% malt agar.

Number of days after pairing	Treatment	Peak heights (mm)											
		Acetaldehyde Assembly No.			Ethanol Assembly No.			Isobutanol Assembly No.			Pentanol Assembly No.		
		1	2	3	1	2	3	1	2	3	1	2	3
0	F. noxius	5	5	5	6	8	10	2	2	2	2	2	2
1		19	14	20	2	2	2	4	3	3	0	0	2
2		14	11	27	2	2	2	3	2	2	0	0	0
3		15	13	10	3	3	6	2	2	1	0	0	2
4		12	13	16	8	4	0	11	10	15	2	0	0
6		8	10	15	2	4	3	7	10	5	0	0	0
7		22	9	7	1	2	1	6	4	4	0	1	0
0	Control	0	3	0	1	20	1	0	0	0	0	0	0
1		3	2	-	5	8	-	0	0	0	0	0	0
2		3	4	-	6	8	-	0	0	-	0	0	-
3		2	3	-	2	4	-	0	0	-	0	0	-
4		5	2	-	23	-	-	0	-	-	0	-	-
6		3	3	-	7	7	-	0	0	-	0	0	-
7		0	3	0	1	20	0	0	0	0	0	0	0

- = no measurement made.

Table XXV. Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with 20 ml aqueous solutions of acetaldehyde, or ethanol, or isobutanol, or pentanol, and in controls paired with 20 ml. sterile deionized water.

Experiment I

Treatment*	Assembly No.	Peak height of authentic component of each solution at end of experiment (mm)	% germination	Mean lengths of roots (mm)	Mean lengths of shoots (mm)
0.05% acetaldehyde	1	64	80	5.5	10.4
	2	177	90	6.4	11.9
	3	267	90	6.7	8.6
0.05% ethanol	1	53	100	5.4	8.1
	2	46	80	8.6	24.6
	3	17	90	6.1	10.4
0.05% isobutanol	1	57	90	5.8	14.0
	2	109	80	3.8	9.4
	3	47	90	6.3	6.1
0.05% pentanol	1	20	90	4.4	8.3
	2	10	80	8.3	10.9
	3	24	80	5.0	12.4
H_2O	1	Traces of ethanol and acetaldehyde	90	6.6	14.0
	2		100	7.1	10.5
	3		90	11.1	13.4

* 20 ml aqueous solutions in all treatments.

Differences between means of numbers of germinated seeds and between means of lengths of roots for each treatment were not significant ($P = 0.05$).

Table XXVI. Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with 20 ml aqueous solutions of acetaldehyde, or ethanol, or isobutanol, or pentanol, and in controls paired with 20 ml sterile deionized water.

Experiment II

Treatment	Assembly No.	Peak height of authentic component of each solution at end of experiment (mm)	% germination	Mean lengths of roots (mm)	Mean lengths of shoots (mm)
* 0.05% acetaldehyde	1	15	90	5.9	16.7
	2	14	80	7.0	11.6
	3	17	80	6.5	14.6
0.05% ethanol	1	111	80	6.6	12.9
	2	42	80	5.6	16.6
	3	141	80	6.4	20.8
0.05% isobutanol	1	35	90	7.5	12.8
	2	15	70	4.4	12.9
	3	61	70	7.9	19.6
0.05% pentanol	1	145	70	6.3	13.3
	2	92	90	7.6	24.7
	3	31	70	4.9	11.3
H_2O	1	Traces of ethanol and acetaldehyde	90	8.1	23.7
	2		90	5.0	13.3
	3		80	6.5	16.1

* 20 ml aqueous solution in all treatments

Differences between means of numbers of germinated seeds and between means of lengths of roots for each treatment were not significant (P = 0.05).

Table XXVII. Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with 20 ml aqueous solutions of acetaldehyde, or ethanol, or isobutanol, or pentanol, and in controls paired with 20 ml sterile deionized water.

Experiment III

Treatment	Assembly No.	Peak height of authentic component of each solution at end of experiment (mm)	% germination	Mean lengths of roots (mm)	Mean lengths of shoots (mm)
*0.05% acetaldehyde	1	880	70	5.0	10.0
	2	60	90	7.2	21.7
	3	31	90	7.3	24.6
0.05% ethanol	1	260	60	7.0	8.5
	2	640	80	9.0	26.4
	3	1040	90	5.4	11.8
0.05% isobutanol	1	140	90	7.3	28.0
	2	280	70	7.1	17.4
	3	123	80	6.5	12.1
0.05% pentanol	1	12	70	6.7	21.1
	2	92	90	7.1	21.8
	3	61	50	8.0	21.0
H_2O	1	Traces of ethanol and acetaldehyde	70	5.4	9.4
	2		50	8.8	22.0
	3		50	7.6	15.6

* 20 ml aqueous solution in all treatments

Differences between means of numbers of germinated seeds and between means of lengths of roots for each treatment were not significant (P=0.05).

Table XXVIII. Peak heights produced by gases from standard aqueous solutions of each of acetaldehyde, ethanol, isobutanol, and pentanol in paired bottle assemblies.

Volume of pure chemical added to 20 ml sterile deionized water	Assembly No.	Acetaldehyde Peak height (mm)	Ethanol Peak height (mm)	Isobutanol Peak height (mm)	Pentanol Peak height (mm)
1 μl	1	532	140	171	102
	2	404	162	116	78
	3	458	216	162	125
	4	608	219	191	103
	5	376	156	196	101
	6	554	131	212	164
	7	312	117	126	169
	8	468	141	126	187
	9	572	136	162	167
	10	488	137	172	127
	Average:	477	155	163	133

Table XXIX. Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with standard 20 ml solutions of acetaldehyde, ethanol, isobutanol and pentanol and in controls, paired with 20 ml sterile deionized water.

Experiment I

Treatment	Assembly No.	Peak heights of authentic constituents (mm)								% germination		Mean lengths of shoots (mm)	Mean lengths of roots (mm)	
		Immediately after pairing				At end of biological tests								
		Acetaldehyde	Ethanol	Isobutanol	Pentanol	Acetaldehyde	Ethanol	Isobutanol	Pentanol		Mean			Mean
I	1	175	58	7	4	108	124	27	14	90		13.5	38.7	
	2	179	72	4	3	69	112	26	8	90	93	11.1	43.1	36.9
	3	228	32	6	4	49	54	16	14	100		11.2	28.8	
II	1	3232	108	209	82	840	142	33	14	100		11.0	27.7	
	2	1728	117	32	13	740	192	44	18	100	93	12.7	32.3	34.2
	3	1776	592	74	24	800	159	32	15	80		11.4	42.5	
III	1	13568	1568	244	91	3008	438	66	24	70		3.7	1.9	
	2	14336	848	175	56	6400	564	103	40	80	80	5.5	1.2	2.3
	3	7552	680	108	28	2026	400	111	45	90		4.8	3.7	
H_2O	1									100		13.6	26.6	
	2	Traces of acetaldehyde and ethanol								100	100	13.4	46.9	41.0
	3									100		12.8	49.6	

Least significant difference between means of lengths of roots in different treatments = 16.4 (P = 0.05)

Differences between means of numbers of germinated seeds for each treatment were not significant (P = 0.05)

*20 ml aqueous mixtures as described in text Figure (xviii).

Table XXX. Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with standard 20 ml solutions of acetaldehyde, ethanol, isobutanol and pentanol and in controls, paired with 20 ml sterile deionized water.

Experiment II

*Treatment	Assembly No.	Peak heights of authentic constituents (mm)								% germination		Mean lengths of shoots (mm)	Mean lengths of roots (mm)	
		Immediately after pairing				At end of biological tests								
		Acetaldehyde	Ethanol	Isobutanol	Pentanol	Acetaldehyde	Ethanol	Isobutanol	Pentanol		Mean			Mean
I	1	155	3	8	13	270	294	32	14	90		16.8	50.2	
	2	70	7	11	4	26	142	26	8	90	93	17.8	47.0	49.0
	3	240	9	8	7	141	170	58	15	100		15.2	49.8	
II	1	3120	109	12	18	888	318	51	16	100		10.6	20.3	
	2	952	60	10	16	616	250	50	16	100	100	17.3	35.6	32.3
	3	1136	57	18	18	420	700	119	27	100		17.2	21.1	
III	1	5544	564	191	86	3680	1248	217	70	100		5.6	5.7	
	2	5182	528	162	77	2688	1416	260	105	100	100	9.2	8.5	6.8
	3	5280	488	140	64	3216	1536	167	67	100		5.8	6.2	
Control	1									100		18.7	75.2	
	2	Traces of acetaldehyde and ethanol								100	100	20.2	52.5	53.9
	3									100		11.7	37.0	

Least significant difference between means of lengths of roots in different treatments = 21.7 (P = 0.05)

Differences between means of numbers of germinated seeds for each treatments were not significant (P = 0.05)

*20 ml aqueous mixtures as described in text Figure (xix).

Table XXXI. Germination of lettuce seeds and growth of lettuce seedlings in paired bottle assemblies containing lettuce paired with 20 ml standard solutions of acetaldehyde, ethanol, isobutanol and pentanol and in controls, paired with 20 ml sterile deionized water.

Experiment III

*Treatment	Assembly No.	Peak heights of authentic constituents (mm)								% germination		Mean lengths of shoots (mm)	Mean lengths of roots	
		Immediately after pairing				At end of biological tests								
		Acetaldehyde	Ethanol	Isobutanol	Pentanol	Acetaldehyde	Ethanol	Isobutanol	Pentanol		Mean		(mm)	Mean
I	1	214	94	14	6	78	140	30	12	100		13.4	42.4	
	2	400	161	40	27	132	137	26	12	90	97	10.8	33.6	36.0
	3	35	60	22	7	42	47	17	9	100		10.9	32.0	
II	1	380	384	115	56	18	125	28	13	100		14.4	45.9	
	2	1216	484	138	52	172	182	41	19	80	90	8.8	16.6	30.4
	3	2560	258	168	59	784	158	74	32	90		11.1	28.6	
III	1	12544	1448	340	151	3968	908	164	79	100		4.6	2.3	
	2	6016	864	200	109	2112	856	170	80	100	97	5.2	3.4	2.5
	3	12544	1408	370	152	2572	407	148	64	90		4.4	1.9	
Control	1	Traces of acetaldehyde and ethanol								100		13.6	26.6	
	2									100	100	13.4	46.9	41.0
	3									100		12.8	49.6	

Least significant difference means of lengths of roots in different treatments = 22.8 mm (P = 0.05)

Differences between means of numbers of germinated seeds for each treatment are not significant (P = 0.05)

*20 ml aqueous mixtures as described in text Figure (xviii).

Table XXXII. Changes in concentrations of authentic compounds from standard aqueous solutions in paired bottle assemblies containing lettuce paired with standard 20 ml solutions of acetaldehyde, ethanol, isobutanol and pentanol.

Mixture	Number of days after pairing	Acetaldehyde concentration* Assembly No. 1	2	3	Ethanol concentration Assembly No. 1	2	3	Isobutanol concentration Assembly No. 1	2	3	Pentanol concentration Assembly No. 1	2	3
I	0	100	100	100	100	100	100	100	100	100	100	100	100
	1	37	21	137	72	82	79	38	130	48	100	66	158
	2	43	56	43	31	140	33	43	89	30	25	64	42
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	28	1	35	33	46	35	57	35	32	50	41	29
	5	21	7	40	22	52	50	12	35	30	50	43	38
	6	-	-	-	-	-	-	-	-	-	-	-	-
	7	24	22	14	51	46	40	11	65	16	42	45	29
II	0	100	100	100	100	100	100	100	100	100	100	100	100
	1	2.1	-	77	32	35	138	33	21	103	36	47	119
	2	0.9	49	46	35	40	75	31	26	58	16	37	86
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	0.95	21	19	38	39	49	18	37	35	11	29	51
	5	1.1	15	20	36	30	45	16	34	30	6	30	55
	6	-	-	-	-	-	-	-	-	-	-	-	-
	7	1.3	14	30	33	38	63	24	30	44	23	37	54
III	0	100	100	100	100	100	100	100	100	100	100	100	100
	1	1.8	3.8	6.1	15	95	104	13	84	90	12	62	91
	2	2.3	3.1	4.6	30	144	64	23	100	60	21	72	52
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	4.9	1.4	4.2	45	50	31	31	50	55	23	68	29
	5	3.2	1.4	3.6	29	63	41	43	77	24	46	50	28
	6	-	-	-	-	-	-	-	-	-	-	-	-
	7	2.1	0.8	2.1	41	67	29	48	85	40	52	73	42

*Concentrations are expressed as a percentage of the concentration of each immediately after the assemblies were paired.
For composition of mixtures see text Figure (XIX).

- = No measurement made.

Initial Peak Heights (mm)

	Acetaldehyde			Ethanol			Isobutanol			Pentanol		
Mixture I	214	400	70	180	161	120	28	40	44	8	27	14
Mixture II	1380	1216	2560	324	484	258	115	138	168	56	52	59
Mixture III	12544	16016	12544	1448	864	1408	340	200	370	151	109	152

Table XXXIII. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with 10 ml standard aqueous solutions of acetaldehyde and in controls paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Expt. No.	Treatment	Assembly No.	Peak height of acetaldehyde at end of experiment (mm)	% germination	Mean Lengths of shoots (mm)	Mean Lengths of roots (mm)	Mean
I	F. noxius	1	12	90	9.2	7.0	8.4
		2	16	90	13.0	12.9	
		3	12	80	10.2	5.2	
		4	-	-	-	-	
	Uninoculated 5% malt agar	1	48	90	16.3	21.8	20.8
		2	32	1000	11.2	18.9	
		3	-	-	-	-	
		4	20	100	9.7	21.8	
	0.002% acetaldehyde	1	-	80	11.0	17.1	18.8
		2	124	90	13.3	25.8	
		3	-	80	10.4	16.5	
		4	36	80	18.8	15.6	
	0.01% acetaldehyde	1	80	80	11.1	17.1	20.7
		2	48	80	11.4	17.0	
		3	-	100	13.9	23.8	
		4	16	100	14.2	25.0	
	H_2O	1	-	-	-	-	22.2
		2	12	80	14.5	19.4	
		3	8	100	11.9	20.6	
		4	-	100	12.5	26.7	
II	F. noxius	1	40	80	9.8	5.9	7.9
		2	-	90	10.9	3.6	
		3	-	100	11.9	9.6	
		4	-	100	11.2	12.6	
	uninoculated 5% malt agar	1	2	100	12.3	20.3	19.8
		2	7	90	9.1	16.9	
		3	-	-	-	-	
		4	-	90	9.1	22.2	
	0.002% acetaldehyde	1	-	100	12.4	23.1	23.8
		2	6	100	9.6	23.2	
		3	15	100	11.8	23.1	
		4	-	90	16.4	25.2	
	0.01% acetaldehyde	1	24	90	13.8	25.7	24.1
		2	6	100	13.2	25.2	
		3	9	100	11.1	20.5	
		4	-	80	13.7	25.1	
	0.1% acetaldehyde	1	9	90	10.8	16.0	20.7
		2	18	100	13.2	26.2	
		3	-	90	10.0	21.0	
		4	-	100	12.5	19.7	
	H_2O	1	-	100	10.3	22.0	22.7
		2	3	100	10.7	22.1	
		3	-	-	-	-	
		4	-	100	11.3	24.1	
III	F. noxius	1	16	90	10.2	10.0	8.8
		2	27	80	9.6	6.2	
		3	20	90	10.6	7.2	
		4	16	100	12.6	11.7	
	Uninoculated 5% malt agar	1	13	90	14.6	26.6	21.3
		2	15	90	7.2	18.3	
		3	10	100	9.1	20.9	
		4	13	100	7.4	19.5	
	0.002% acetaldehyde	1	-	90	10.4	12.7	19.9
		2	22	90	10.7	26.1	
		3	4	100	10.5	13.4	
		4	-	100	10.1	20.1	
	0.01% acetaldehyde	1	16	90	8.8	14.6	17.1
		2	11	90	7.0	17.2	
		3	19	100	8.4	17.8	
		4	19	100	8.2	18.7	
	H_2O	1	-	100	8.3	10.0	16.2
		2	8	90	9.8	14.6	
		3	9	100	12.0	23.6	
		4	9	100	8.0	16.6	

Least significant differences between the means of lengths of roots for each treatment in experiments I, II and III are 6.2mm, 6.3mm and 8.1mm respectively. Differences between means of numbers of germinated seeds for each treatment are not significant. (P=0.05).

- = No measurement made.

Table XXXIV. Germination of lettuce seeds and growth of lettuce seedlings in paired petri dish assemblies containing lettuce paired with *F. oxius* cultures of 10 ml standard aqueous solutions of ethanol and in controls paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Expt. No.	Treatment	Assembly No.	Peak height of ethanol at end of experiment (mm)	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
I	*F. oxius*	1	13	100	15.6	20.0	13.2
		2	13	100	11.1	7.9	
		3	10	100	11.5	10.9	
		4	20	100	13.4	13.8	
	Uninoculated 5% malt agar	1	20	90	12.2	23.3	25.6
		2	-	100	19.6	32.9	
		3	-	-	-	-	
		4	20	90	15.4	20.6	
	0.01% ethanol	1	13	100	10.0	24.3	25.3
		2	24	100	11.9	21.9	
		3	12	100	14.0	28.9	
		4	-	100	13.2	26.1	
	0.05% ethanol	1	18	90	12.6	17.2	24.7
		2	24	100	12.4	20.0	
		3	16	90	19.7	31.1	
		4	20	100	14.6	30.5	
	0.1% ethanol	1	19	100	14.1	35.0	29.6
		2	20	100	13.2	30.1	
		3	24	90	17.2	23.7	
		-	-	-	-	-	
	H_2O	1	17	90	19.3	22.8	22.0
		2	-	80	16.1	23.6	
		3	22	100	12.2	21.0	
		4	14	100	14.8	20.6	
II	*F. oxius*	1	9	100	12.1	13.2	12.9
		2	3	100	9.4	8.2	
		3	-	-	-	-	
		4	7	100	11.1	17.2	
	Uninoculated 5% malt agar	1	-	-	-	-	
		2	-	-	-	-	
		3	-	-	-	-	
		4	-	-	-	-	
	0.01% ethanol	1	2	100	11.4	27.7	23.8
		2	3	90	14.4	26.3	
		3	4	70	10.5	18.1	
		4	2	100	10.0	23.1	
	0.05% ethanol	1	4	100	10.8	25.0	22.8
		2	4	80	11.2	23.9	
		3	2	80	11.5	18.4	
		4	2	80	12.1	23.8	
	0.1% ethanol	1	5	100	11.2	22.6	23.9
		2	2	100	11.5	32.8	
		3	3	100	12.7	22.2	
		4	2	90	11.3	17.9	
	H_2O	1	5	90	10.6	21.1	24.7
		2	3	100	12.7	25.3	
		3	4	100	13.2	24.6	
		4	3	100	14.3	27.8	
III	*F. oxius*	1	26	100	9.2	13.2	16.5
		2	-	-	-	-	
		3	14	100	10.8	19.0	
		4	20	90	17.2	17.3	
	uninoculated 5%malt agar	1	15	100	12.2	27.8	26.0
		2	-	100	10.3	24.2	
		3	-	-	-	-	
		4	9	-	-	-	
	0.01% ethanol	1	26	100	12.4	23.1	24.4
		2	3	90	10.8	16.1	
		3	6	90	11.9	22.6	
		4	36	90	16.0	35.8	
	0.05% ethanol	1	14	100	12.2	21.0	27.5
		2	-	90	13.1	26.0	
		3	9	100	15.3	31.5	
		4	9	100	15.8	31.6	
	0.1% ethanol	1	-	100	10.5	22.1	26.0
		2	38	100	13.0	25.0	
		3	15	100	14.8	28.4	
		4	-	80	14.2	28.4	
	H_2O	1	9	100	13.8	29.0	24.3
		2	-	100	13.3	19.0	
		3	13	100	11.8	22.8	
		4	11	100	13.8	26.4	

Least significant differences between the means of lengths of roots for each treatment in experiments I, II and III are 7.8, 10.9 and 10.6 respectively.
Differences between means of numbers of germinated seeds for each treatment are not significant.
(P=0.05)
- = no measurement made.

Table XXXV. Germination of lettuce seeds and growth of lettuce seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures or 10 ml standard aqueous solutions of isobutanol, and in controls, paired with uninoculated 5% malt agar, or 10 ml sterile deionized water.

Expt. No.	Treatment	Assembly No.	Peak height of isobutanol at end of experiment (mm)	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
I	*F. noxius*	1	4	90	7.1	4.4	6.1
		2	6	100	10.4	9.4	
		3	-	80	8.0	8.0	
		4	-	80	5.6	2.5	
	Uninoculated 5% malt agar	1	-	90	12.0	16.7	16.2
		2	2	-	-	-	
		3	-	90	9.7	15.6	
		4	-	-	-	-	
	0.002% isobutanol	1	2	80	13.6	16.6	13.7
		2	4	100	11.7	14.0	
		3	-	90	10.6	12.4	
		4	2	80	11.4	11.8	
	0.01% isobutanol	1	8	100	8.7	12.7	14.9
		2	9	90	11.9	17.0	
		3	-	90	11.9	13.9	
		4	-	90	9.9	16.0	
	0.1% isobutanol	1	14	70	15.9	19.3	18.0
		2	15	-	-	-	
		3	6	90	10.0	16.2	
		4	-	80	12.4	18.6	
	H_2O	1	-	90	9.6	14.3	16.0
		2	-	100	11.0	18.9	
		3	2	90	10.6	18.3	
		4	-	80	10.9	12.5	
II	*F. noxius*	1	18	80	4.9	4.4	5.3
		2	11	40	4.8	3.0	
		3	-	90	8.9	9.4	
		4	-	50	3.0	1.4	
	Uninoculated 5% malt agar	1	-	100	11.7	25.0	25.9
		2	3.5	90	12.1	19.2	
		3	-	80	9.5	23.2	
		4	-	90	12.6	36.4	
	0.002% isobutanol	1	-	90	9.2	20.1	20.2
		2	-	90	9.4	21.5	
		3	-	90	7.3	18.2	
		4	1.5	90	10.4	20.9	
	0.01% isobutanol	1	-	100	7.5	25.6	28.4
		2	3.0	100	9.8	20.7	
		3	3.5	90	10.7	25.0	
		4	-	70	10.3	22.3	
	0.1% isobutanol	1	-	70	7.0	27.4	24.2
		2	6	90	8.4	29.6	
		3	5	80	8.1	16.8	
		4	4	80	8.8	23.2	
	H_2O	1	-	100	11.7	22.6	20.0
		2	0	90	10.0	19.8	
		3	0	90	6.1	19.4	
		4	-	90	8.4	18.2	
III	*F. noxius*	1	13	50	7.2	1.2	1.1
		2	3	60	5.5	1.0	
		3	-	70	7.4	1.1	
		4	-	70	0.7	1.0	
	Uninoculated 5% malt agar	1	3	90	8.7	11.9	16.1
		2	1	90	11.1	20.4	
		3	-	100	9.6	16.1	
		4	2	-	-	-	
	0.002% isobutanol	1	9	90	9.2	15.9	15.0
		2		80	8.2	14.0	
		3		90	8.0	10.4	
		4	2	90	12.3	19.7	
	0.01% isobutanol	1	9	90	8.1	12.3	14.1
		2	15	90	8.0	13.5	
		3	-	100	9.2	15.3	
		4	-	100	12.4	15.3	
	0.1% isobutanol	1	4	90	7.7	10.7	10.9
		2	6	80	11.4	12.9	
		3	14	80	6.8	7.3	
		4	-	80	8.4	12.9	
	H_2O	1	2	80	5.9	10.6	9.8
		2	3	80	7.9	9.4	
		3	2	70	6.4	7.3	
		4	2	80	7.9	11.8	

Least significant difference between the means of lengths of roots for each treatment in experiments I, II and III are 5.5 mm, 5.7 mm and 5.2 mm respectively.

Differences between means of numbers of germinated seeds in each treatment were not significant in experiment I. Least significant differences in experiments II and III are = 17.6, 13.6 ($P = 0.05$)

seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures or standard 10 ml aqueous solutions of pentanol and in controls, paired with uninoculated 5% malt agar or sterile deionized water.

Expt. No.	Treatment	Assembly No.	Peak height of pentanol at end of experiment (mm)	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
I	F. noxius	1	1	90	9.0	1.0	
		2	0	90	8.4	1.6	1.8
		3	-	100	10.9	2.5	
		4	-	90	12.6	2.3	
	Uninoculated 5% malt agar	1	0	90	16.0	22.0	
		2	-	100	12.6	21.7	22.1
		3	-	100	11.5	22.6	
		4	-	-	-	-	
	0.002% pentanol	1	1	100	13.1	21.8	
		2	10	90	14.6	20.1	16.8
		3	3	100	12.4	17.6	
		4	-	90	11.2	15.8	
	0.01% pentanol	1	3	100	16.0	17.8	
		2	2	100	14.9	20.0	18.9
		3	1	100	14.7	18.7	
		4	9	100	14.4	19.4	
	0.1% pentanol	1	3	100	11.5	21.0	
		2	1	90	9.8	19.3	18.0
		3	2	90	12.7	14.4	
		4	1	80	16.3	17.4	
	H_2O	1	-	90	14.4	26.4	
		2	0	90	15.7	19.8	19.9
		3	0	100	10.0	12.3	
		4	0	100	14.9	21.0	
II	F. noxius	1	1	90	9.7	3.9	
		2	1	80	.0	2.5	3.5
		3	3	90	7.1	4.1	
		4	2	100	8.4	3.7	
	Uninoculated 5% malt agar	1	1	80	12.4	23.5	
		2	0	80	10.9	20.8	21.7
		3	-	90	9.9	18.7	
		4	1	90	10.1	23.8	
	0.002% pentanol	1	1	100	10.3	23.7	
		2	0	90	9.2	22.3	21.0
		3	0	90	14.0	25.3	
		4	0	100	9.4	15.9	
	0.01% pentanol	1	0	90	9.7	16.7	
		2	1	90	7.7	6.6	13.9
		3	3	100	13.1	17.9	
		4	2	90	10.0	14.6	
	0.1% pentanol	1	2	100	12.5	17.7	
		2	3	100	8.6	6.3	11.4
		3	2	80	8.4	9.0	
		4	3	90	10.9	12.6	
	H_2O	1	1	100	11.2	11.8	
		2	0	100	12.4	17.3	10.8
		3	0	90	9.0	9.7	
		4	0	100	5.5	4.3	
III	F. noxius	1	0	70	6.0	2.4	
		2	4	80	5.6	2.9	7.0
		3	2	70	11.9	9.4	
		4	3	100	9.2	13.1	
	Uninoculated 5% malt agar	1	0	90	7.6	13.7	
		2	-	-	-	-	15.6
		3	-	-	-	-	
		4	-	90	10.2	17.4	
	0.002% pentanol	1	-	100	9.2	15.2	
		2	-	80	9.8	19.4	16.3
		3	0	80	9.6	14.0	
		4	0	80	8.6	16.7	
	0.01% pentanol	1	0	90	8.2	14.8	
		2	-	80	11.8	15.4	15.8
		3	3	100	11.0	14.8	
		4	2	100	11.6	18.2	
	0.1% pentanol	1	3	80	9.1	13.0	
		2	4	90	17.1	22.3	17.4
		3	3	80	9.4	18.6	
		4	2	70	10.6	15.7	
	H_2O	1	0	80	10.0	14.9	
		2	-	90	10.6	13.7	15.2
		3	-	100	10.7	17.1	
		4	-	-	-	-	

Least significant differences between the means of lengths of roots for each treatment in experiments I, II and III are 4.9 mm, 9.6 mm, and 6.1 mm respectively (P = 0.05).

Differences between the mean numbers of germinated seeds for each treatment are not significant (P = 0.05).

Table XXXVII.

Appendix

Peak heights produced by gases from 10 ml standard aqueous solutions of each of acetaldehyde, ethanol, isobutanol and pentanol in paired petri dish assemblies.

Concentration of standard solution as % by volume of authentic component.	Assembly No.	Acetaldehyde Peak height (mm)	Ethanol Peak height (mm)	Isobutanol Peak height (mm)	Pentanol Peak height (mm)
0.0002	1	49		23	60
	2	64		26	52
	3	76		11	34
	4	36		21	28
	5	85		23	38
	6	58		20	64
	7	43		52	52
	8	85		20	78
	9			16	32
	10			44	
	11			15	
	12			17	
	13			27	
	14			26	
	15			25	
0.001	1	824	37	41	74
	2	616	71	66	132
	3	672	31	116	100
	4	640	36	58	136
	5	672	29	96	80
	6	698	75	52	126
	7	624	105	105	164
	8	992	56	92	160
	9		33	115	146
	10		62	116	
	11		31	77	
	12			76	
0.01	1	4352	260	116	111
	2	3840	194	90	64
	3	8064	149	60	97
	4	6272	196	230	95
	5	3520	122	205	96
	6	4928	220	150	93
	7	2624	133	135	80
	8	4480	155	76	88
	9	2112	192	96	
	10	2432	210	131	
	11	3680	218	167	
	12	6144	112	180	
	13	5440	161	78	
	14		165	112	
	15		220	190	
0.1	1	26856	652		
	2	30704	876		
	3	31616	640		
	4	18336	916		
	5	19572	752		
	6	30146	520		
	7	28441	860		
	8	30068	720		
	9		1060		
	10		840		

Table XXXVIII. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures, standard 10 ml aqueous mixtures of acetaldehyde, ethanol, isobutanol and pentanol and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Experiment I

Treatment	Assembly No.	Peak heights at end of experiment (mm) Acetaldehyde	Ethanol	Isobutanol	Pentanol	% germination	Mean	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	8	12	2	3	100		10.2	5.1	
	2	4	10	4	0	90	95	10.8	9.1	9.2
	3	8	10	11	4	100		11.2	10.0	
	4	6	4	3	4	90		11.8	12.7	
Uninoculated 5% malt agar	1	0	5	0	0	100		8.9	28.0	
	2	4	1	1	1	100	95	9.8	23.7	25.9
	3	2	2	3	2	90		9.0	21.3	
	4	1	2	0	0	90		11.4	30.5	
I	1	46	11	3	1	80		13.5	26.1	
	2	135	12	0	0	90	88	10.3	20.2	23.1
	3	56	12	3	3	100		8.3	21.5	
	4	82	10	4	2	80		10.5	24.4	
II	1	59	96	17	7	100		6.1	17.4	
	2	115	48	5	3	90	95	6.1	21.2	20.1
	3	51	61	12	4	100		6.6	21.9	
	4	97	23	8	5	90		6.3	19.9	
III	1	85	144	37	16	0		0.0	0.0	
	2	98	83	24	12	30	15	3.6	0.7	0.4
	3	59	121	47	17	30		1.0	1.0	
	4	83	263	67	41	0		0.0	0.0	
H_2O	1	10	0	0	0	100		10.9	22.6	
	2	4	1	0	2	90	97	10.6	16.9	22.4
	3	2	2	1	2	100		10.7	25.0	
	4	-	-	-	-	100		11.0	25.1	

Least significant difference between means of lengths of roots for each treatment is 4.1 mm ($P = 0.05$)
Least significant difference between means of numbers of germinated seeds for each treatment is 13% ($P = 0.05$)
- = no measurement made.

Composition of mixtures -

Mixture No.	Volume in 10 ml deionized water (µl) Acetaldehyde	Ethanol	Isobutanol	Pentanol
I	10	10	1	1
II	1	1	0.1	0.1
III	0.1	0.1	0.02	0.02

Table XXXIX. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures, standard 10 ml aqueous mixtures of acetaldehyde, ethanol, isobutanol and pentanol and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Experiment II

Treatment	Assembly No.	Peak heights at end of experiment (mm) Acetaldehyde	Ethanol	Isobutanol	Pentanol	% germination	Mean	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	12	17	6	2	80		15.2	20.7	
	2	6	14	4	1	100	90	9.4	11.3	15.3
	3	4	10	1	4	100		11.2	18.4	
	4	7	12	2	4	80		9.0	10.7	
Uninoculated 5% malt agar	1	4	4	1	0	100		11.4	23.5	
	2	4	2	2	0	90	95	19.9	24.7	26.5
	3	7	3	0	0	90		11.0	25.6	
	4	2	6	0	1	100		11.9	32.1	
I	1	15	366	0	0	100		8.0	20.3	
	2	75	1040	17	8	100	97	10.4	24.4	19.8
	3	29	1152	28	10	90		11.3	16.0	
	4	38	1704	23	11	100		8.5	18.5	
II	1	38	760	8	2	100		6.2	10.9	
	2	112	1240	31	11	100	97	5.6	3.0	6.1
	3	38	700	11	4	90		6.0	4.2	
	4	472	864	28	14	100		5.0	6.5	
III	1	18	388	33	8	0		0.0	0.0	
	2	92	840	30	25	0	0	0.0	0.0	0.0
	3	56	1684	62	28	0		0.0	0.0	
	4	50	640	60	28	0		0.0	0.0	
H_2O	1	0	0	2	0	100		11.5	20.3	
	2	0	0	0	0	100	100	9.0	23.4	20.8
	3	-	-	-	-	100		10.5	19.8	
	4	-	-	-	-	100		10.3	19.9	

Least significant difference between means of lengths of roots for each treatment = 5.4 mm (P = 0.05)
Least significant difference between means of numbers of germinated seeds for each treatment = 10% (P = 0.05)
- = no measurement made.
For composition of mixtures see appendix Table XXXVIII.

Table XL. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures, standard 10 ml aqueous mixtures of acetaldehyde, ethanol, isobutanol and pentanol and in controls, paired with uninoculated 5% malt agar or 10 ml sterile deionized water.

Experiment III

Treatment	Assembly No.	Peak heights at end of experiment (mm) Acetaldehyde	Ethanol	Isobutanol	Pentanol	% germination	Mean	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	1	6	2	1	100		13.5	15.6	
	2	3	5	4	0	100	100	12.8	16.2	14.9
	3	2	2	1	2	100		12.3	13.3	
	4	6	12	2	3	100		10.1	14.4	
Uninoculated 5% malt agar	1	2	6	2	0	100		12.8	20.4	
	2	2	2	0	0	90	95	9.3	26.8	22.7
	3	3	2	0	0	90		10.2	22.4	
	4	-	-	-	-	100		10.8	21.2	
I	1	2	5	2	1	100		12.0	18.8	
	2	2	2	1	0	100	100	12.4	28.2	23.1
	3	2	12	2	1	100		12.1	22.7	
	4	2	10	6	1	100		10.4	22.8	
II	1	6	18	3	4	80		6.8	14.0	
	2	7	98	11	12	90	92	7.3	21.4	15.3
	3	10	67	14	4	100		6.9	16.1	
	4	21	75	15	6	100		6.9	9.8	
III	1	7	186	46	19	10		2.0	1.0	
	2	4	204	38	24	40	15	5.2	2.5	1.4
	3	7	52	8	7	10		3.0	1.0	
	4	130	154	54	26	0		0.0	0.0	
H_2O	1	1	4	0	1	100		12.3	19.0	
	2	3	2	0	0	100	100	9.3	24.2	20.7
	3	2	5	0	0	100		10.2	18.3	
	4	-	-	-	-	100		10.8	20.4	

Least significant difference between means of lengths of roots for each treatment = 2.8 mm (P = 0.05)
Least significant difference between means of numbers of germinated seeds for each treatment = 13.7% (P = 0.05)
- = no measurement made.
For composition of mixtures see appendix Table XXXVIII.

Table XLI. Changes in *concentrations of authentic compounds in paired petri dish assemblies containing lettuce paired with 20 ml standard aqueous solutions of acetaldehyde, ethanol, isobutanol and pentanol.

Treatment	Number of days after pairing	Acetaldehyde concentration			Ethanol concentration			Isobutanol concentration			Pentanol concentration		
		Assembly No.			Assembly No.			Assembly No.			Assembly No.		
		1	2	3	1	2	3	1	2	3	1	2	3
I	0	100	100	100	100	100	100	100	100	100	100	100	100
	1	43	27	40	554	303	855	139	212	760	350	233	350
	2	25	78	-	22	350	-	200	212	-	200	133	-
	3	9	37	34	14	505	1388	154	350	1700	200	467	2400
	4	-	-	-	-	-	-	-	-	-	-	-	-
	5	3	4	8	31	172	31	69	75	140	150	75	150
	6	5	12	1	108	244	51	25	150	400	550	133	900
	7	2	32	4	42	938	978	77	487	640	200	567	550
II	0	100	100	100	100	100	100	100	100	100	100	100	100
	1	9	33	13	120	60	189	170	137	316	160	129	316
	2	11	5	6	41	58	165	103	96	185	80	136	-
	3	1	3	3	34	68	115	60	147	185	80	143	193
	4	-	-	-	-	-	-	-	-	-	-	-	-
	5	0.2	0.2	1	20	30	96	37	54	116	27	43	93
	6	18	0.1	.3	56	56	65	143	100	66	113	100	50
	7	13	0.1	.5	59	80	99	70	96	89	67	79	57
III	0	100	100	100	100	100	100	100	100	100	100	100	100
	1	17	36	39	231	105	133	138	115	204	197	83	234
	2	18	33	11	59	72	103	69	86	-	106	67	3
	3	-	2	1	58	192	-	78	153	137	139	273	104
	4	-	-	-	-	-	-	-	-	-	-	-	-
	5	2	1	1	34	186	-	30	21	38	47	21	44
	6	2	.1	.1	58	453	42	46	75	41	78	76	52
	7	42	.4	6	63	338	27	81	60	85	119	54	102

* Concentrations are expressed as a percentage of the concentration of each immediately after the assemblies were paired.

- = no measurement made.

For composition of mixtures see appendix Table XXXVIII.

Mixture No.	Initial peak heights/assembly (mm)											
	Acetaldehyde			Ethanol			Isobutanol			Pentanol		
I	205	215	220	17	21	18	13	8	5	2	3	2
II	1440	1120	1408	182	201	141	30	30	26	15	14	14
III	7680	9024	6048	512	856	808	116	150	146	36	76	64

Table XLII. Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with 4 week old F. noxius cultures and in paired controls with uninoculated 5% malt agar.

Expt. No.	Treatment	% germination							Mean lengths of shoots (mm)							Mean lengths of roots (mm)						
		Assembly No						Mean	Assembly No.						Mean	Assembly No.						Mean
		1	2	3	4	5	6		1	2	3	4	5	6		1	2	3	4	5	6	
I	F. noxius	100	70	90	90	100	100	92	17.9	27.2	20.9	18.7	12.7	18.5	19.2	16.9	18.9	18.6	15.2	17.5	21.9	18.2
	Control	100	90	100	100	100	100	98	19.9	18.3	16.9	15.5	18.7	20.3	18.3	23.0	24.2	20.7	20.9	22.1	25.3	22.7
II	F. noxius	100	90	90	90	100	100	95	18.1	16.2	15.2	15.1	15.2	17.2	16.2	21.0	18.2	13.6	12.8	16.4	16.2	16.4
	Control	100	100	100	100	100	90	98	21.1	12.6	15.6	16.0	21.0	23.8	18.0	27.4	21.6	26.9	23.1	31.3	24.9	25.9
III	F. noxius	80	100	90	100	90	100	93	11.5	13.2	14.4	15.5	12.8	11.9	13.2	19.2	33.0	19.1	13.1	17.0	17.3	19.8
	Control	100	100	100	90	90	100	97	11.4	11.5	14.5	10.7	10.4	19.4	11.3	28.2	17.8	29.0	18.7	15.4	19.4	21.4

Table XLIII.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of oxalic acid.

Experiment I

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	90	13.1	16.3	
	2	100	8.9	10.8	
	3	100	10.6	6.3	11.5
	4	100	12.2	12.6	
Control	1	100	10.9	27.1	
	2	100	10.6	20.8	
	3	100	9.8	19.0	22.1
	4	100	9.0	21.3	
F. noxius + 2 ml 1N oxalic acid	1	70	7.9	10.9	
	2	100	13.4	30.2	
	3	80	9.9	19.8	18.8
	4	90	10.8	14.3	
Control + 2 ml 1N oxalic acid	1	90	10.9	11.1	
	2	100	10.0	19.6	
	3	90	10.9	10.1	13.6
	4	100	8.4	13.6	

Least significant differences between means of lengths of roots for each treatment = 4.8 mm ($P = 0.05$).

Differences between means of numbers of germinated seeds for each treatment are not significant ($P = 0.05$).

Table XLIV.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of oxalic acid.

Experiment II

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	90	4.9	10.8	
	2	100	8.3	9.5	10.8
	3	100	7.3	11.1	
	4	100	9.0	11.8	
Control	1	90	7.6	18.0	
	2	90	8.4	24.3	22.0
	3	100	7.7	26.8	
	4	100	7.0	18.8	
F. noxius + 2 ml oxalic acid	1	90	5.8	13.3	
	2	100	5.2	13.1	13.8
	3	80	6.0	11.1	
	4	100	8.1	17.6	
Control + 2 ml oxalic acid	1	100	8.5	16.6	
	2	90	7.9	18.2	15.0
	3	90	7.7	9.9	
	4	100	7.6	15.4	

Least significant difference between means of lengths of roots for each treatment = 5.7 mm ($P = 0.05$).

Differences between means of number of germinated seeds in each treatment are not significant ($P = 0.05$).

Table XLV.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of oxalic acid.

Experiment III

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	80	9.0	2.6	
	2	90	5.7	3.3	3.1
	3	90	9.0	3.2	
	4	80	7.1	3.5	
Control	1	80	8.5	9.5	
	2	80	6.5	15.4	12.7
	3	90	6.7	8.1	
	4	90	11.6	17.7	
F. noxius + 2 ml 1N oxalic acid	1	70	3.9	1.1	
	2	90	6.2	3.1	5.0
	3	90	9.6	4.6	
	4	90	10.0	11.3	
Control + 2 ml 1N oxalic acid	1	90	8.3	14.0	
	2	90	6.6	19.1	15.4
	3	80	6.1	10.8	
	4	80	9.2	17.8	

Least significant difference between the means of lengths of roots for each treatment = 3.5 mm (P = 0.05).

Differences between means of numbers of germinated seeds for each treatment are not significant (P = 0.05).

Table XLVI.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of silver nitrate.

Experiment I

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	100	8.5	7.8	7.7
	2	100	8.6	9.5	
	3	100	6.2	6.6	
	4	100	6.7	6.8	
Control	1	90	7.6	11.9	16.8
	2	80	9.8	17.8	
	3	100	7.9	23.2	
	4	100	6.8	14.4	
F. noxius + 2 ml 0.1M $AgNO_3$	1	100	9.7	16.3	17.1
	2	100	10.6	18.5	
	3	90	12.6	19.2	
	4	100	10.2	14.4	
Control + 2 ml 0.1M $AgNO_3$	1	100	6.3	21.7	21.5
	2	100	14.6	26.5	
	3	90	6.8	20.2	
	4	100	9.7	17.7	

Least significant differences between the means of lengths of roots for each treatment = 7.2 mm (P = 0.05).

Differences between means of numbers of germinated seeds for each treatment are not significant (P = 0.05).

Table XLVII.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of silver nitrate.

Experiment II

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	100	13.4	7.8	
	2	100	12.5	11.7	10.8
	3	90	15.1	14.1	
	4	100	9.3	9.8	
Control	1	100	15.7	21.2	
	2	100	11.9	16.2	19.6
	3	90	12.3	22.5	
	4	100	9.2	18.4	
F. noxius + 2 ml 0.1M $AgNO_3$	1	80	11.8	22.0	
	2	100	10.1	18.8	20.4
	3	100	10.9	23.4	
	4	90	9.1	17.3	
Control + 2 ml 0.1M $AgNO_3$	1	90	10.7	15.2	
	2	90	13.6	20.2	17.7
	3	100	10.6	17.6	
	4	-	-	-	

Least significant differences between the means of lengths of roots for each treatment = 3.2 mm ($P = 0.05$).

Differences between means of numbers of germinated seeds for each treatment are not significant ($P = 0.05$).

- = no measurement made.

Table XLVIII.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of silver nitrate.

Experiment III

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	90	6.2	1.6	
	2	90	7.2	3.7	3.9
	3	90	8.9	6.1	
	4	100	12.0	4.3	
Control	1	90	9.4	18.6	
	2	90	10.2	15.0	17.2
	3	90	9.0	15.0	
	4	90	9.8	20.2	
F. noxius + 2 ml 0.1M $AgNO_3$	1	80	8.9	10.2	
	2	90	5.6	6.4	9.6
	3	80	7.4	7.5	
	4	80	9.1	14.2	
Control + 2 ml 0.1M $AgNO_3$	1	90	8.0	17.0	
	2	90	11.0	19.7	17.1
	3	80	6.5	14.7	
	4	80	8.0	16.8	

Least significant differences between the means of lengths of roots for each treatment = 4.1 mm ($P = 0.05$).

Differences between means of numbers of germinated seeds for each treatment are not significant ($P = 0.05$).

Table XLIX.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of silver nitrate.

Experiment I

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	100	12.3	6.2	
	2	40	1.2	1.3	
	3	80	12.5	21.0	9.5
	4	100	7.7	9.7	
Control	1	100	18.5	25.4	
	2	100	18.7	30.9	
	3	100	13.5	25.9	25.1
	4	80	10.6	18.2	
F. noxius + 1 ml 0.1M $AgNO_3$	1	80	11.1	17.9	
	2	100	12.9	17.6	
	3	100	7.2	11.1	15.5
	4	-	-	-	
Control + 1 ml 0.1M $AgNO_3$	1	90	15.8	24.6	
	2	-	-	-	
	3	100	14.2	22.2	22.4
	4	90	14.3	20.4	

Least significant difference between the means of lengths of roots for each treatment = 9.7 mm ($P = 0.05$).

Differences between means of numbers of germinated seeds in each treatment are not significant ($P = 0.05$).

- = no measurement made.

Table I.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with *F. noxius* cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of silver nitrate.

Experiment II

Treatment	Assembly no.	% germination	Mean	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	50		2.4	0.2	
	2	100		3.3	1.1	
	3	20	45	5.0	0.5	0.9
	4	10		6.0	2.0	
Control	1	90		9.7	17.0	
	2	100		8.5	13.0	
	3	100	98	9.1	25.2	18.3
	4	100		9.0	17.9	
F. noxius + 1 ml 0.1M $AgNO_3$	1	50		6.8	3.8	
	2	70		3.7	3.1	
	3	90	68	10.6	18.2	6.5
	4	60		4.0	1.1	
Control + 1 ml 0.1M $AgNO_3$	1	80		5.4	13.8	
	2	80		9.0	16.5	
	3	60	78	8.0	11.0	13.4
	4	90		6.7	12.1	

Least significant difference between means of lengths of roots for each treatment = 7.7 mm ($P = 0.05$).

Least significant difference between means of numbers of germinated seeds for each treatment = 38%.

Table LI.

Germination of lettuce seeds and growth of germinated seedlings in paired petri dish assemblies containing lettuce paired with F. noxius cultures and in controls paired with uninoculated 5% malt agar in the presence or absence of silver nitrate.

Experiment III

Treatment	Assembly no.	% germination	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean
F. noxius	1	100	12.9	14.3	16.3
	2	100	11.3	20.8	
	3	100	9.5	10.6	
	4	100	10.3	19.5	
Control	1	90	11.3	28.3	29.1
	2	100	11.6	32.8	
	3	100	11.5	25.0	
	4	90	14.8	30.2	
F. noxius + 1 ml 0.1M $AgNO_3$	1	100	14.0	28.8	16.9
	2	100	12.0	13.6	
	3	100	8.4	16.8	
	4	100	10.3	8.5	
Control + 1 ml 0.1M $AgNO_3$	1	100	10.0	21.7	24.1
	2	90	11.3	25.3	
	3	100	7.6	20.0	
	4	100	12.2	29.5	

Least significant difference between means of lengths of roots for each treatment = 9.3 mm ($P = 0.05$).

Differences between the means of numbers of germinated seeds for each treatment are not significant ($P = 0.05$).

Table LII.

Clitocybe species examined for the production of volatile metabolites.

Fungus.	Origin.
C. candida Bres.	+C.B.S. 166.48.
C. cyanthiformis Fr.	C.B.S. 150.36.
C. dealbata Sow.	C.B.S. 168.48.
C. diatetra Fr.	C.B.S. 125.46.
C. flaccida Sowerb.	C.B.S. 105.12.
C. fragrans (Sow.) Fr.	C.B.S. 151.37.
C. geotropa (Bull.) Fr.	C.B.S. 110.21.
C. gigantea (Sow.) Quel.	C.B.S. 165.59.
C. illudens Schw.	C.B.S. 141.34.
C. infundibuliformis (Schaeff.) Fr.	C.B.S. 129.44.
C. odora (Bull.) Fr.	C.B.S. 128.46.
C. rivulosa (Pers.) Fr.	C.B.S. 152.37.

+C.B.S. = Centraalbureau voor Schimmelcultures.

Table LIII.

Germination of lettuce seeds and growth of germinated seedlings in assemblies containing lettuce paired with Clitocybe cultures and in controls, paired with uninoculated 5% malt agar.

Treatment	% germination							Mean lengths of shoots of germinated seedlings (mm)							Mean lengths of roots of germinated seedlings (mm)						
	Assembly no.							Assembly no.							Assembly no.						
	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean
C. candida	90	100	90	90	100	90	93.3	7.4	6.3	4.6	7.1	6.0	5.9	6.2	24.3	21.8	20.6	21.2	23.1	26.1	22.8
Control	100	90	90	100	100	100	96.7	11.4	19.9	11.0	11.9	11.9	11.2	12.9	23.5	24.7	25.6	32.1	22.8	23.4	25.3
C. candida	100	80	100	90	80	100	91.7	4.4	4.1	4.2	3.7	5.0	5.5	4.5	7.2	7.8	7.1	3.9	6.1	7.3	6.6
Control	80	90	80	100	100	90	90.0	10.1	10.7	4.4	7.7	9.3	10.2	8.7	15.6	21.3	9.5	15.8	20.0	17.7	16.6
C. candida	80	80	100	100	90	90	90.0	5.6	3.8	4.3	4.1	4.4	6.0	4.7	13.5	8.9	5.6	6.2	8.0	13.7	9.3
Control	100	100	90	100	100	100	98.3	11.5	8.3	12.1	11.7	8.4	13.4	10.9	20.5	14.9	24.1	16.9	14.7	16.8	18.0
C. cyanthiformis	100	80	0	100	10	100	65.0	2.1	3.4	0.0	7.8	2.0	9.6	4.2	21.6	18.5	14.9	18.3	14.7	20.5	18.1
Control	80	100	100	90	100	100	95.0	1.4	2.0	0.0	2.8	1.0	6.4	2.3	30.5	27.7	14.9	22.6	17.5	27.3	23.4
C. cyanthiformis	90	90	90	70	90	90	86.7	16.6	6.6	3.6	8.4	4.1	8.7	8.3	16.5	6.7	2.6	6.3	1.7	13.6	7.9
Control	90	90	90	100	100	90	93.3	14.0	7.2	8.0	9.9	17.1	15.4	11.9	26.2	21.3	11.4	24.8	36.6	28.0	24.7
C. cyanthiformis	100	90	100	100	90	90	95.0	13.6	10.1	9.9	5.5	9.3	13.3	10.3	24.7	15.9	10.4	9.7	20.8	30.7	18.7
Control	90	80	100	90	90	90	90.0	8.9	3.6	12.4	12.7	11.6	14.4	11.3	28.4	27.5	24.8	28.6	25.9	31.8	27.8
C. dealbata	100	70	100	90	80		88.0	5.8	5.1	4.8	6.6	5.4		5.5	12.6	11.0	17.7	18.7	17.8		15.6
Control	90	70	80	100	80		84.0	6.4	4.3	5.0	6.1	6.5		5.7	23.0	11.7	11.9	14.5	15.4		15.3
C. diatetra	100	100	100	100	100	100	100	9.8	12.4	12.4	10.9	14.1	16.3	12.6	23.6	22.4	28.0	25.3	20.7	32.7	25.4
Control	100	100	100	90	100	90	96.7	10.9	10.6	9.8	9.0	12.8	9.4	10.4	27.1	20.8	19.0	21.3	19.4	25.8	22.2
C. flaccida	100	90	100	90	100	100	96.7	13.0	10.6	10.1	12.4	10.2	9.3	10.9	25.0	20.1	24.7	26.7	22.4	21.2	23.3
Control	100	100	100	100	100	100	100	9.4	13.1	9.5	9.8	7.6	11.1	10.1	16.9	21.4	24.0	21.3	18.8	21.2	20.6
C. fragrans	100	100	100	100	100		100	14.2	17.9	13.8	18.6	15.3		16.0	18.8	21.5	18.3	21.1	21.2		20.2
Control	80	100	100	100	100		96.0	15.2	15.6	15.6	12.8	16.3		15.1	15.9	21.4	23.6	22.3	21.3		20.9
C. geotropa	0	0	0	0	0	0	0														
Control	90	70	80	100	80		84.0	6.4	4.3	5.0	6.1	6.5		5.7	23.0	11.7	11.9	14.5	15.4		15.3
C. geotropa	30	40	20	100			47.5	0.0	0.8	0.0	1.6			0.6	1.0	1.0	1.0	1.6			1.2
Control	100	100	100	100	100	100	100	9.4	13.1	9.5	9.8	7.6	11.1	10.1	16.9	21.4	24.0	21.3	18.8	21.2	20.6
C. geotropa	0	0	0	30	0	0	5.0				3.0			3.0				3.0			3.0
Control	100	90	100	100	100	100	98.3	14.0	8.4	13.7	10.1	9.8	15.0	11.8	23.1	19.2	19.8	19.9	17.0	17.0	19.3
C. gigantea	100	70	100	100	100	80	91.7	5.5	4.1	5.3	5.9	4.9	5.5	5.2	16.2	6.3	13.7	10.8	12.2	17.0	12.7
Control	100	80	90	100	100	100	95.0	7.4	11.0	8.0	12.8	8.8	11.6	9.9	27.9	24.4	19.8	21.4	22.8	19.3	22.6
C. gigantea	100	70	100	100	100	80	91.7	6.5	5.3	7.0	8.7	6.4	3.5	6.2	9.3	9.0	12.7	20.0	8.6	2.8	10.4
Control	100	100	90	70	80	100	90.0	9.1	8.9	8.9	10.7	11.0	8.3	9.5	12.5	17.4	13.0	20.9	18.2	9.9	15.3
C. gigantea	90	100	80	80	90	100	90.0	6.7	6.4	6.6	3.5	3.2	3.1	4.9	16.1	9.8	14.1	3.8	6.9	5.0	9.3
Control	80	90	80	100	100	90	90.0	10.1	10.7	4.8	7.7	9.3	10.1	8.8	15.6	21.3	9.5	15.8	20.0	17.7	16.6
C. illudens	80	90	80	90	100	70	85.0	5.8	6.8	4.8	8.2	7.0	4.9	6.3	5.8	7.8	5.0	9.9	8.4	5.7	7.1
Control	70	100	90	90	80	80	85.0	8.7	6.3	6.7	11.6	6.5	8.1	8.0	11.3	15.7	8.1	17.7	12.9	9.5	12.5
C. illudens	100	80	100	100	90		94.0	10.4	11.1	17.3	11.7	20.8		14.3	17.4	18.2	20.6	18.9	27.8		17.1
Control	90	90	90	100	100	90	93.3	14.0	7.2	8.0	9.9	17.1	15.4	11.9	26.2	21.3	11.4	24.8	36.6	28.0	24.7
C. illudens	80	100	100	100	90	100	95.0	8.5	9.0	7.0	11.0	7.9	11.6	9.2	20.9	23.3	13.9	26.0	28.0	31.0	23.9
Control	90	80	100	90	90	90	90.0	8.9	8.6	11.4	12.7	11.6	14.4	11.3	28.4	27.5	24.8	28.6	25.9	31.8	27.8
C. infundibuliformis	80	60	80	50	90	50	68.3	3.2	1.1	5.4	1.8	1.7	1.4	2.4	3.1	0.7	1.5	1.0	1.7	0.6	1.4
Control	70	100	90	90	80	80	85.0	8.7	6.3	6.7	11.6	6.5	8.1	8.0	11.3	15.7	8.1	17.7	12.9	9.5	12.5
C. infundibuliformis	90	80	90	100	90	100	91.7	7.1	5.8	14.4	5.2	6.7	4.4	7.8	5.8	8.9	14.4	5.2	6.7	2.4	7.2
Control	100	100	100	100	100	100	100	9.4	13.1	9.5	9.8	7.6	11.1	10.1	16.9	21.4	24.0	21.3	18.8	21.2	20.6
C. infundibuliformis	90	100	90	90	100	100	95.0														

Table LIV.

Diameters and sporulation of *A. niger* colonies in assemblies containing *A. niger* paired with *Clitocybe* cultures and in controls, paired with uninoculated 5% malt agar.

Treatment	Colony diameter after 7 days (mm) Assembly no. 1	2	3	4	5	6	Mean	Sporulation after 7 days
C. candida	50	50	50	51	52	51	50.7	2.0+
Control	46	45	50	44	44	45	45.7	2.5+
C. candida	49	48	48	44	48		47.4	2.0+
Control	43	41	42	36	40	39	40.2	3.0+
C. candida	40	38	40	42			40.0	3.6+
Control	45	44	43	44	43	44	43.8	3.4+
C. cyanthiformis	45	45	46	46	44	45	45.2	3.0+
Control	50	45	46	45	44	44	45.7	3.0+
C. dealbata	40	40	42	41	41		40.8	3.0+
Control	40	40	40	44	43	42	41.5	3.0+
C. diatetra	42	43	45	45	43	46	44.0	3.0+
Control	46	50	47	47	47	48	47.5	3.0+
C. diatetra	43	43	40	41	44	43	42.3	3.2+
Control	45	44	43	44	43	44	43.8	3.2+
C. diatetra	45	45	44	45	43	45	44.5	3.0+
Control	45	45	44	45	46	46	45.2	3.0+
C. flaccida	44	44	44	44	45	46	44.5	3.0+
Control	46	50	47	47	47	48	47.5	3.0+
C. flaccida	45	43	44	46	45	46	44.8	2.0+
Control	48	45	44	46	45		45.6	2.0+
C. flaccida	45	45	44	45	44		44.6	2.0+
Control	48	45	44	46	45		45.6	2.0+
C. fragrans	46	45	45	45	44	45	45.0	2.0+
Control	45	44	46	45	48		45.6	2.0+
C. geotropa	38	39	38	39	39	40	38.8	3.0+
Control	41	40	40	41	44	43	41.5	3.0+
C. geotropa	43	42	42	42	43	43	42.5	3.0+
Control	46	46	45	44	45	45	45.2	3.0+
C. geotropa	44	42	44	41	43	43	42.8	3.0+
Control	47	47	48	47	47	48	47.3	3.0+
C. gigantea	52	50	46	47	48	54	49.5	3.0+
Control	47	45	46	46	45	47	46.0	3.0+
C. illudens	51	55	52	54	51		52.6	2.0+
Control	56	60	56	55	53		56.0	2.0+
C. illudens	42	40	41	40	40	40	40.5	3.5+
Control	45	43	42	43	43	45	43.5	2.8+
C. illudens	42	41	40	42	40	41	41.0	3.2+
Control	46	46	45	45	44	45	45.2	2.8+
C. infundibuliformis	57	55	55	51	54		54.4	2.5+
Control	56	60	56	55	53		56.0	2.0+
C. infundibuliformis	40	40	40	40	40	40	40.0	3.6+
Control	45	44	43	44	43	45	44.0	3.2+
C. infundibuliformis	45	45	45	45			45.0	3.1+
Control	47	47	48	47	47	48	47.3	3.0+
C. odora	42	41	40	41	40	41	40.8	2.0+
Control	42	41	46	44	48	43	44.0	2.0+
C. odora	42	41	40	41	40	41	40.8	2.0+
Control	42	41	46	44	48	43	44.0	2.0+
C. odora	40	43	40	40	40	41	40.7	3.0+
Control	42	41	43	42	40	41	42.2	3.0+
C. rivulosa	40	40	40	40	40	40	40.0	3.0+
Control	45	43	42	43	43	45	43.5	3.0+
C. rivulosa	45	45	45	45	45		45.0	2.8+
Control	47	45	46	46	45	47	46.0	2.8+
C. rivulosa	42	42	40	42	41	41	41.3	2.8+
Control	45	44	43	44	43	44	43.8	2.8+

Table LV.

Growth of bacteria in assemblies containing bacteria paired with Clitocybe cultures and in controls, paired with uninoculated 5% malt agar.

Species having no apparent effect on bacteria.

C. candida	
C. cyanthiformis	C. gigantea
C. dealbata	C. illudens
C. diatetra	C. infundibuliformis
C. flaccida	C. odora
C. fragrans	C. rivulosa

Growth in assemblies with C. geotropa cultures and in controls.

Treatment	Bacterial growth.							
	M.ph.	M.r.	C.x.	St.a.Ox.	B.s.	Ps.a.	K.a.	Rh.2001
	Assembly no.							
	123	123	123	123	123	123	123	123
C. geotropa	---	--+	+++	---	---	+++	++-	---
Control	+++	+++	+++	+++	+++	+++	+++	+++
C. geotropa	---	---	+++	++-	---	---	+++	---
Control	+++	+++	+++	+++	+++	+++	+++	+++
C. geotropa	---	--+	+++	++-	---	+++	++-	---
Control	+++	+++	+++	+++	+++	+++	+++	+++

Table LVI.

Differences in mean % germination of lettuce seeds and mean growth of germinated seedlings, mean diameters and sporulation of A. niger colonies and growth of bacteria in assemblies containing assay species paired with Clitocybe cultures compared with controls, paired with uninoculated 5% malt agar.

Assay species / Test species	Lettuce								A. niger			Bacteria
	% Germination		Shoot length (mm)		Root length (mm)		Total length (mm)		Colony diameter (mm)		Sporulation	
	Observed mean difference	L.S.M.D.	Observed mean difference	L.S.M.D.	Observed mean difference	L.S.M.D.	Observed mean difference	L.S.M.D.	Observed mean difference	L.S.M.D.	Observed difference	Observed difference
C. candida	- 3.4	6.6	- 6.7	3.3	- 2.5	3.6	- 9.0	4.6	+ 5.0	2.1	- 0.5	None
C. candida	+ 1.7	12.0	- 4.2	2.2	-10.0	3.9	-14.1	5.8	+ 7.2	2.9	- 1.0	
C. candida	- 8.3	8.5	- 6.2	1.6	- 8.7	4.6	-14.9	6.1	- 3.8	1.6	+ 0.2	
C. cyanthiformis	-30.0	9.1	-14.0	4.2	-21.2	5.9	-35.1	9.2	- 0.5	2.1	0.0	"
C. cyanthiformis	- 6.6	8.7	- 3.6	6.0	-16.8	9.2	-20.4	1.5				
C. cyanthiformis	+ 5.0	7.6	- 1.0	3.4	- 9.1	7.7	-10.1	16.5				
C. dealbata	+ 4.0	18.3	- 0.2	1.3	+ 0.3	5.9	- 0.1	5.3	- 0.7	2.0	0.0	"
C. diatetra	+ 3.3	4.7	+ 2.2	2.4	+ 3.2	5.0	+ 5.4	6.1	- 3.5	1.7	0.0	"
C. diatetra									- 1.5	1.6	0.0	
C. diatetra									- 0.7	1.1	0.0	
C. flaccida	- 3.3	4.7	+ 0.8	2.1	+ 2.7	3.9	+ 3.4	7.1	- 3.0	0.9	0.0	"
C. flaccida									- 0.8	1.9	0.0	
C. flaccida									- 1.0	1.4	0.0	
C. fragrans	+ 4.0	8.2	+ 0.9	2.6	- 0.7	2.3	+ 0.2	4.3	- 0.6	1.5	0.0	"
C. geotropa	-84.0	11.8	- 5.7	0.8	-15.3	3.7	-21.1	4.4	- 2.7	1.7	0.0	—
C. geotropa	-52.5	15.2	- 9.5	2.1	-19.4	2.6	-29.0	3.9	- 4.5	1.0	0.0	—
C. geotropa	-95.3	11.3	-10.8	2.6	-15.7	2.4	-26.5	4.1	- 3.5	0.1	0.0	—
C. gigantea	- 3.3	14.2	- 4.7	2.1	- 9.9	3.8	-13.6	8.7	+ 3.4	5.0	0.0	None
C. gigantea	+ 1.7	16.6	- 3.3	1.9	- 4.9	6.4	- 8.2	8.1				
C. gigantea	0.0	11.4	- 3.9	2.6	- 7.6	5.9	-11.5	7.1				
C. illudens	0.0	13.4	- 1.7	2.2	- 5.4	3.7	- 7.1	5.3	- 3.4	2.7	0.0	"
C. illudens	+ 0.7	11.7	- 2.4	5.4	- 7.6	8.7	-10.0	12.9	- 3.5	1.3	+ 0.7	
C. illudens	+ 5.0	11.1	- 2.1	2.9	- 2.9	6.3	- 5.0	7.2	- 4.2	1.1	+ 0.4	
C. infundibuliformis	-18.7	13.0	- 5.6	2.3	-11.1	3.4	-16.7	4.7	- 1.6	3.3	+ 0.5	"
C. infundibuliformis	- 8.3	9.8	- 2.4	3.3	-13.4	8.6	-15.8	7.3	- 4.0	1.0	+ 0.4	
C. infundibuliformis	- 3.2	5.3	- 2.3	2.8	- 5.0	3.4	- 7.3	8.5	- 2.3	0.3	+ 0.1	
C. odora	- 1.7	8.9	- 0.4	2.8	- 1.8	8.1	- 2.2	3.5	- 3.2	1.5	0.0	"
C. odora									- 3.2	6.8	0.0	
C. odora									- 1.5	2.1	0.0	
C. rivulosa	+ 2.5	9.8	- 2.4	2.6	- 3.0	5.6	- 5.4	11.2	- 3.5	1.1	0.0	"
C. rivulosa									- 1.0	0.9	0.0	
C. rivulosa									- 2.5	1.2	0.0	

- = growth less than in control treatment.

Table LVII.

Times taken (min) for pallid violet blue colour to develop in paired bottle assemblies containing lettuce paired with cultures of C. candida, C. cyanthiformis, C. geotropa and C. infundibuliformis.

a)

C. candida

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	* > 150	> 150	> 150						
1				> 150	> 150	60			
2							> 150	16.0	> 150
3	> 150	20	> 150						
5				> 150	80	26			
6							> 150	14.0	> 150
7	> 150	20	> 150	> 150	75	24	> 150	18.0	> 150

b)

C. cyanthiformis

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	> 150	> 150	> 150						
1				> 150	> 150	> 150			
2							18.0	> 150	100
3	> 150	28	> 150						
5				80	> 150	> 150			
6							16.0	> 150	60
7	> 150	30	> 150	80	> 150	> 150	20	> 150	70

c)

C. geotropa

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	> 150	> 150	> 150						
1				60	70	100			
2							21	16.0	19.0
3	7.0	8.0	10.0						
5				8.0	10.0	11.0			
6							9.0	10.0	8.0
7	4.0	10.0	12.0	17.0	6.0	8.0	8.0	8.0	7.0

d)

C. infundibuliformis

Assembly no. / Day no.	1	2	3	4	5	6	7	8	9
2/3	> 150	> 150	> 150						
1				50	50	80			
2							10.0	18.0	20
3	8.0	9.0	9.0						
5				8.0	9.0	14.0			
6							6.0	10.0	12.0
7	5.0	7.0	9.0	6.0	13.0	14.0	10.0	7.0	8.0

* > 150 = no development of colour by 150 min after insertion of test paper.

For illustration, see text Figure (xxxiii).

Table LVIII.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *C. candida* cultures and in controls, paired with uninoculated 5% malt agar.

Expt. no.	Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
I	*C. candida*	1	100	12.1	32.6	10
		2	90	7.7	35.3	6
		3	100	8.3	84.7	180
	Control	1	100	36.9	99.5	
		2	90	27.3	76.2	
		3	90	19.6	88.0	
II	*C. candida*	1	90	9.9	38.3	20
		2	100	7.9	33.9	25
		3	100	7.8	29.7	23
	Control	1	100	15.1	56.9	
		2	100	15.2	64.0	
		3	100	11.3	47.7	
III	*C. candida*	1	100	9.7	56.3	> 150
		2	90	9.2	45.7	80
		3	100	9.0	40.1	22
	Control	1	100	12.5	107.3	
		2	100	11.3	104.3	
		3	100	14.4	106.1	

>150 = no development of colour by 150 min after insertion of test paper.

For illustration, see text Figure (xxxiv).

Table LIX.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with C. cyanthiformis cultures and in controls, paired with uninoculated 5% malt agar.

Expt. no.	Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
I	+C. cyan.	1	100	12.6	37.9	*> 200
		2	90	21.0	39.3	> 200
		3	100	17.7	47.3	> 200
	Control	1	100	19.8	57.2	
		2	100	23.8	69.8	
		3	100	20.2	63.0	
II	C. cyan.	1	100	11.3	83.0	> 200
		2	100	14.7	64.0	> 200
		3	90	15.9	73.7	> 200
	Control	1	100	36.9	99.5	
		2	90	27.3	76.2	
		3	90	19.6	88.0	
III	C. cyan.	1	100	11.9	72.5	*> 150
		2	100	13.9	68.0	> 150
		3	100	14.2	55.2	36
	Control	1	100	12.5	107.3	
		2	100	11.3	104.3	
		3	100	14.4	106.1	

* > 200 and >150 = no development of colour by 200 or 150 min respectively after the insertion of test paper.

+ C. cyan. = C. cyanthiformis.

For illustration, see text Figure (xxxiv).

Table LX.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *C. geotropa* cultures and in controls, paired with uninoculated 5% malt agar.

Expt. no.	Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
I	*C. geotropa*	1	100	6.5	13.7	4.0
		2	100	5.4	16.2	7.0
		3	80	5.8	16.6	7.0
	Control	1	100	10.0	60.2	
		2	90	21.3	71.8	
		3	100	6.9	69.9	
II	*C. geotropa*	1	100	10.1	22.1	12.0
		2	100	17.9	26.8	15.0
		3	100	10.0	28.6	16.0
	Control	1	100	19.8	57.2	
		2	100	23.8	69.8	
		3	100	20.2	63.0	
III	*C. geotropa*	1	100	13.3	42.0	14.0
		2	90	16.8	47.9	24
		3	100	12.0	45.1	12.0
	Control	1	100	36.9	99.5	
		2	90	27.3	76.2	
		3	90	19.6	88.0	

For illustration, see text Figure (xxxiv).

Table LXI.

Germination of lettuce seeds and growth of germinated seedlings in paired bottle assemblies containing lettuce paired with *C. infundibuliformis* cultures and in controls, paired with uninoculated 5% malt agar.

Expt. no.	Treatment	Assembly no.	% germination	Mean lengths of shoots of germinated seedlings (mm)	Mean lengths of roots of germinated seedlings (mm)	Time taken for pallid violet blue colour to develop (min)
I	+*C. inf.*	1	80	3.8	8.9	5.0
		2	90	4.9	10.2	7.0
		3	100	5.4	12.7	9.0
	Control	1	100	10.0	60.2	
		2	90	21.3	71.8	
		3	100	6.9	69.9	
II	*C. inf.*	1	90	4.7	17.3	6.0
		2	100	7.1	24.7	14.0
		3	100	4.7	9.3	8.0
	Control	1	100	15.1	56.9	
		2	100	15.2	64.0	
		3	100	11.3	47.7	
III	*C. inf.*	1	100	19.0	35.9	12.0
		2	100	19.3	43.2	18.0
		3	100	19.7	47.9	26
	Control	1	100	19.8	57.2	
		2	100	23.8	69.8	
		3	100	20.2	63.0	

+ *C. inf.* = *C. infundibuliformis*.

For illustration, see text Figure (xxxiv).

Table LXII. Numbers of zygospores produced in petri dish colonies of R.sexualis at 10°C and 20°C.

Dish no.	Average number of zygospores per transect					
	10°C			20°C		
Expt. no.	I	II	III	I	II	III
1	31	22	5	162	114	174
2	2	19	24	124	162	156
3	15	2	11	115	167	164
4	10	33	16	126	128	129
5	14	8	14	157	130	141
6	13	21	12	130	181	192

For results of statistical treatment see Table 21 in text.

Table LXIII. Numbers of zygospores produced at 10°C in paired petri dish assemblies by assay colonies of *R. sexualis* paired with mature colonies of the same species and in controls paired with uninoculated 1% malt agar.

Expt. no.	Assembly no.	Average number of zygospores per transect: Test fungus present — 2 day culture	Test fungus present — 5 day culture	Test fungus absent — 2 day control	Test fungus absent — 5 day control
I	1	5	30	23	28
	2	7.5	38	29	18.5
	3	8	44	8.5	11.5
	4	21.5	42	15	2.5
	5	21	55.5	45.5	18.5
	6	13	48	20.5	22.5
II	1	3.5	26	0	0
	2	0	6	0	0
	3	12	12	0	0
	4	0	26.5	0	3
	5	0	-	0	19.5
	6	0	-	0	-
III	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0

For results of statistical analysis see text Table 22.

- = no measurement made.

Table LXIV. Number of zygospores produced in petri dish cultures of three subcultured strains of R. sexualis at several temperatures.

Strain	Expt. no.	Average number of zygospores per transect in each petri dish					
		10°C	11°C	12°C	13°C	14°C	20°C
A	I	0	0	5.0	10.0	82.5	140.0
		0	0	17.5	19.5	59.0	150.0
		0	0	7.5	20.0	80.5	176.0
	II	0	0	22.0	19.5	99.0	152.0
		0	0	9.0	15.5	75.0	165.5
		0	0	3.0	22.5	94.0	176.0
	III	0	0	2.5	9.5	24.5	127.5
		0	0	21.5	16.0	59.0	145.0
		0	0	4.0	26.5	50.0	147.0
B	I	0	0.5	19.5	7.5	52.5	156.0
		0	0	5.0	3.5	64.0	143.0
		0	2.0	1.5	12.5	56.0	170.0
	II	0	0	2.5	21.0	34.0	155.0
		0	0	5.0	35.0	35.0	170.0
		0	0	7.5	39.0	25.0	180.0
	III	0	0	3.5	4.5	21.5	157.5
		0	0	2.0	5.5	31.5	145.5
		0	0	2.5	3.0	45.5	158.5
C	I	0	0	0	2.0	20.5	138.5
		0	1.5	7.5	3.0	32.5	185.0
		0	0.5	0.5	5.5	13.5	144.0
	II	0	0	1.5	26.0	45.5	167.0
		0	0	0.5	25.5	54.0	162.5
		0	0	2.5	9.5	72.5	182.0
	III	0	0	2.5	14.0	46.5	165.0
		0	0	5.0	19.5	28.0	172.5
		0	0	0.5	33.5	50.5	156.5

For statistical treatment of results see text Table 23.

Table LXV. Results of variance analyses on number of zygospores produced by colonies of *R. sexualis* at each of various temperatures in three replicate experiments at each temperature.

Strain	Temperature treatment	Between replicate experiments at each temperature (F_4^2 observed)	Between replicate plates in each experiment (F_4^2 observed)
A	12°C	1.2	0.04
	13°C	1.9	2.7
	14°C	4.1	0.2
	20°C	1.5	1.6
B	11°C	1.4	0.8
	12°C	0.7	0.6
	13°C	18.2	1.1
	14°C	8.0	0.6
	20°C	2.2	2.9
C	11°C	1.1	0.5
	12°C	0.1	1.4
	13°C	3.51	0.1
	14°C	5.2	0.3
	20°C	0.6	0.7

F_4^2 sig = 6.9 at P = 0.05.

Table LXVI. Number of zygospores produced at 13°C in paired bottle assemblies by 5 day old assay colonies of R. sexualis paired with 5 day old mature colonies of the same species and in controls, paired with uninoculated 1% malt agar and incubated for 7 days after pairing.

Assembly no.	Average number of zygospores per transect					
	Test fungus present			Test fungus absent		
Expt. no.	I	II	III	I	II	III
1	30	61	41	54	36	56
2	34.5	35.5	79	27	70	65.5
3	26.5	56.5	69	34	46	59
4	29	51.5	61	25	-	56
5	28.5	39.5	51	44.5	44	70
6	29.5	-	79	21.5	36	66.5

- = no measurement made.

Differences between means of numbers of zygospores produced in different treatments in each experiment are not significant (P = 0.05).

Table LXVII. Numbers of zygospores produced by assay colonies of *R. sexualis* in paired petri dish assemblies with mature cultures of *R. sexualis* and in controls with uninoculated 1% malt agar at 20°C under two conditions of ventilation.

	Assembly no.	Average number of zygospores per transect: Test fungus present Expt. I	Expt. II	Expt. III	Test fungus absent Expt. I	Expt. II	Expt. III
	1	115.5	114.5	188.0	126.5	132.0	158.0
Assemblies	2	101.0	102.5	192.5	98.5	188.0	178.5
bound with	3	118.5	102.5	141.5	109.5	108.0	200.5
rubber	4	86.0	126.5	197.0	88.0	141.5	185.0
bands	5	82.5	127.0	235.0	96.0	114.5	254.5
	6	79.0	135.0	-	-	-	172.5
	1	91.0	99.0	250.0	124.5	120.5	210.5
Assemblies	2	101.5	59.5	277.0	87.5	98.0	264.5
bound with	3	80.0	70.5	270.0	95.0	134.5	261.5
sellotape	4	111.5	89.0	241.0	99.5	99.5	203.5
	5	-	91.5	233.0	96.0	107.0	188.0
	6	-	78.5	-	102.0	103.5	176.0

- = no measurements made.

Differences in mean number of zygospores produced in each treatment were not significant in any experiment ($P = 0.05$).

Table LXVIII. Numbers of zygospores produced at 13°C by assay colonies of R. sexualis in paired petri dish assemblies with mature colonies of three species of Zygomycete and in controls with uninoculated potato dextrose agar.

Expt. no.	Assembly no.	Average number of zygospores per transect			
		Test fungus present			Test fungus absent
		M. plumbens	A. spinosa	R. stolonifer	
I	1	101.0	60.0	46.0	18.5
	2	100.5	11.5	33.0	8.5
	3	87.0	15.0	37.5	2.5
	4	107.0	32.5	17.5	45.0
	5	71.5	40.0	51.0	14.5
	6	100.0	20.5	23.0	14.5
II	1	92.0	70.5	83.0	41.0
	2	124.5	66.5	85.0	49.5
	3	105.0	88.5	68.0	52.0
	4	69.5	94.0	99.0	60.5
	5	69.0	61.5	-	59.0
	6	-	88.0	-	61.5
III	1	94.5	83.0	119.5	58.5
	2	107.5	85.0	12.1	63.0
	3	93.0	68.0	120.0	47.5
	4	81.0	99.0	123.0	59.5
	5	-	-	-	66.5
	6	-	-	-	-

- = no measurements made

The results of statistical tests on these data are given in text Table 24.

Table LXIX.

Growth of lettuce seedlings on 3 soils in assemblies containing lettuce paired with F. scutellatus cultures and in controls, paired with uninoculated 5% malt agar.

Soil type	Expt. no.	Treatment	Assembly no.	Mean lengths of shoots (mm)	Mean lengths of roots (mm)	Mean lengths of shoots + roots (mm)
Peat	I	F. scutellatus	1	3.0	2.3	5.3
			2	2.0	1.2	3.2
			3	0.8	0.7	1.5
		Control	1	3.5	3.8	7.3
			2	7.5	4.0	11.5
			3	6.5	4.5	11.0
	II	F. scutellatus	1	6.5	1.0	7.5
			2	4.2	1.0	5.2
			3	0.0	0.0	0.0
		Control	1	9.0	3.5	12.5
			2	9.0	3.5	12.5
			3	9.0	13.3	22.3
	III	F. scutellatus	1	0.0	5.0	5.0
			2	1.5	4.0	5.5
			3	1.8	3.2	5.0
		Control	1	10.0	5.5	15.5
			2	6.0	4.0	10.0
			3	9.2	5.8	15.0
Peralite	I	F. scutellatus	1	4.0	2.8	6.8
			2	2.5	0.7	3.2
			3	2.8	1.5	4.3
		Control	1	6.5	3.0	9.5
			2	7.8	8.8	16.5
			3	6.7	5.0	11.8
	II	F. scutellatus	1	1.0	1.0	2.0
			2	0.0	0.0	0.0
			3	1.7	1.5	3.2
		Control	1	23.3	29.5	52.8
			2	10.0	14.5	24.5
			3	17.0	14.8	32.7
	III	F. scutellatus	1	8.0	5.5	13.5
			2	2.5	3.5	6.0
			3	7.0	4.8	11.8
		Control	1	11.5	10.5	22.0
			2	5.0	5.5	10.5
			3	–	–	–
Sand	I	F. scutellatus	1	3.8	1.3	5.0
			2	1.2	1.5	2.8
			3	2.3	1.3	3.5
		Control	1	11.0	7.0	18.0
			2	14.5	10.5	25.0
			3	7.8	7.8	15.6
	II	F. scutellatus	1	4.0	4.8	8.8
			2	7.2	3.0	10.2
			3	3.5	0.7	4.2
		Control	1	26.0	19.0	45.0
			2	17.0	14.5	31.5
			3	18.0	33.2	51.3
	III	F. scutellatus	1	8.7	1.5	10.2
			2	3.2	0.8	4.0
			3	6.0	1.0	7.0
		Control	1	23.5	24.5	48.0
			2	10.0	13.0	23.0
			3	32.8	40.7	73.5

For results of statistical tests see text Table 26. --no measurement made.

Table LXX.

Growth of lettuce seedlings on 3 soils in assemblies containing lettuce paired with F. noxius cultures and in controls, paired with uninoculated 5% malt agar.

Soil type	Expt. no.	Treatment	Assembly no.	Mean lengths of shoots (mm)	Mean lengths of roots (mm)
Peat	I	F. noxius	1	3.0	1.0
			2	6.7	2.7
			3	3.3	2.5
		Control	1	3.5	3.8
			2	6.5	4.5
			3	7.5	4.0
	II	F. noxius	1	7.5	3.5
			2	-	-
			3	6.2	3.0
		Control	1	7.7	5.0
			2	5.0	7.2
			3	10.7	8.0
	III	F. noxius	1	6.0	0.2
			2	3.0	0.3
			3	3.5	0.7
		Control	1	9.0	3.5
			2	9.0	13.2
			3	9.0	3.5
Peralite	I	F. noxius	1	10.0	3.5
			2	6.5	2.0
			3	3.5	1.0
		Control	1	6.5	3.0
			2	7.8	8.7
			3	6.7	5.0
	II	F. noxius	1	14.2	4.5
			2	8.0	2.8
			3	9.2	3.2
		Control	1	5.5	6.0
			2	8.5	6.5
			3	13.2	9.8
	III	F. noxius	1	8.5	3.2
			2	20.5	6.5
			3	-	-
		Control	1	23.3	29.5
			2	10.0	14.5
			3	17.0	14.8
Sand	I	F. noxius	1	7.0	6.2
			2	14.3	5.0
			3	10.5	4.3
		Control	1	20.5	14.3
			2	22.0	9.0
			3	9.7	13.2
	II	F. noxius	1	14.2	3.9
			2	17.2	6.0
			3	10.0	2.5
		Control	1	22.5	15.0
			2	11.2	13.0
			3	21.5	10.5
	III	F. noxius	1	4.7	1.7
			2	4.0	1.5
			3	7.3	2.0
		Control	1	11.0	7.0
			2	14.5	10.5
			3	7.8	7.7

For results of statistical tests see text Table 27. - = no measurement made.

STUDIES IN THE PRODUCTION AND EFFECTS OF VOLATILE FUNGAL METABOLITES.

by

Alison May Marshall

Summary

Previous surveys in this Department have been extended by examining 33 species of *Fomes* and 12 species of *Clitocybe*.

Seventeen *Fomes* species showed biological activity against at least one of the assay organisms (lettuce, *Aspergillus niger* and 8 bacteria). There was no particular pattern of activity in this group. Fifteen species showed conspicuous peaks on G.L.C. traces. Of these, only two groups of species, one of 5 and one of 4, produced similar G.L.C. patterns. The conspicuous peaks produced by one of these groups, (*F. pomaceus et al.*) was subsequently shown to be due to the presence of methyl chloride. No other patterns were produced by more than one species.

There was no consistent correlation between biological activity and the production of conspicuous peaks on G.L.C. traces and no consistent correlation of pattern production with any existing taxonomic arrangement. Hexatriyne, previously reported from *F. annosus* was not produced by any species examined here.

The inhibition of lettuce seedling growth by the culture gases of

F. scutellatus has been shown to be attributable to the production of gaseous hydrogen cyanide. A new adaption of Feigl & Anger's technique was developed for quantitative measurement of HCN in this study.

The factor responsible for characteristic inhibition of lettuce root growth by F. noxius has not been identified. None of the compounds, acetaldehyde, ethanol, isobutanol or a mixture of pentanols which gave conspicuous peaks on G.L.C. traces produced comparable inhibition either individually or in mixtures. There are indications that a sulphur-containing compound may be involved. Derivatives of the suspected compound are awaiting analysis.

Nine of the Clitocybe species showed biological activity against at least one of the assay species. A pattern of activity was observed which correlated with the production of hydrogen cyanide. All 4 species, C. geotropa, C. infundibuliformis, C. candida and C. cyanthiformis, producing HCN inhibited lettuce seedling growth, the first two of these also consistently inhibited growth and stimulated sporulation of A. niger; C. geotropa inhibited the growth of bacteria. The concentrations of HCN produced by these species were shown to be sufficient to account for the inhibitory effects produced on lettuce; other effects of these species have not been examined further.

Nine Clitocybe species produced conspicuous peaks on G.L.C. traces.

Of these, only two produced similar patterns. No other pattern was produced by more than one speices, although there are indications that several species may be producing similar compounds.

Earlier work was extended by examining the factor reported to stimulate production of zygospores in Rhizopus sexualis at low temperatures. This factor could not be identified. Zygospore production was found to be variable over the critical temperature range and differences of one Centigrade degree had a large effect on the numbers of zygospores produced. Because of this, it was suspected that physical factors could contribute to the stimulation effect under certain circumstances.

The effect of F. scutellatus and F. noxius gases acting through soil was studied briefly with a view to examining the possible effects of volatile metabolites in nature. The inhibition produced by these species through soil layers of three types was similar to that demonstrated on agar medium.