STUDIES OF SOME EFFECTS OF VOLATILE FUNGAL METABOLITES ON THE GROWTH AND ECOLOGY OF SOIL FUNGI AND OF PLANT PATHOGENIC BACTERIA

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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C O N T E N T S

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to

my wife Sabiha Al-Tamimi and both daughters Sausan and Suha.

SUMMARY

The thesis reports an investigation of the effects of volatile metabolites produced by cultures of a range of species of Trichoderma on the growth of some other fungi in agar culture and on some bacteria in agar culture and in soil.

The effects of the culture gases on other species of fungi could be accounted for by the amounts of carbon dioxide produced by the Trichoderma cultures in the conditions used. The differences between the effects of different species could be accounted for by differences in the rate of production of CO_2 in the early stages of interactions. The amounts of acetaldehyde and of ethanol produced could also contribute to the effects in some conditions. No other metabolites were found in sufficient concentrations to affect the interactions; this does not discount the possibility of other unidentified metabolites contributing to the interactions in the conditions examined. Those identified were all primary metabolites; small changes in the environment might also result in differences in the production and concentrations of these constituents of the culture gas cloud. This could result in differences in their individual contributions to the total effects.

The effects of the Trichoderma culture gases on five species of plant pathogenic bacteria were strongly affected by the medium and conditions in which the tests were carried out.

The techniques did not reveal any effects of Trichoderma culture gases on bacterial growth on Bouillon agar or clay soil. In tests with authentic material, Erwinia tracheiphila was found to be inhibited by lower concentrations of primary gaseous metabolites than those needed to inhibit E.aroideae; but in these cases the inhibitory concentrations were greater than those found in Trichoderma culture gases. In other soil cultures the Trichoderma culture gases inhibited the bacteria. The inhibition was greatest on a loam soil and less on a sand, and greater with E.tracheiphila than with E.aroideae. By tests with authentic material in the concentrations found in Trichoderma culture gases, it was shown that

effects and the differences between the effects of gases from cultures of T.viride 1 and T.longibranchiatum WBC 4576 could be accounted for by the amounts of carbon dioxide present in the different conditions.

GENERAL INTRODUCTION

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Previous work in this department and elsewhere has shown that cultures of Trichoderma species may produce voltaile metabolites which can affect the growth of other living things. Various species and strains differ in this character.

This thesis reports on an investigation of these differences in biological activity on a selection of soil fungi, and an extension of the study into their effects on some phytopathogenic soil bacteria.

The first part comprises an examination of the effects on fungi, it follows the sequence of:

(a) Demonstration of the effects of culture gases.
(b) Analysis of culture gases by G.L.C.
(c) Tests of effects of mixtures of authentic material of identified metabolites in air in concentrationscomparable to those found in culture gases.

These tests are carried out in detail for two representative species of Trichoderma, then extended in varying depth to a range of other species.

The second part comprises a similar examination of the effects on bacteria, and it is extended into a study of the effects in soil as well as on agar media.

The appendices give some experimental work involved in the design of suitable test apparatus, and the detailed experimental measurements which were made throughout.

A report on the first part has been accepted for publication (cf. Trans. Br. Mycol. Soc. vol.64(3), 1975. In Press).

INTRODUCTORY REVIEW

INTRODUCTORY REVIEW

Fries (1973) and Hutchinson (1973) have reviewed the earlier work on the effects of volatile fungal metabolites on the growth and other development of other living things. These reviews are comprehensive and recent, and it seems inappropriate to cover the same ground again. This introduction will therefore be restricted to a critical summary and comparison of these reviews and an extension into the work on effects on bacteria.

Hutchinson deals only with metabolites liberated by fungi. He presents the review under the themes of the effects of known volatile primary metabolites and those of known volatile secondary metabolites or of unidentified volatile factors. "Primary metabolites" is used to refer to those produced by and subsequently involved in the common processes of energy exchange and synthesis of all substance. He points out that, as these processes are believed to follow the same pathways in all living things, many of these metabolites are likely to be present in most living environments, to be produced by linked reactions, and to show patterns of activity which may support widely applicable generalisations. "Secondary metabolites" refers to these produced by living cells which have no known function in subsequent metabolism. They may be produced by unusual changes in basic metabolism or by idiosyncratic processes; hence they may be uniquely interesting compared with primary metabolites but they are less likely to be present in most living environments and less likely be a basis for widely applicable generalisations.

He also distinguishes between recordsof investigations in closely controlled laboratory conditions, and those of analyses of less controlled field conditions. He points out, however, that it could be misleading to restrict the consideration to case in which concentrations of gaseous metabolite in air spaces above fungal colonies have been known to contribute to biological interactions. This would not permit proper emphasis to be put on the interesting cases in which biological effects of culture gases have been correlated with the analysis of potential activity of volatile substance identified in dead mycelial extracts.

He emphasises that examination in controlled laboratory conditions is only an examination of "potentials". How far such potentials are expressed in an ecosystem will depend on the balance which develops between production and removal of the metabolite and the sensitivity of the organisms concerned. Hence this discussion of each metabolite covers:-

(1) Records of its effects on fungi and on other organisms in controlled conditions in concentrations known to be produced by fungi.

(2) Records of the existence or likely existence of conditions which could support such activity in less controlled environments in which fungi are likely to grow, and of known effects of such compounds on the ecology of fungi in these conditions.

PRIMARY METABOLITES

He discusses CO_2 separately, because of its universal occurrence in living environments. At higher concentrations it is generally inhibitory; a large proportion of the fungi tested have been inhibited but not killed under about 10% to 20% volume/volume of CO_2 in air. The gas may affect the type of growth as well as the amount, and its effects maybe greatly limited by the balances of other factors in the environment. In field conditions it has frequently been implicated in the distribution of soil fungi, particularly in relation to differences in distribution in depth. Interesting applications of the effects have been developed in commercial methods of fruit storage.

The other main primary metabolites are acetaldehyde, ethanol, acetone, ethyl acetate, n-propanol, isobutanol and pentanols. There are many examples of cases in which each of these substances may beproduced by fungal cultures in concentrations which may affect the growth of other living things in the environment. He emphasises, however, the problem of identifying the effects of any particular constituent of a mixture in which

all are produced by linked reactions. In many cases it seems likely that the major effects of such mixtures may be due to the CO_2 content, but the precise proportions will vary greatly. Similarly environmental factors may change the proportions of mixtures after they have been liberated from cells, e.g. by differential effects on solution or other inactivation of particular constituents during the movement of the mixture from the site of origin to the site of reaction.

He presents separate discussions of work on a variety of secondary metabolites, the choice of substances and order of discussion being based mostly on the amount known about each. This includes reports on HCN as a common metabolite, on a variety of partly identified odorous compounds which have been implicated in interactions with insects in particular and on the complex and unexplained phenomena associated with some Phycomycetes reproduction. He comments on reports that ethylene is a common product from fungal cells, but notes that the experimental procedure in some of the surveys reported does not enable one to distinguish between products of healthy cells and those from damaged cells or those in starvation conditions. He also comments on the problems of determining how much of the ethylene produced during disease development is produced by host cells and how much by fungus concerned.

The report by Fries differs from that by Hutchinson in two main ways:-

(a) He discusses volatile products from fungi, volatile products from other living plants and volatile products from dead material of higher plants, particularly wood, and he shows many examples of all these substances affecting the growth and development of fungi, particularly in soil.

(b) He puts more emphasis on the biochemical mechanisms involved in their physiological effects. He comments on the stimulations of growth and development being "more puzzling and thus more interesting, then the inhibitions ..... the particularly intriguing question is how compounds as simple and trivial as these aliphatic acids, aldehydes and alchohols can cause an increase in growth that is open quite out of proportion to their small active quantities."

He states that "...one could characterise many, if not most of these compounds as regulators of intermediary metabolism." He mentions as examples

(1) activation (or blocking) of an enzyme reaction;
(2) removal or neutralization of an inhibitor;
(3) influencing nutrient uptake from the medium;
(4) action as a derepressor or otherwise affecting enzyme synthesis at the nuclear or ribosomal level;
(5) changing membrane structure;
(6) substitution of a limiting factor in intermediary metabolism.

He illustrates further cases in which these substances have been shown to be active in ecology in the field. Both authors have raised the question of the value of basing discussion or study on the property of volatility. They note that "outside the cell the distinction between volatility and non-volatility is only qualitative in the magnitude of the vapor pressures in particular conditions. They agree, however, that it has been a useful concept in practice, particularly in the following ways: (a) The ability to approach an organism through the gas phase may be particularly relevant for that majority of fungi that develop part of their mycelium and their entire reproductive structure in air above wet or liquid substrates; (b) many lipophilic compounds produced directly into the air and almost insoluble in water may, even at some distance from a donor source, accumulate faster in the plasma membrane of an acceptor cell if the transfer takes place via the gas phase instead of via the liquid phase; (c) during movement in the gas phase, metabolites will be exposed only to gaseous and physical inactivating or stimulating factors. Those diffusing in complex liquid solutions are likely to be exposed to more concentrated chemical inactivating or stimulating factors, and their movements will be limited by discontinuities in water films; (d) because the substances are active as gases, they are likely to be relatively simple molecules; with modern methods their identification is probably less of a problem than their measurement and control; this leads to a common experimental approach, particularly for their chemistry; (e) the very low concentrations in which some of the identified ones are active suggests a comparison with antibiotics, growth factors and vitamins, all areas of knowledge in which a loosely defined concept has promoted inquiry and discovery; (f) perhaps the most important practical point; that their volatility leads to impermanence in particular situations, and to risks of escape from observation." (Quoted from Hutchinson, 1973, p.223.)

EFFECTS ON BACTERIA

The effects of volatile metabolites in general on bacteria have been studied even less intensively than their effects on fungi.

A big proportion of the studies which have been made relate to the effects of CO_2. A classical report was made by Pasteur and Joubert (1877); Frankel (1889) demonstrated that an alkaline gelatine medium became acid when exposed to CO_2, and Valley and Rettger (1927), concluded that the bacteriostatic or bactericidal effects of CO_2 on 109 different species were due to these effects on the pH of the medium; they also showed that small amounts of CO_2 were needed for the normal growth of all the strains which they examined. Others have found that CO_2 may have specific effects which are distinct from its effects on pH, e.g. Stalons et al. (1974) concluded that the growth of anaerobic bacterial pathogens can be stimulated by addition of up to 5% CO_2 to a mixture of H_2 and N_2 and inhibited by the addition of 10% CO_2, and that this effect is not correlated directly with any pH change in the medium.

CO_2 has a beneficial effect on the growth of many organisms in concentrations up to 10%. Davies (1940) showed most growth of Mycobacterium tuberculosis was optimum when exposed to concentration of 2.5% CO_2 volume/volume, whereas 20% CO_2 inhibited the three strains of this species was tested. He believed that the effect on growth is due to a specific action of CO_2 itself and not due to the presence of a particular concentration of ions in the medium. Schaefer et al. (1955) found that concentrations of 0.03%, 1% and 10% CO_2 had no significant effect on growth of these bacteria. Most workers agreed, however, that common concentrations up to 10% CO_2 may stimulate the growth of bacteria but above this level CO_2 may inhibit the growth (Rockwell & Highberger, 1927; Whitcomb et al.,1962; James-Holmquest et al., 1973; Marshall et al., 1973).

Similarly the effects of CO_2 on bacteria in soil through changing the pH have been distinguished from specific effects of the gas itself (Russell, 1968). Generally soil bacteria have been found to be less sensitive than fungi to CO_2; differences in concentration associated with water logging and anaerobiosis, and with differences in the depth of the soil, have not had

great effects on the bacterial population (Leach, 1940; Griffin, 1963(a), 1972).

There is very little information available on the effects of high concentrations of CO_2 on phytopathogenic bacteria. King (1966) found that concentrations of 50% volume/volume CO_2 in air were stimulatory to the growth of Pseudomonas aeruginosa (Sch.) Migula. Most recently, Wells (1974), reported on the effects of high concentrations of CO_2 and lower concentrations of O_2, and a combination of low O_2 and high CO_2 concentrations on growth of species of Erwinia and Pseudomonas in culture. He found that the growth of Erwinia atroseptica, E.carotovora and P.fluorescens was inhibited when exposed to atmospheres containing different concentrations of CO_2 above 10% and either 3% or 21% O_2. The addition of 30% CO_2 to air above cultures reduced the growth to 15% to 34% of that in air.

OTHER COMPOUNDS

For convenience discussion of the other compounds is grouped into the following themes:-

(i) Volatile authentic Chemicals in soil sterilization.

A big range of compounds have been studied; many are known to be produced by Microbial cells, but the concentrations in which they are usually produced are not likely to affect the growth of other organisms, (e.g. Dalton & Hurwitz, 1948; Kreutzer, 1963; Russell, 1968). Kholodny and his co-workers (1943-1945) showed that the application of ethanol, methanol, iso-butonal, acetone, methane, naphthalene "paraffin" and "pine gallipot" as vapours to soil affected the bacterial population. 5% to 10% ethanol stimulated the growth of species of Corynebacterium and of Azatobacter. The species of these bacteria were inhibited by higher concentrations. Many common soil fumigants apparently have relatively little inhibitory effect on the bacterial population in the concentrations in which they are commonly used; e.g. methyl bromide, chloropicrin and dichloropropene treatment increased the bacterial populations studied by Klemmer (1957), but in classical studies Wagner (1895) found a decrease in nitrification in soil treated with carbon disulphide vapour. From empirical observations Wensley (1953) concluded

that methyl bromide at fungicidal levels is more toxic to nitrifying and cellulose decomposing bacteria than to denitrifiers and ammonium producing species. It is shown that bacteria possess a broader range of variability than the fungi in their tolerance to methyl bromide and some other volatile metabolites. eg. Xanthomonas vesicatoria was slightly inhibited by concentrations of 1.5 ml of methyl bromide per cubic foot of soil, and completely inhibited by concentrations of 10 ml/cubic foot. Rhizoctonia solani was completely inhibited by concentrations of 8 ml per cubic foot (McKeen, 1954).

There have been several reports on the relationship of physico chemical characters of soil and its effects on gas mixtures (e.g. Fuhr et al., 1948; Kreutzer, 1960). It has for example, been taken for granted that increased organic matter in soil increases the degree of sorption (Siegel et al., 1951).

(ii) Control of bacterial plant disease by treatment with volatiles.

Phytopathogenic bacteria in the soil appear to be less sensitive than fungi to many toxic or inhibitory substances e.g. Munnecke & Ferguson (1953) found that the gas concentration of methyl bromide, chloropicrin or Na-N-methyl dithiocarbamate required to inhibit Agrobacterium tumefaciens, Cornyebacterium michiganense, and Xanthomonas pelargonii was higher than that needed to inhibit fungal species. There are very few records of the effects of ethylene on bacterial ecology, but Freebairn & Buddenhagen (1964) and Abeles (1973) reported on it affecting the disease syndromes associated with some species.

Winfree et al. (1958) found that fumigation with unspecified concentrations of chloropicrin did not control soft-rotting species of Erwinia and Pseudomonas, in naturally infested soil. Nesmith and Dowler (1975) found that fumigation of field soil with 6 gallon of 1, 2-dibromo-3-chloropropane per acre twice a year resulted in no significant differences in defoliation caused by Xanthomonas pruni. Owens et al. (1969) found that fumigation with a mixture of 0.8% volume/volume acetaldehyde and 0.25% volume/volume of methanol in air stimulated the growth of the bacterial population which they measured; a range of other volatile aldehydes and alcohols in

concentrations which they identified in gases from alfalfa roots produced similar effects. They suggested that since these are commonly available at relatively low cost they might well be useful in stimulating the growth of saprophyte non-pathogenic soil bacteria. This could lead to the control of the pathogenic species, which are not generally good competitors with the free living saprophytes.

(iii) Studies of the ecological effects of concentrations of metabolites identified in gas mixtures produced by living cells.

There have been few studies on these lines. The work of Owens et al. (1969) reported above was based on amounts of material emanating from alfalfa and other plant residues in soils. Russian workers, particularly Bilai (1956, 1963) reported on antibacterial properties of gases from Trichoderma cultures, using in vitro tests with paired Petri dishes; found that gases from cultures of strains of T.koningii were more inhibitory than those from strains of T.viride; Marshall & Hutchinson (1970) and Hutchinson & Cowan (1972) did not find any significant effects of gases from cultures of species of Fomes and of Trichoderma on a few saprophyte bacteria. McGain (1966) reported that some species of Streptomyces are able to produce a volatile "antibiotic" or "antibiotics" which can inhibit the growth of bacteria in soil, but he did not identify the active material. The concept of volatile "antibiotics" has been referred to in other cases (e.g. Fries, 1973). There have been many studies in the use of antibiotics in general for the control of soil borne bacterial plant pathogens (e.g. Patrick, 1954; Wood & Tveit, 1955; Pridham et al., 1956; Dekker, 1963; Brown, 1973; Schroth et al., 1974); these comps. generally of very low volatility, however, and outwith the scope of this review.

The total of these and the few other quantitative studies of interactions with bacterial pathogens is slight, however, and further work seems to be justified.

GENERAL METHODS

GENERAL METHODS.

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

Throughout this work, the term 'test' organism or 'test' material refers to the material 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 authentic chemicals.

1. TEST METHODS

(a) Cultural media and conditions.

The following media were used:-

(i) 2% malt agar (20 g Oxoid malt-extract, 20 g Oxoid agar in 1 litre deionized water).

(ii) Bouillon agar (10 g Lab.Lemco beef extract, 10 g Difco peptone, 5 g Na Cl, 20 g Oxoid agar in 1 litre deionized water, adjusted to pH7 with Na OH).

Media were autoclaved at 120°C for 20 minutes, unless otherwise stated. Fungal cultures were incubated at 24°C and 70-80 % R.H. in intermittent diffuse light in an air-conditioned room. Experimental assemblies with bacteria were held in the same room in the dark. Stock cultures of bacteria were kept on Bouillon agar, in a dark incubator at 24° -25°C.

(b) Apparatus.

The following types of assay assemblies were used:-

(i) Paired Petri dish assemblies.

The fungus to be tested for volatile production was inoculated centrally on a 9 cm Petri dish of 2% malt agar and after the specific period of incubation the culture was paired with another Petri dish containing agar inoculated with the appropriate assay organism. The

two dishes were separated by a sterile cellophane disc (Fig. 1). After further incubation of the paired dishes, observations of the assay colony were compared with those for similar assemblies in which the assay organism was paired with a dish containing uninoculated agar; all tests were carried out in at least three-fold replicates, and held at laboratory temperature (23^{o} - 24^{o}) under intermittent light.

(ii) Paired bottle assemblies.

These consist of two one litre Roux bottles, connected by a glass 'T' piece (bore diameter 1 cm; 'T' bar length of 9 cm; single arm length, 2 - 5 cm) fitted into the necks of the bottles with rubber bungs at either end of the long arm. The short (vertical arm of the 'T' piece) is fitted with a suba rubber cap (Fig. 2), to allow gas samples to be withdrawn for analysis. In all tests, one bottle contained a culture of the fungus being tested, or uninoculated malt agar; the other one contained a culture of the assay organism.

(iii) Single bottle assemblies.

These were used for tests with mixtures of air with authentic organic chemicals. They consist of a single bottle containing the assay organism inoculated on the appropriate medium and sealed during the experiment with a Suba rubber cap.

The known concentrations of authentic liquid chemicals were placed (using a Hamilton micro gas tight syringe) on a sterile - Whatman filter paper suspended in the neck of Roux bottle. 3-4 hours were allowed for volatilization and diffusion in the vessels before gas samples were taken for analysis unless otherwise stated.

(iv) CO_2/air mixture - apparatus.

The assay cultures were aerated with CO_2 and air mixtures. The development and use of a special apparatus for this is described in appendix - I.

2. ORGANISMS USED.

Organisms used in these tests are:-

(a) Species of Trichoderma.

Professor J. Webster and Dr. C. Dennis courteously sent us the following species and strains of Trichoderma. The reference numbers were those which they supplied and which they used in their publications.

T. viride 1
T. viride 3
T. viride 14
T. viride A
T. viride B/45553 ii
T. viride C/16198
T. viride D/109551
T. koningii 6
T. koningii 7
T. koningii A/73022
T. koningii B/62429
T. koningii C/54693
T. koningii SHD/M/2629
T. pseudokoningii A/196-1
T. polysporum 2
T. polysporum 5
T. polysporum 74
T. polysporum C/306
T. piluliferum SHD/M/2636
T. hamatum 127
T. hamatum JMD/12
T. harzianum 1
T. harzianum 129
T. longibranchiatum WBC 4576

(b) Assay Fungi.

The assay fungi used in this course of study are:

Rhizoctonia solani, Kühn.
*Pyronema domesticum, (Sow. ex.Fr.) Sacc.
*Pythium ultimum, Trow.
*Fomes annosus, (Fr.) Cooke.
*Mucor hiemalis, Wehm.
*Fusarium oxysporum, Schlecht. ex Fr.

*Dr. Dennis supplied these to us and he said that they were those which he had used in his work. He was unable to supply his strain of R.solani and the one in the Glasgow University Collection No.1, Mycology Laboratories, was used instead.

Uniform inoculae were made by cutting 5mm discs from the periphery of a young Petri dish culture of the required assay fungi on 2% malt agar. Each disc was used to inoculate one Roux bottle or paired assembly as required. By this procedure, colonies of regular shape were formed. Daily observations of linear growth were assessed in all cases by taking the mean value of two previously marked diameters at right angles.

(c) Assay Bacteria.

The assay bacteria were obtained from Department of Agriculture and Fisheries of Scotland, Edinburgh. They are listed below with the Dept. of Agriculture catalogue numbers.

Erwinia amylovora 595/V
Erwinia aroideae 929/II
Erwinia atroseptica 549/VI
Erwinia carotovora 312/VIII
Erwinia tracheiphila 2133/I

All cultures were inoculated on Bouillon agar as described above.

3. GAS-LIQUID CHROMATOGRAPHIC ANALYSIS

(a) Conditions.

Micro cross-section detectors were used in the analysis of CO_2 and O_2. The analyses were carried out using an Aerograph, Model 200 Gas-Liquid Chromatograph. Levels of CO_2 were measured, using a stainless steel column ($\frac{1}{4}$ inch x 6 feet) packed with Porapak R, at 75-80°C, helium flow-rate was 45-55 mls/min (38 p.s.i.). Oxygen levels were measured using a stainless steel column ($\frac{1}{4}$ inch x 10 feet) packed with Molecular Sieve 5A, 30/60 mesh, at 75°C, helium flow-rate was 45-55 mls/minute.

Ammonia, acetaldehyde, acetone, and ethanol were measured and other organic volatiles were looked for using a Pye Model 104 Gas-Liquid Chromatograph with a flame ionization detector, a stainless steel column ($\frac{1}{8}$ inch x 6 feet) packed with Carbowax 1500, or with a glass column ($\frac{1}{8}$ inch x 6 feet) packed with Carbowax 20 M at 78°C, hydrogen flow-rate 40 mls/min. (15 p.s.i.); nitrogen flow-rate 40 mls/minute. Ethylene was measured using the same detector, a glass column ($\frac{1}{4}$ inch x 6 feet) packed with Porapak R, at 65°C, hydrogen flow-rate 40 mls/min. (16 p.s.i.), nitrogen flow-rate 36 mls/minute. Air was used with measurements with the F.I.D. at flow-rate 40 mls/min. (10-15 p.s.i.).

(b) Identification of culture gases.

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

Concentrations of CO_2 were estimated by comparison with peak heights produced by the known mixtures of CO_2 in air made up in the apparatus, illustrated and explained in detail in Appendix - I below.

Concentrations of other components of the identified volatile metabolites were measured by comparison of peak heights given by culture gases with those given by samples from sealed Roux bottles in which known amounts of authentic analar metabolites had been allowed to volatilize (cf. Section 1.b.iii above). A measured volume of ethylene gas had been introduced by a micro gas syringe.

All gas samples were taken for analyses from replicate bottles by "5 ml" polyethylene- disposable syringe. The syringe was flushed between each sample and no residual traces of samples were found on test injections after the analysis.

Peak heights were measured at convenient attenuations. For ease of comparison in the appendix tables, these have been converted to the equivalent heights at 1/32 attenuation on the Aerograph 200 (CO_2 and O_2), or 5×10^2 on the Pye 104 (other metabolites).

(c) Standardisations

Means of peak heights of known concentrations of identified metabolites were recorded under typical conditions. G.L.C. units were checked every six months regularly and every time when they were repaired and/or standard operational conditions were changed.

Details of maximum, minimum, and typical peak heights of known concentrations of different volatile materials measured under uniform conditions are recorded in Table 1 , and illustrated in Figures (3 to 7).

It can be seen from the table that the consistency of peak heights was greatest with the lower concentrations measured. These concentrations covered the critical ranges in these tests; the greater variation with high concentrations was not therefore critical in this work.

Table 1 G.L.C. peak heights obtained from samples of known concentrations of identified volatile metabolites measured at different periods during these studies.

authentic compound	cocn. added ml/litre air	Peak height (mm) at 5 x 10^2 [(a)] sensitivity at any time 1	2	3	4	5	6	Range and mean of peak heights
ethylene	0.001	6	3	10	12	8	5	7 + 5 - 4
	0.01	19	14	16	28	26	18	20 + 8 - 6
	0.1	303	220	300	380	260	344	301 +79 -81
	1.0	2200	2100	2300	2420	2280	2290	2265 +155 -165
acetaldehyde	0.0005	26	16	22	35	24	19	24 +11 - 8
	0.001	70	52	68	90	67	72	70 +20 -18
	0.005	190	150	188	200	182	163	179 +21 -29
	0.01	274	260	290	330	286	300	290 +40 -30
	0.05	960	900	1009	1080	1000	932	980 +100 -80
acetone	0.0005	20	11	26	30	14	18	20 +10 - 9
	0.001	45	40	50	66	42	59	50 +16 -10
	0.005	136	120	164	154	126	139	140 +24 -20
	0.01	258	220	230	290	282	280	260 +30 -40
ethanol	0.01	30	25	40	50	28	36	35 +15 -10
	0.03	63	60	62	88	84	65	70 +18 -10
	0.05	121	100	112	140	130	117	120 +20 -20
	0.1	260	200	220	290	280	252	250 +40 -50
	0.3	570	510	520	660	650	570	580 +80 -70
carbon dioxide	5% v/v	9	6	5	10	5	7	7 + 3 - 2
	10% "	18	20	14	21	18	16	18 + 3 - 4
	15% "	26	22	19	26	26	25	24 + 2 - 5
	20% "	31	33	25	34	28	28	30 + 4 - 5
	25% "	41	42	30	44	42	40	40 + 4 -10
	30% "	49	52	35	55	52	55	50 + 5 -15

(a) All readings are the mean of peak heights of samples taken from 3 replicate Roux bottles, given similar readings.

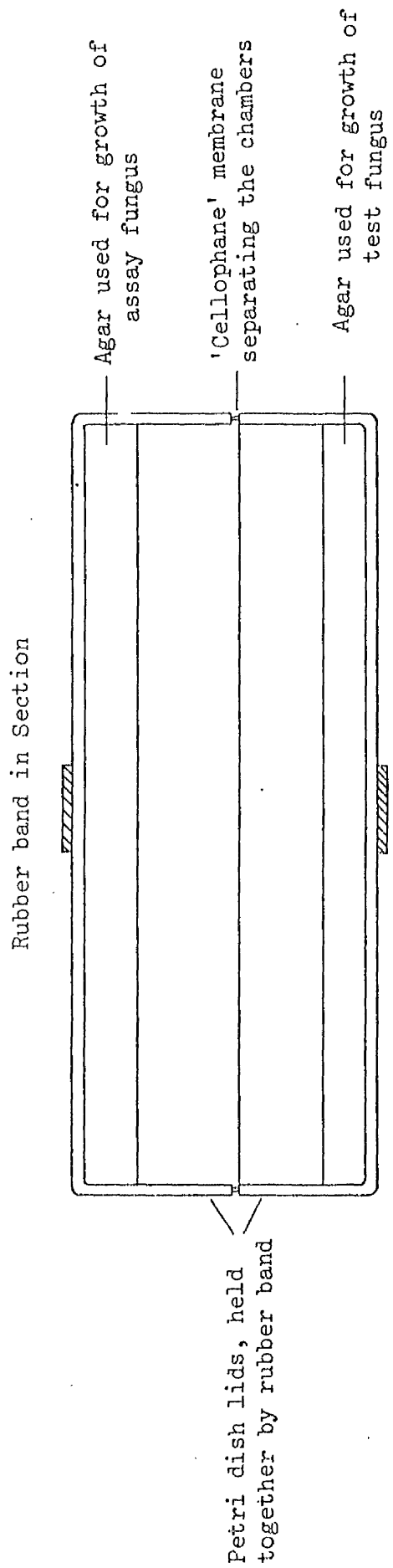


Figure 1 Assembly used for the tests of effects of volatile fungal metabolites

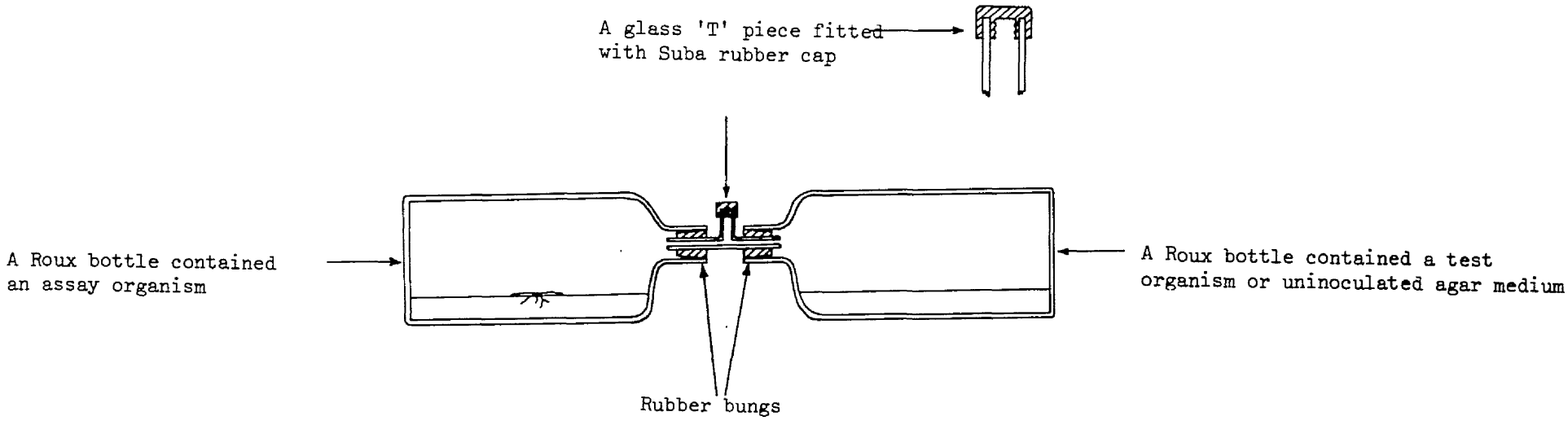


Figure 2 Assembly used for the tests of effects of volatile fungal metabolites

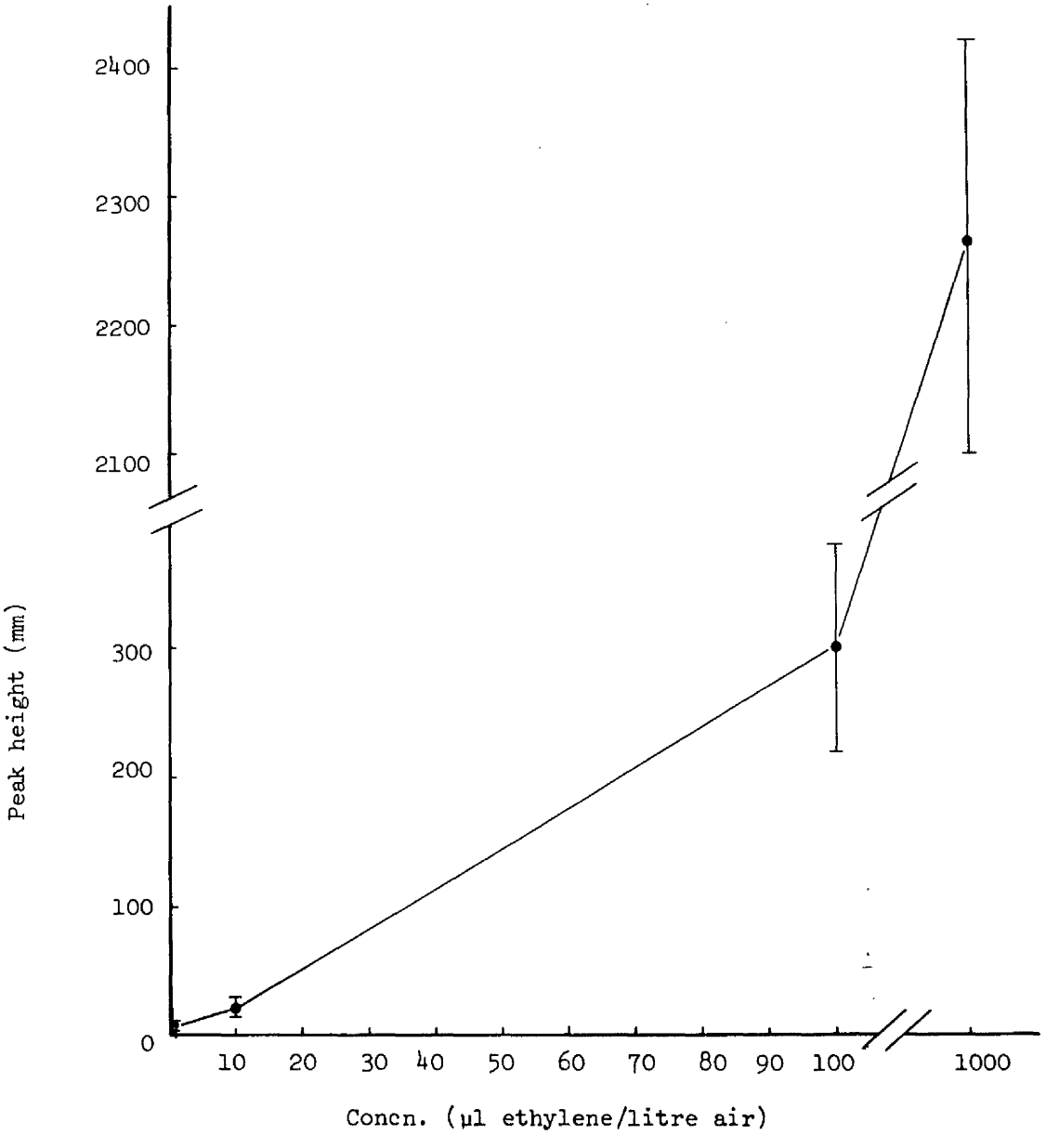


Figure 3 Mean and range of G.L.C. peak heights obtained from gas samples of known concentrations of ethylene at different times during the study

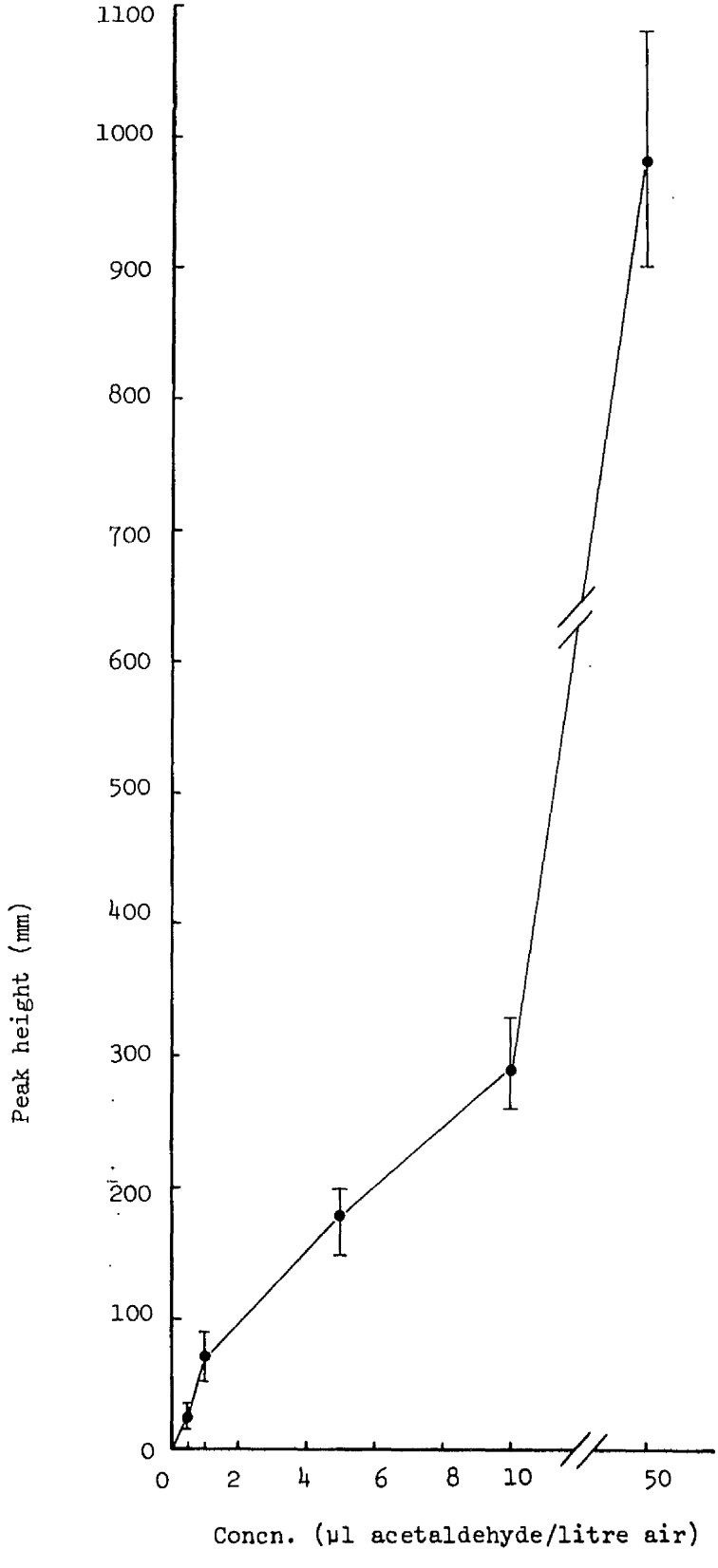


Figure 4 Mean and range of G.L.C. peak heights obtained from gas samples of known concentrations of acetaldehyde at different times during the study

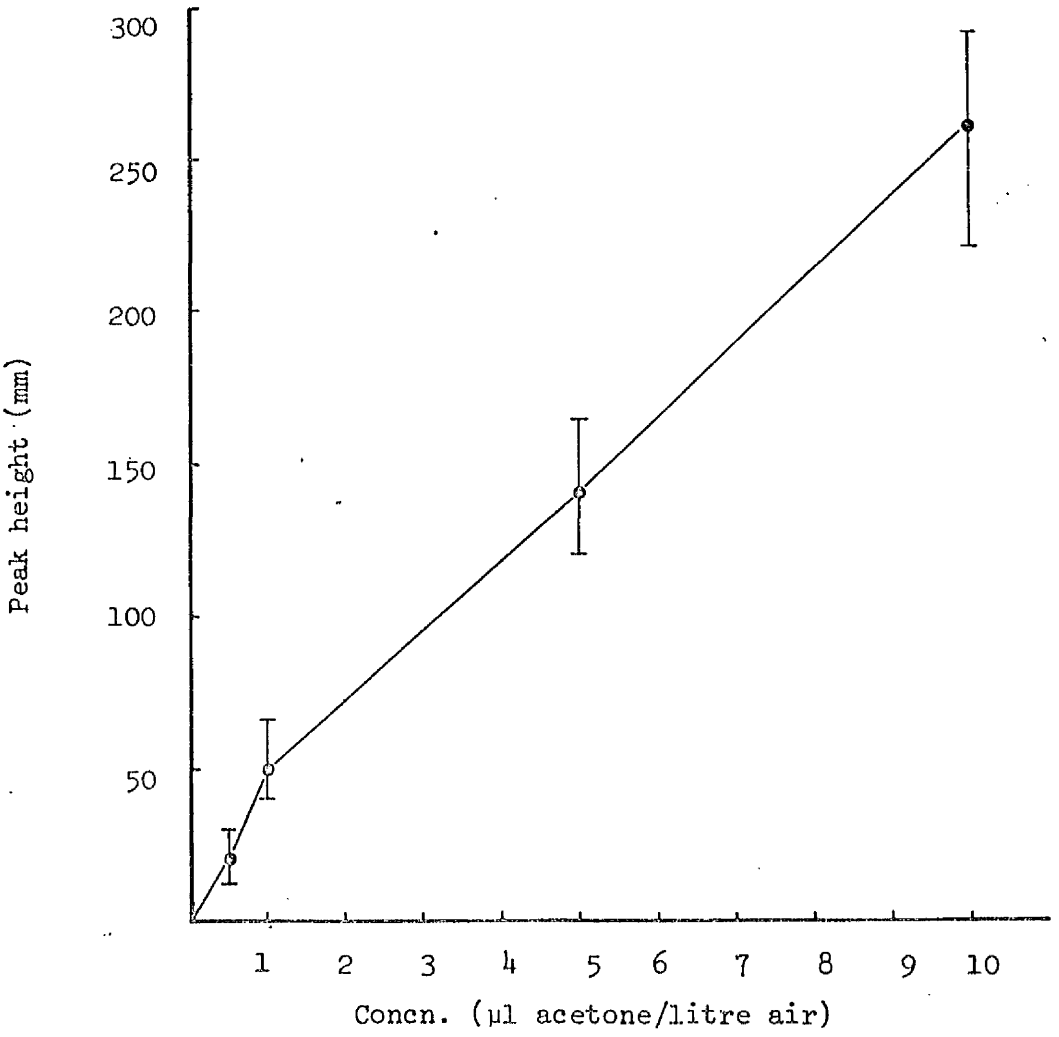


Figure 5 Mean and range of G.L.C. peak heights obtained from gas samples of known concentrations of acetone at different times during the study

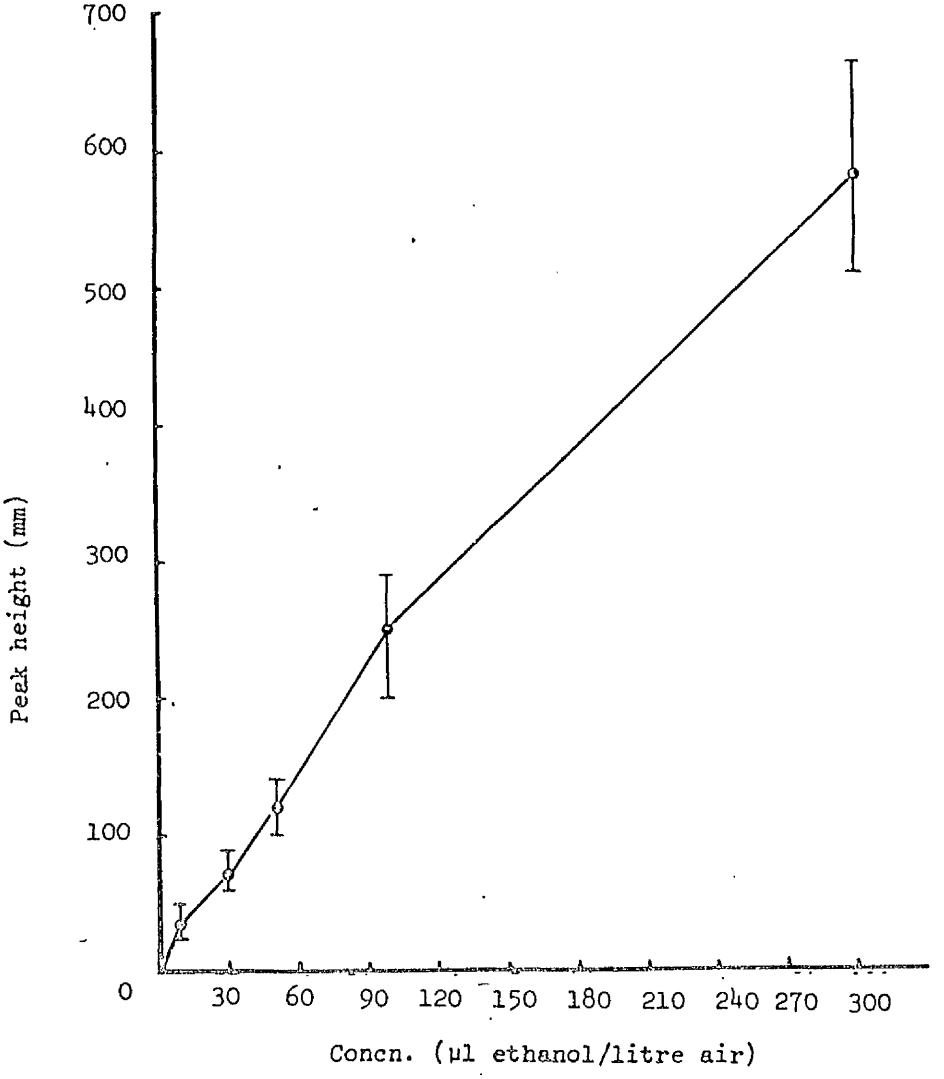


Figure 6 Mean and range of G.L.C. peak heights obtained from gas samples of known concentrations of ethanol at different times during the study

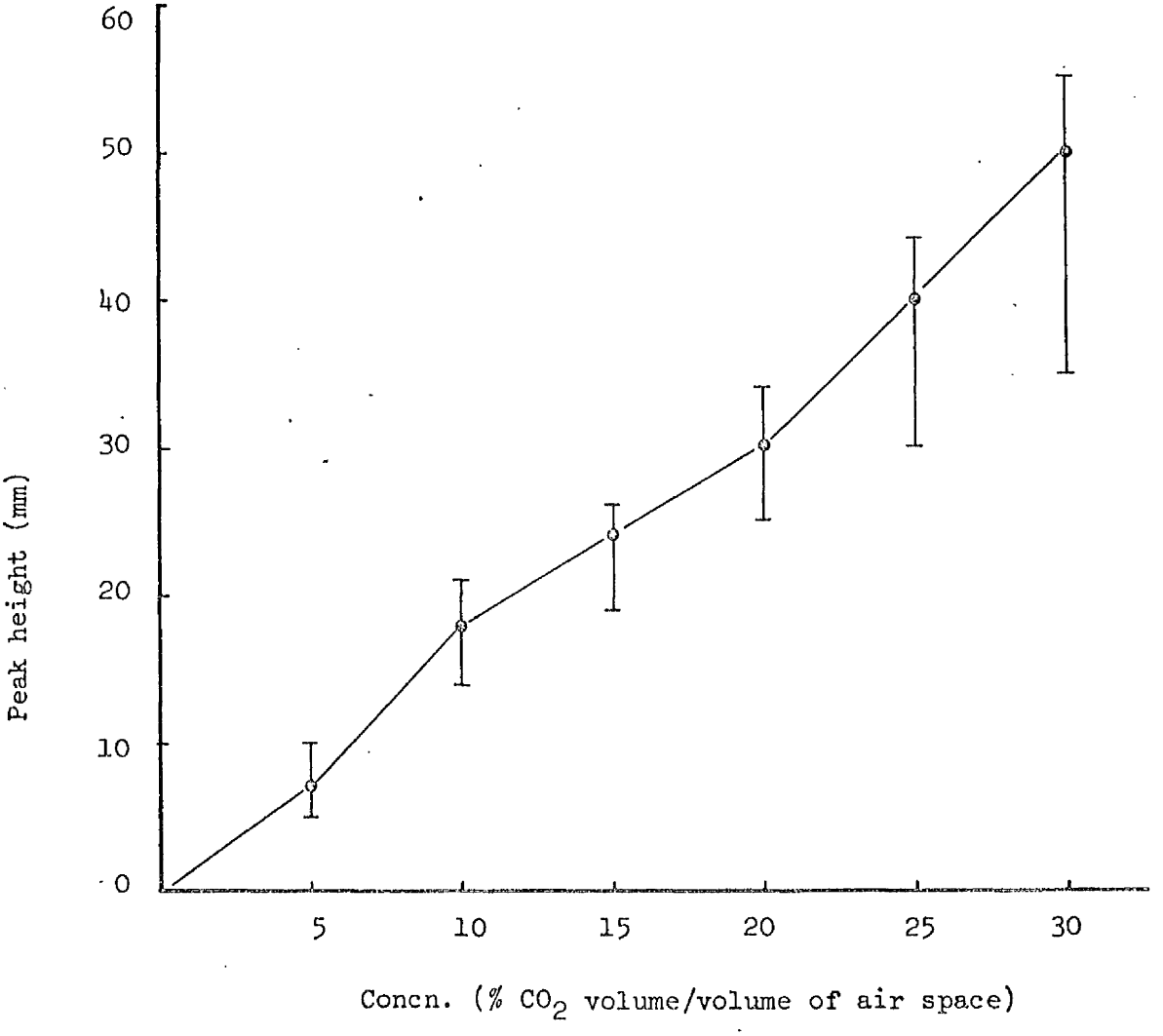


Figure 7 Mean and range of G.L.C. peak heights obtained from gas samples of known concentrations of CO_2/air mixtures at different times during the study

PART I

AN INVESTIGATION OF THE DIFFERENCES BETWEEN THE BIOLOGICAL EFFECTS OF CULTURE GASES FROM VARIOUS SPECIES OF *TRICHODERMA* ON GROWTH OF ASSAY FUNGI

PART I.1

INTRODUCTION AND REVIEW OF WORK ON *TRICHODERMA* SPP.

I.1 INTRODUCTION AND REVIEW OF WORK ON TRICHODERMA SPP.

The production of biologically active volatile metabolites by cultures of species of Trichoderma Pers. has been reported frequently. Russian workers, notably Kholodny et al. (1945), Bilai (1956, 1963), Dymovich (1960), Khasanov (1962) and Sarymsakova (1967), have reported that volatile metabolites (antibiotics) of Trichoderma species are antagonistic to other fungi and bacteria; but they did not study their nature and biological properties in detail. To avoid uncertainties in nomenclature the names used in this review are those given by the individual authors in each paper.

Bilai (1956) used four different strains which were distinguishable from other soil isolates by a specific odour. This odour named as a coconut smell produced by active strains of the genus (Weindling, 1932, 1934; Rifai, 1969; Dennis & Webster, 1971).

Dymovich (1960) found that the volatile fractions from cultures of T.lignorum (Tod.) Harz. and T.koningii Oud., were inhibitory to growth of many species of phytopathogenic bacteria and fungi, of the bacteria tested by him, Pseudomonastumifaciens, P.tabacum, P.andropogonis, P.fluorescens and Xanthomonas phaseoli proved to be the most sensitive; Pseudomonas pisi was resistant. He also found that results varied according to the age of the culture, the nutrient medium, the characters of the test organism, etc. The most active volatile metabolites for instance, were found from 10-day-old cultures. Khasanov (1962), found the volatile antibiotic substances from three strains (Trichoderma sp.105, T.lignorum 118, T.koningii 97) to be inactive against the whole spectrum of test bacteria. But the same volatile metabolites were found to have a fungistatic effect on all types of tested fungi, the fungicidal character was also common in these tests.

The volatile substances of Trichoderma possess a broad spectrum of antibiotic activity (Bilai, 1956). The same author found that 8-day-old T.lignorum (T.viride) and T.koningii inhibited growth of B.mesentricus and Staphylococcus aureus. The antibiotic properties of the volatile substances vary from one strain to another (Bilai, 1963). Empirical results of Dymovich (1960) showed that T.koningii cultures were more effective than T.lignorum cultures in their inhibitory level to the growth of fungi and phytopathogenic bacteria.

In previous work in this Department the inhibition produced by gases from T.viride cultures was found to be greater than that produced by some of the other fungi which have been tested. (Martin, 1963; Dick & Hutchinson, 1966; Hutchinson, 1971). Hutchinson & Cowan (1972) showed that the amounts of carbon dioxide and/or ethanol in gaseous from cultures of a strain of T.harzianum Rifai could account for the inhibition of growth of fungal cultures and of lettuce (lactuca sativa var. sativa) seedlingsin laboratory tests.

Dennis & Webster (1971) studied the biological properties of Trichoderma species-groups. They found that the gases from cultures of several species of Trichoderma commonly inhibited and in some cases stimulated the rate of increase of colony diameter of several other species of fungi. The amount of effects varied with strains, with species, with the age of different Trichoderma cultures when they were tested, and with the assay fungi subject to test. They concluded that the effects were due to the liberation of biologically active volatile metabolites from the Trichoderma mycelia. They showed that aqueous solutions of condensates of the Trichoderma culture gases inhibited the growth of Fomes annosus cultures placed in contact with them; they identified acetaldehyde as the main carbonyl compound present in these condensates. They found that growth of F.annosus on 2% malt-extract agar discs was reduced if the discs were suspended in a solution of 100 p.p.m. acetaldehyde in water, and completely inhibited in 500 p.p.m. and above. They found that the effect was fungistatic, not fungicidal as growth of the Fomes cultures often occurred after 6-7 days of incubation. They also concluded that the active constituents of the culture gases were unlikely to be CO_2 or ammonia, since "not all strains showed activity and the pattern of activity of the producer strains was not the pattern which would be expected in these primary metabolites were responsible". They state that "experiments were carried out to confirm this" but they did not report the details or the results of these. In particular they did not report any quantitative analysis of

culture gases, and they did not report tests of identified metabolites in the vapour concentrations in which they have been found above the Trichoderma cultures.

This is a report of the identification of metabolites from the Trichoderma cultures, and of the demonstration that their production in these conditions can account for the interactions seen.

PART I.2

A RE-INVESTIGATION OF THE QUALITATIVE WORK OF DENNIS AND WEBSTER, AND COMPARISON WITH GLASGOW TECHNIQUES.

I.2 A re-investigation of the qualitative work of Dennis & Webster, and comparison with Glasgow techniques

As a first step in the investigation of the differences between the biological activities of different strains and species of Trichoderma, it was decided to repeat some of Dennis and Webster's experimental work using their cultures and conditions as far as possible; this was mainly a preliminary check to see whether the interactions which they described could be demonstrated in the Glasgow laboratories. Some minor extensions were done to see whether their methods could be made more convenient for further investigation.

(a) A comparison with their work, using paired Petri dishes.

Materials & Methods

Dr. Dennis sent us cultures of all the available strains of Trichoderma, and of the other assay fungi which he and Professor Webster had tested. The missing cultures are no longer in his collection, and he was unable to identify them in the culture collections at the Commonwealth Mycological Institute or at the Centraal Bureau von Schimmelcultures, Baarn. The strains which they found most active (T. viride 1) and least active (T.longibranchiatum WBC 4576) were chosen for the main investigation.

Paired Petri dishes were set up as described in the General methods section above (l.b.i.). In each test three paired assemblies were set up for each treatment. The only known differences between this work and that of Dennis and Webster are:-

(1) Possible differences which could have developed in the metabolism of the fungi during maintenance in culture since Dennis and Webster completed their work.

(2) The use of a strain of Rhizoctonia solani from Glasgow University, Mycological Culture Collection (R.solani G.U.1) in lieu of their strain, which Dr. Dennis was unable to supply.

(3) Possible unmeasured differences in the environments of the laboratories in which the work was carried out.

(4) The presence of a permeable cellophane disc between the assay and the test fungal dishes in the Glasgow experiments.

(5) In the Glasgow "Control" assemblies, the dishes containing R.solani were paired with dishes containing sterile 2% malt agar. In Dennis & Webster experiments were "The lids of control plates, which had not been inoculated with a Trichoderma strain, were also replaced in the same way.....etc." (Dennis & Webster, 1971; p.41, line 31-32).

(6) 5 ml samples of culture gases were taken from the Glasgow assemblies on the second day after pairing and analysed by G.L. Chromatography.

Dennis and Webster's observations were extended by:-

(a) continuing the investigation of changes in productivity of the Trichoderma culture as they aged from 15 days to 20 days before pairing.

(b) Recording the effects on R.solani cultures at two day intervals from 2 to 8 days after pairing, instead of only at 2 days.

Results

The results of two replicate experiments are given in Appendix-tables 1A-J and 2A-J and summarised in text-table No. 2. Text-figure 8 compares the means of the measurements of both experiments on the second day after pairing with those given by Dennis & Webster for the same incubation time. Text-figure 9 compares the means of measurements of the effects of T.viride 1 cultures of different ages on growth of R.solani after different periods of incubation after pairing with those of T.longibranchiatum WBC 4576 in the same conditions.

Discussion

The results from the two laboratories which are summarised in Figure 8 are similar in that the maximum inhibition is shown by cultures of T.viride 1 aged 6-8 days at the time of pairing, and that T.viride 1 is substantially more active than T.longibranchiatum WBC 4576. The means of the diameters of control colonies of the Glasgow strain of R.solani on 2nd day after

Table 2 Summary of resuls of tests in paired Petri dish assemblies containing colonies of Rhizoctonia solani paired with Trichoderma viride 1, T.longibranchiatum WBC.4576 cultures of different ages, compared with control treatments.

Expt. No.	(Inhibition or Stimulation as % of control on 2nd day after pairing) "Ages of Trichoderma cultures before pairing"																			
	2 days		4 days		6 days		8 days		10 days		12 days		14 days		16 days		18 days		20 days	
	*T.v.	*T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.	T.v.	T.l.
1.	-42	+5	-5	Nil	-67	-5	-55	-50	-11	-28	-13	-13	-27	-15	-25	Nil	Nil	-8	Nil	+10
2.	-10	Nil	-15	Nil	-50	-5	-37	-11	-35	-15	-5	+19	-40	-5	-14	+5	-11	-11	-10	+5
Mean	-26	+3	-10	Nil	-59	-5	-46	-31	-23	-22	-9	+3	-34	-10	-20	+3	-6	-10	-5	+8

*T. v = T.viride 1.

T.l. = T.longibranchiatum WBC 4576

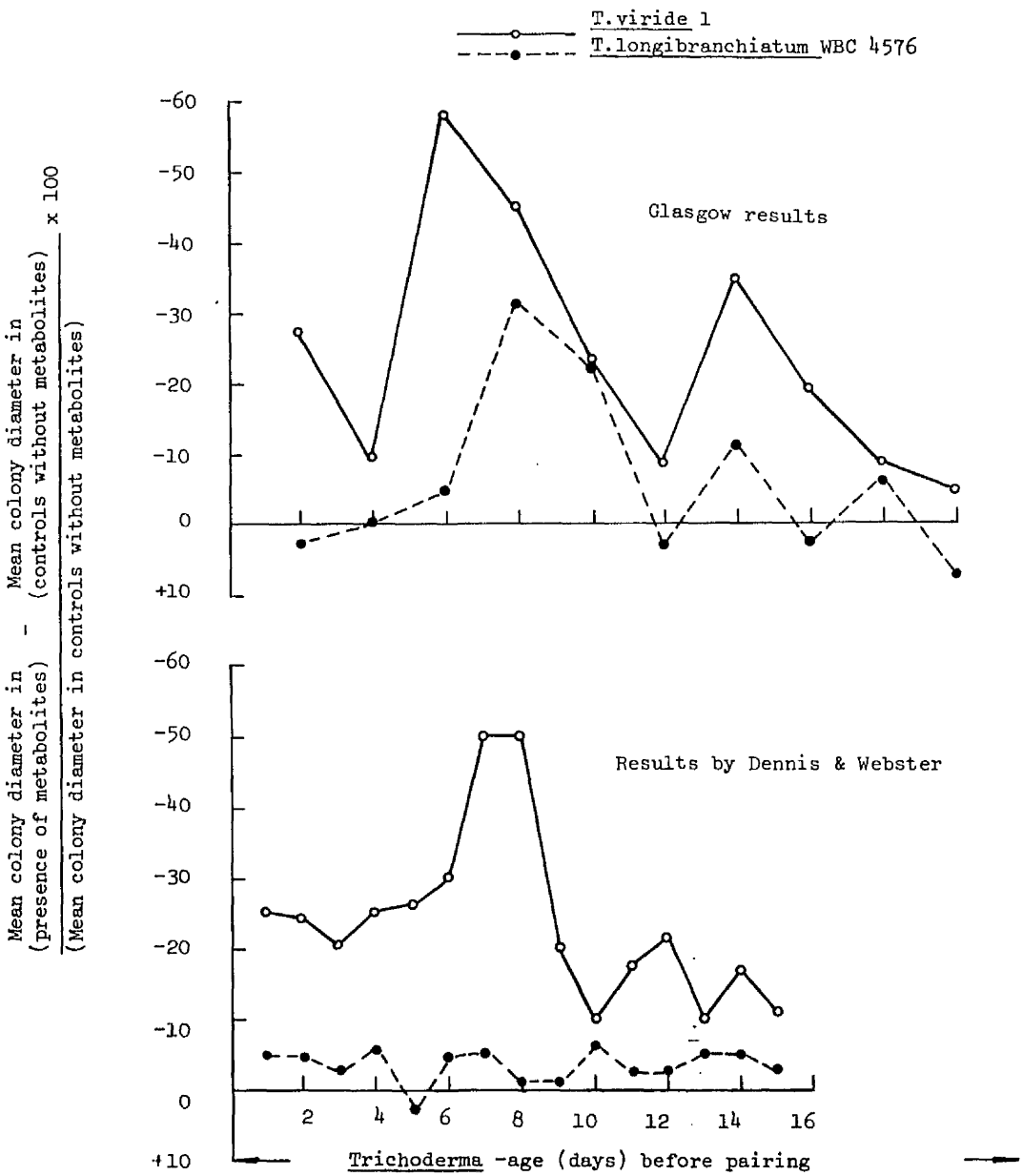


Figure 8 Comparison of Glasgow results on the effects of T.viride 1 and T.longibranchiatum WBC 4576 on growth of R.solani with those of Dennis & Webster. Measurements taken on the 2nd day after pairing.

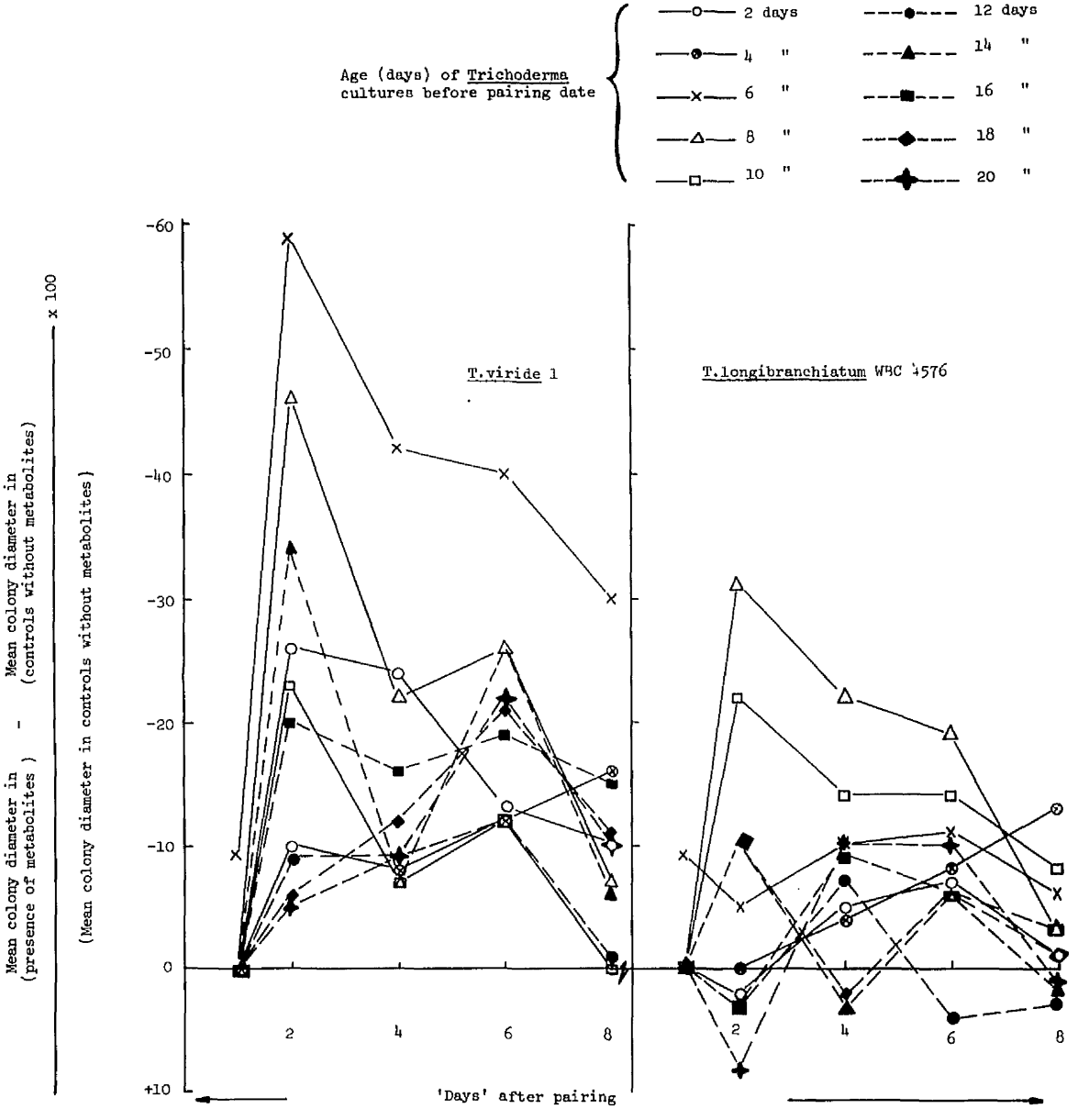


Figure 9 Inhibition or Stimulation of linear growth of Rhizoctonia solani when paired with cultures of two species of Trichoderma of different ages in two replicate experiments.

inoculation was 20 mm in these experiments, compared with the 51 mm reported by Dennis & Webster for their strain (Appendix tables 1A-J and 2A - J).

There is no evidence to suggest that this, or the other minor differences in the techniques, introduced any noteworthy effect on the related amounts of inhibition which were recorded.

From Text-Figure 9, it is concluded that the effects of R.solani of gases from most of the ages of Trichoderma cultures tested was generally greatest at two days after pairing. The only noteworthy difference was that the pattern of activity of T.viride cultures which were 18-and 20-day-old at the time of pairing increased at a steady rate for the first 6 days of the test period. The consistant drop in effects between the second day and the 4th day in other cultures may be associated with the removal of 5 ml samples for G.L.C. analysis on the second day. Samples of head space gases were taken from all assemblies on the second day after pairing. No peaks of more than a trivial height appeared in any trace.

Conclusion

The observations show that the effects on R.solani by the two fungi described by Dennis & Webster (1971) can be demonstrated in the Glasgow Laboratory. Determination of the statistical significance of the differences found in the Glasgow work does not justify the work involved, particularly as Dennis & Webster did not give enough details of replications and variation in their experiments to permit any rigorous comparison. The extension of the range of the investigation does not reveal any more advantageous times to make measurements.

Previous investigations by these methods in this department (Dick & Hutchinson, 1966; Glen et al., 1966; Glen and Hutchinson, 1969, 1973) have shown that it is not a convenient one for analytical work. In particular differences in ventilation inherent in the loose contact between the paired Petri dishes, and the small volumes of the gases involved, lead to big experimental variation. This limits the amount of useful deduction which can be made from results. The work was therefore discontinued as soon as it had actived its purpose of:

(a) demonstrating a convenient degree of comparability with Dennis and Webster investigations.

(b) showing that minor changes in procedure were unlikely to yield a more convenient septum for quantitative work.

(b) A Comparison with their work, using paired Roux bottles

Particular Methods

Cultures of six assay species were paired with cultures of Trichoderma viride 1 which had been set up with Suba rubber caps and incubated for 7 days, by the standard methods for paired assembly tests (General Methods, Paragraph 1.b.ii). Each treatment was examined in five replicate assemblies and compared with five replicate control assemblies set up in normal laboratory air. Measurements of colony diameter were made every day.

Results

The results are given in appendix tables 8A and 10A to 10E. Test-Figure (10) compared the means of this experiment on the second day with these found by Dennis & Webster on the 2nd day too.

Discussion

The results with paired Roux bottle assemblies were similar to those of Dennis and Webster in all important respects. The only noteworthy differences were with Pyronema domesticum, which was the most resistant species to volatile metabolites produced by Trichoderma viride 1 in their laboratory, but the most sensitive in Glasgow Laboratory. The reason for this difference has not been investigated; it may be related to changes in the fungus since it was examined by Dennis and Webster; it is also noted, however, that it is more sensitive to acetaldehyde than the other fungi. This might also affect the

relation in the sealed Roux bottles. It did not seem sensible or profitable to investigate these possibilities further at this stage, as the differences in this particular relationship has relatively small significance in the total work.

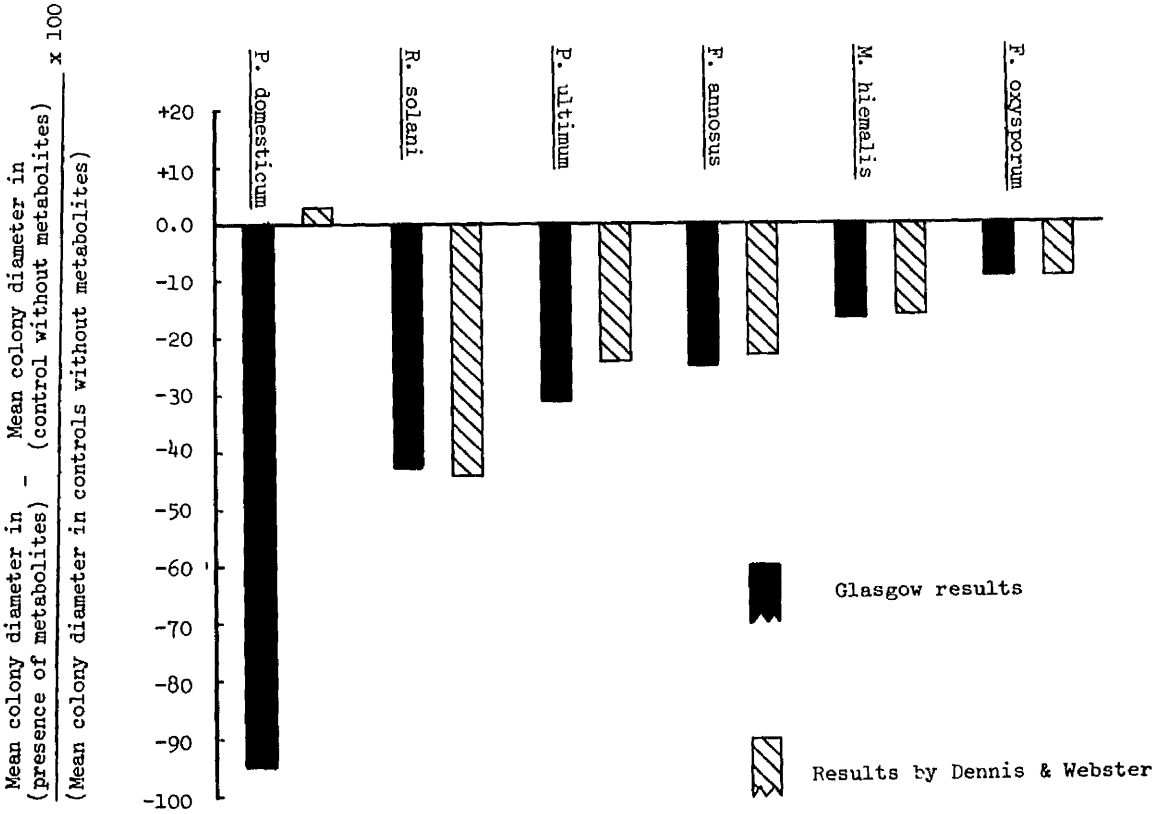


Figure 10 Comparative study of the effects of Trichoderma viride on linear growth of a range of assay fungi used by Dennis & Webster.

PART I.3

QUANTITATIVE EXAMINATION OF THE ACTIVITY OF TWO REPRESENTATIVES OF *TRICHODERMA spp.*

I.3 QUANTITATIVE EXAMINATION OF THE ACTIVITY OF TWO REPRESENTATIVES OF TRICHODERMA SPP.

INTRODUCTION

This report starts with a discussion of the analysis of gases from pure cultures of Trichoderma and of the effects of comparable concentrations of authentic material of identified constituents on other assay species. This was found to be needed to permit judgement of the kind and amounts of metabolites likely to be found in paired cultures of Trichoderma sp. and other fungi. It also gave the information needed for judgement of the scale of replication and precision of analysis likely to be required for the investigation of these less easily controlled interactions between species.

The information from pure culture studies was also needed for the interpretation of the mixed culture interactions in which gases have been produced by the assay fungi as well as from the Trichoderma cultures.

I.3(a) G.L.C. ANALYSIS OF THE HEAD SPACE GASES FROM PURE CULTURES OF T. VIRIDE 1 AND T. LONGIBRANCHIATUM W.B.C. 4576.

METHOD

Cultures in single and in paired sealed Roux bottles were set up, incubated and measured, and gas samples were analysed by the standard techniques which are described in the General Methods (3a, b, c).

RESULTS

Typical G.L.C. traces from analyses of samples from 7-day-old cultures are shown in text-Figures (11-16). Appendix-table (3), records the measurements made in three typical experiments using three replicate single Roux bottle cultures of each species. Text-table (3), shows the concentrations of authentic metabolites required in air mixtures to give closely similar peak heights to the means of those from these experiments. It also shows the concentrations required to give peak heights equal to the maximum recorded in any of the numerous other examinations of gases from pure cultures of these strains made at other times during the work on this project.

Ethylene is identified in this table, but it was determined in 3 separate precisely similar experiments for administrative convenience with the apparatus available.

The response of the chromatograph to mixtures made up by injecting less than 0.01 ml of ammonia liquid into 1 litre of air was too variable to yield useful results. It appears likely that this relates to the reactivity of this gas at these concentrations. Mixtures of 0.05 ml liquid ammonia/litre air gave peak heights at least 10 times larger than those recorded for Trichoderma pure cultures; since R. solani cultures were apparently unaffected by such higher concentrations, the possibility of improvement of the analytical technique was not explored further.

Figure 11 Typical chromatogram of gas samples from 1-week-old cultures of T.viride 1

Compounds

1. ammonia
2. acetaldehyde
3. acetone
4. ethylacetate
5. ethanol
6. n-propanol
7. iso-butanol
8. pentanol

G.L.C. conditions

Column: Carbowax 20M; Temp: 78°C
Detector: F.I.D.; Temp. 150°C
N_2 Flow Rate: 40 mls/minute
Attenuation: 2 x 10^2
Carrier Gas: N_2; Pressure: 38 p.s.i.
H_2 Press.: 15 p.s.i.
Chart speed: 15"/hour.

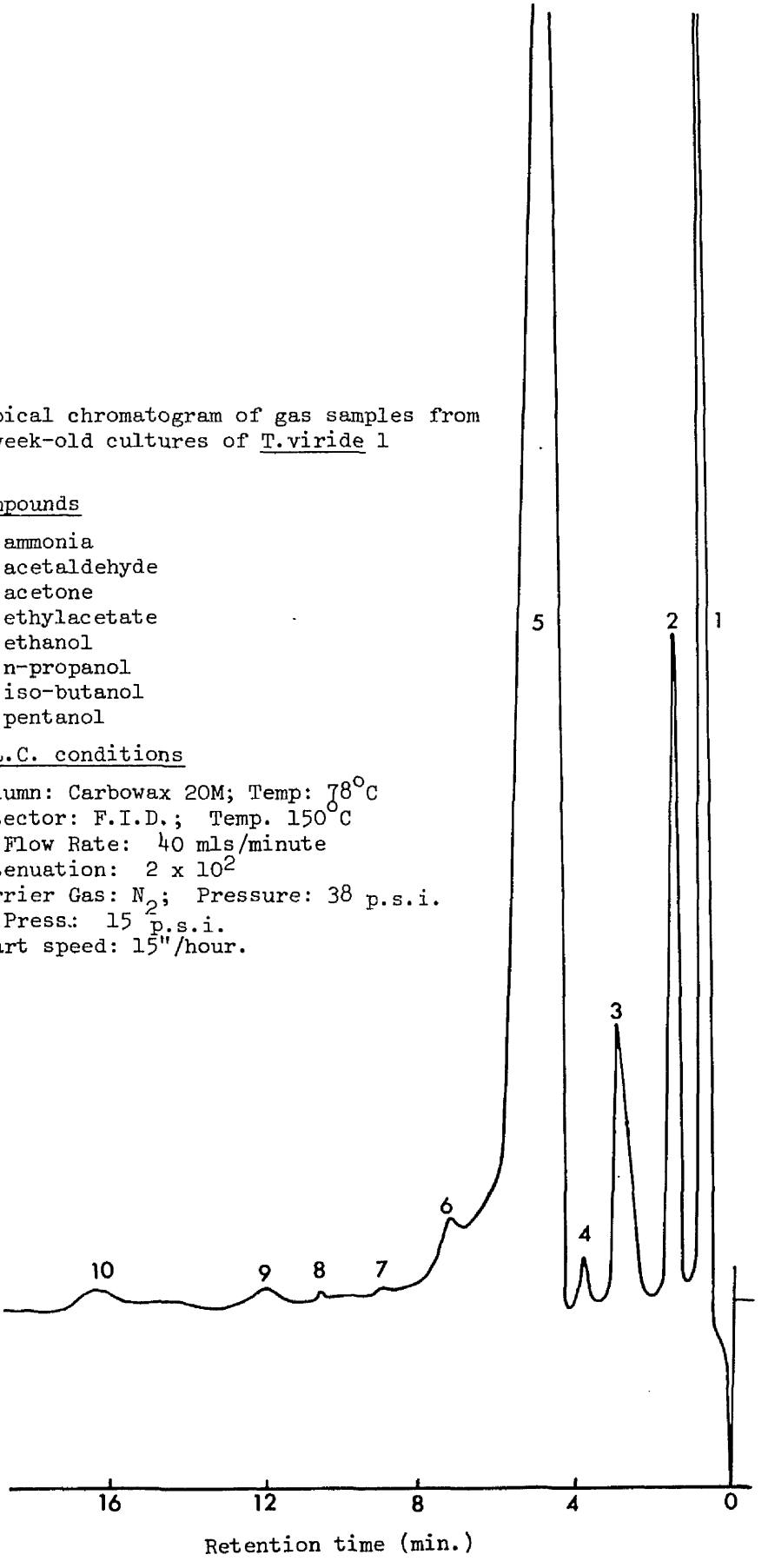


Figure 12 Typical chromatogram of gas samples from 1-week-old cultures of T.viride 1

Compounds
1. ammonia
2. acetaldehyde
3. acetone
4. ethylacetate
5. ethanol
6-10 pentanols

G.L.C. conditions
Column: Carbowax 1500 Temp. 75°C
Detector: F.I.D.: Temp. 150°C
N_2 Flow Rate: 40 mls/minute
Attenuation: 2 x 10^2
Carrier Gas: N_2; Pressure 38 p.s.i.
H_2 Press: 15 p.s.i.
Chart speed: 15"/hour

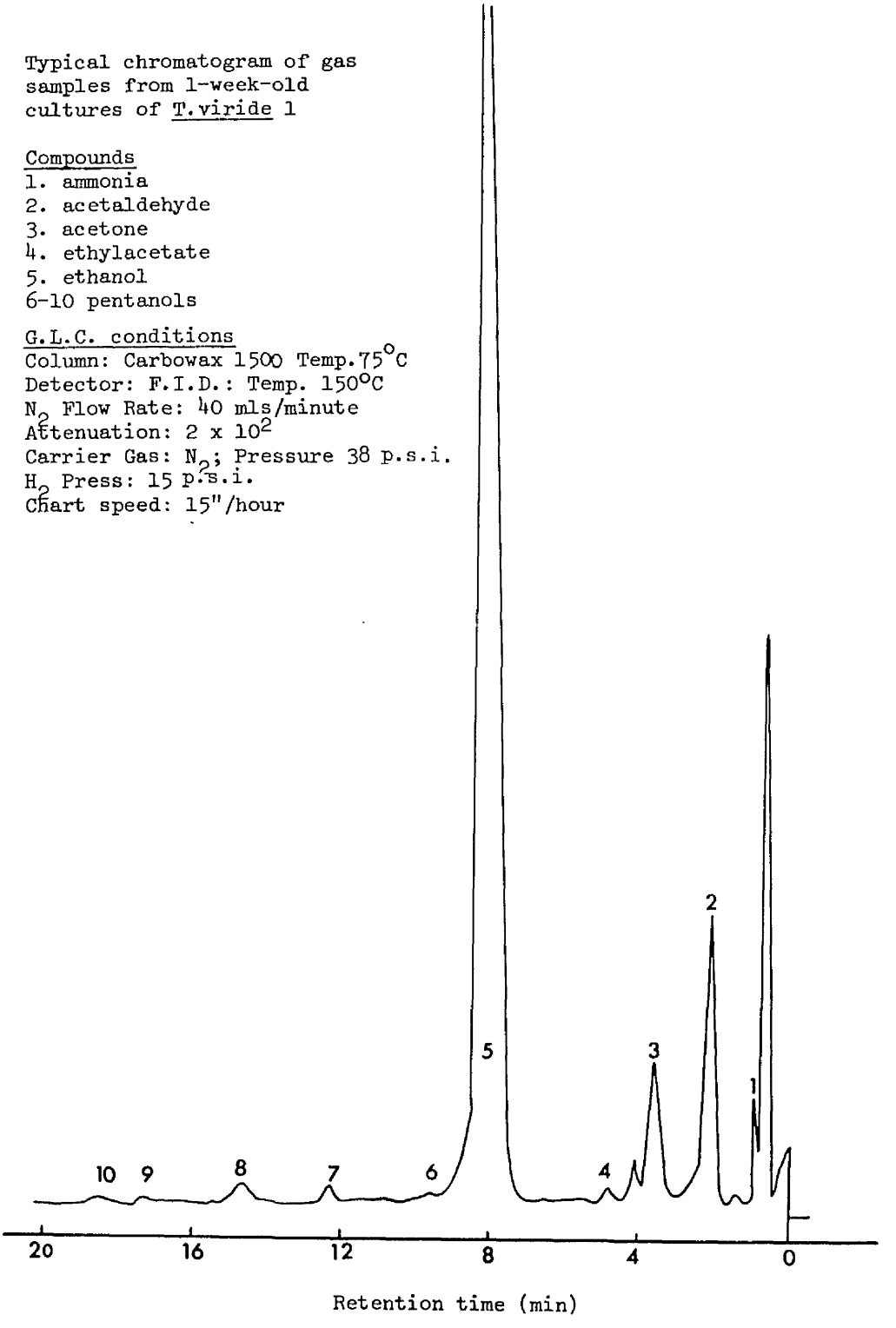


Figure 13 Typical chromatogram of gas samples from 1-week-old cultures of T.viride 1

Compounds

1. ammonia
2. acetaldehyde
3. acetone
4. ethylacetate
5. ethanol
6-10 pentanols

G.L.C. conditions
Column: D.N.P.; Temp. 78°C
Detector: F.I.D.: Temp. 150°C
N_2 Flow Rate: 40 mls/minute
Attenuation: 2 x 10^2
Carrier Gas: N_2 ; Pressure: 38 p.s.i.
H_2 Pressure: 15 p.s.i.
Chart speed: 15"/hour.

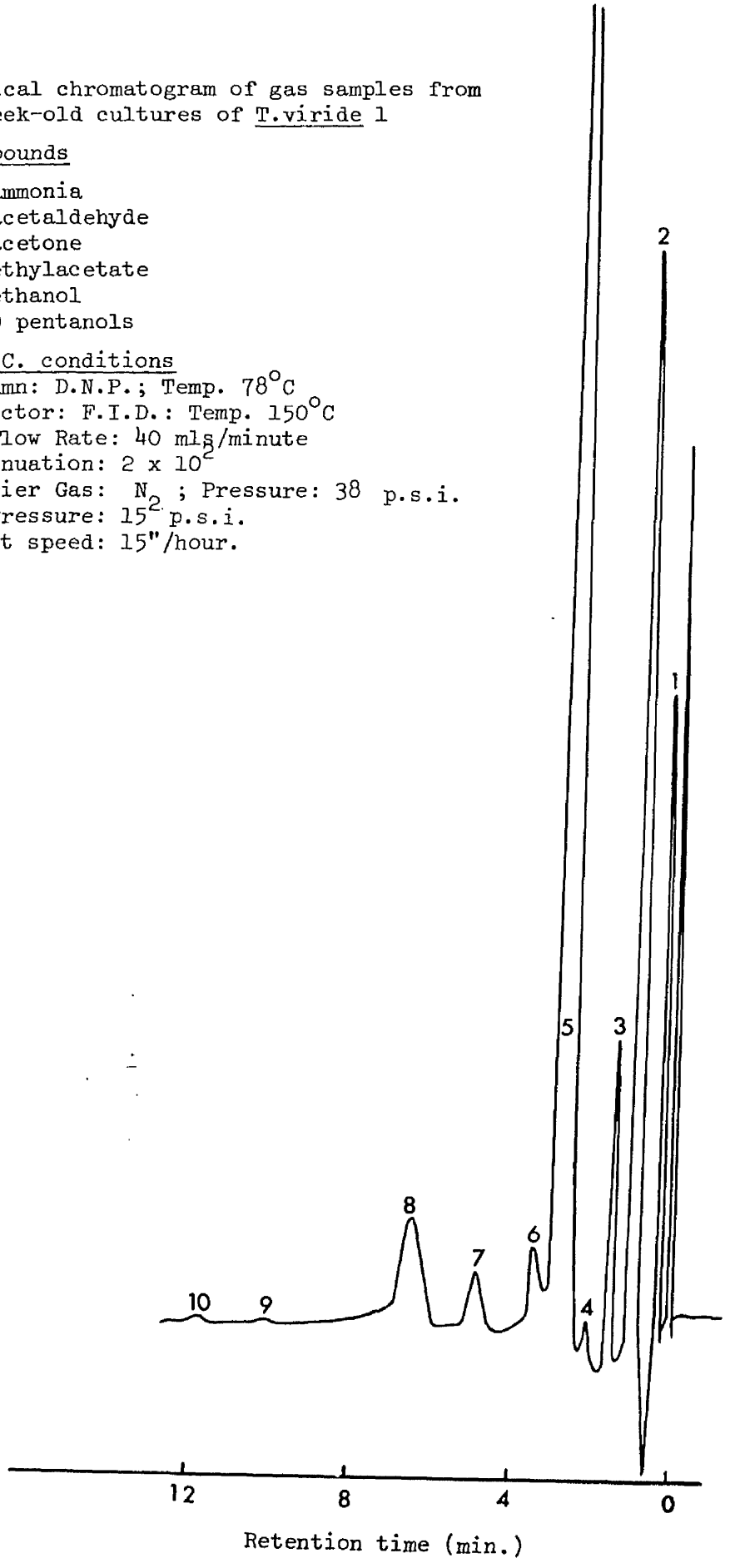


Figure 14 Typical chromatogram of gas samples from 1-week-old cultures of T.viride 1

Compounds: *ethylene
G.L.C. conditions:
Column: Porapak R: Temp. 65°C
Detector: F.I.D.: Temp. 150°C
N_2 Flow rate: 40 mls/minute
Attenuation: 50 x 1
Carrier gas: N_2; Pressure: 36 p.s.i.
H_2 Press: 16 p.s.i.
Chart speed: 15"/hour

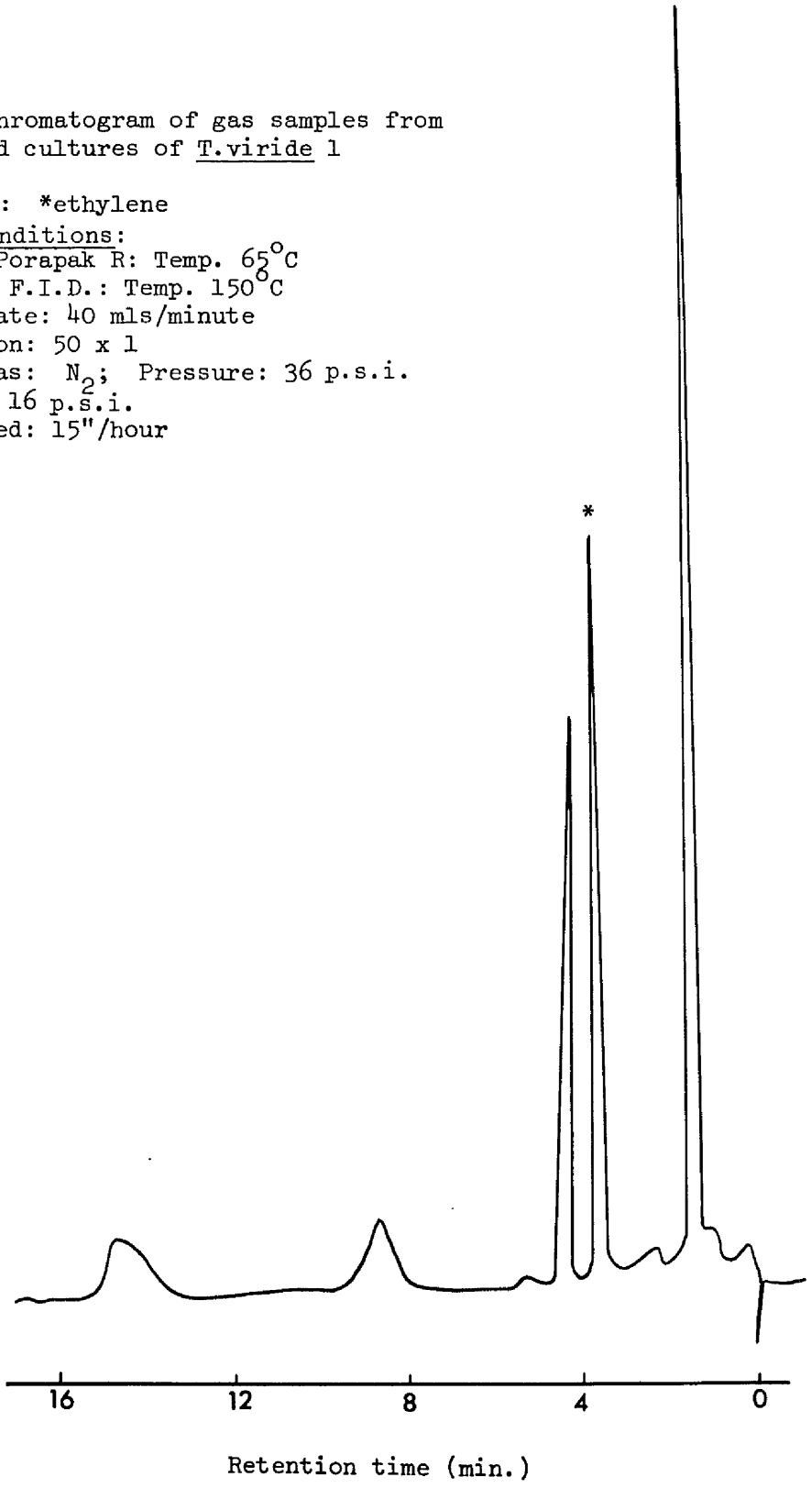


Figure 15 Typical chromatogram of gas samples from 1-week-old cultures of T.viride 1

Compounds: *methanol

G.L.C. conditions:
Column: Porapak Q: Temp. 75°C
Detector: F.I.D.: Temp. 150°C
N_2 Flow rate: 40 mls/minute
Attenuation: 1 x 10^2
Carrier gas: N_2 ; Pressure: 38 p.s.i.
H_2 Press: 15 p.s.i.
Chart speed: 15"/hour

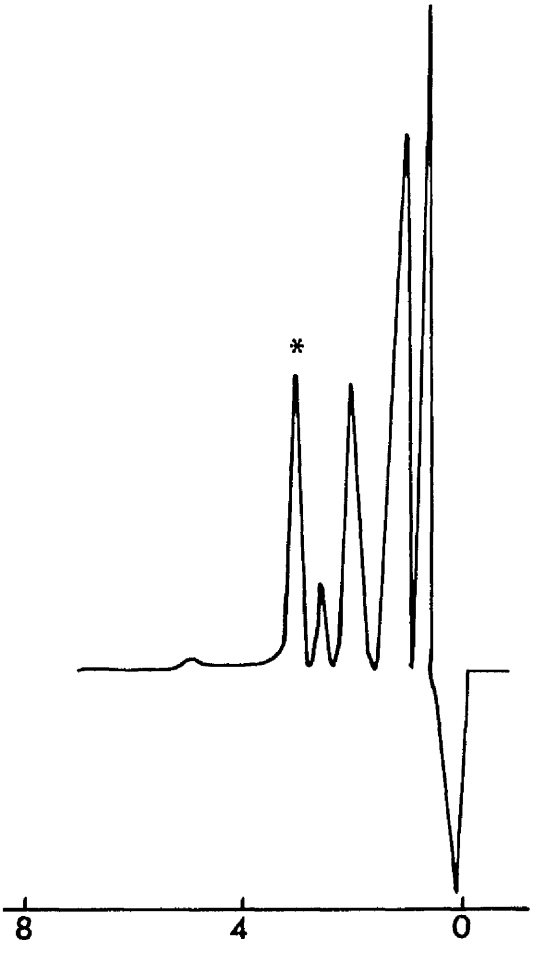
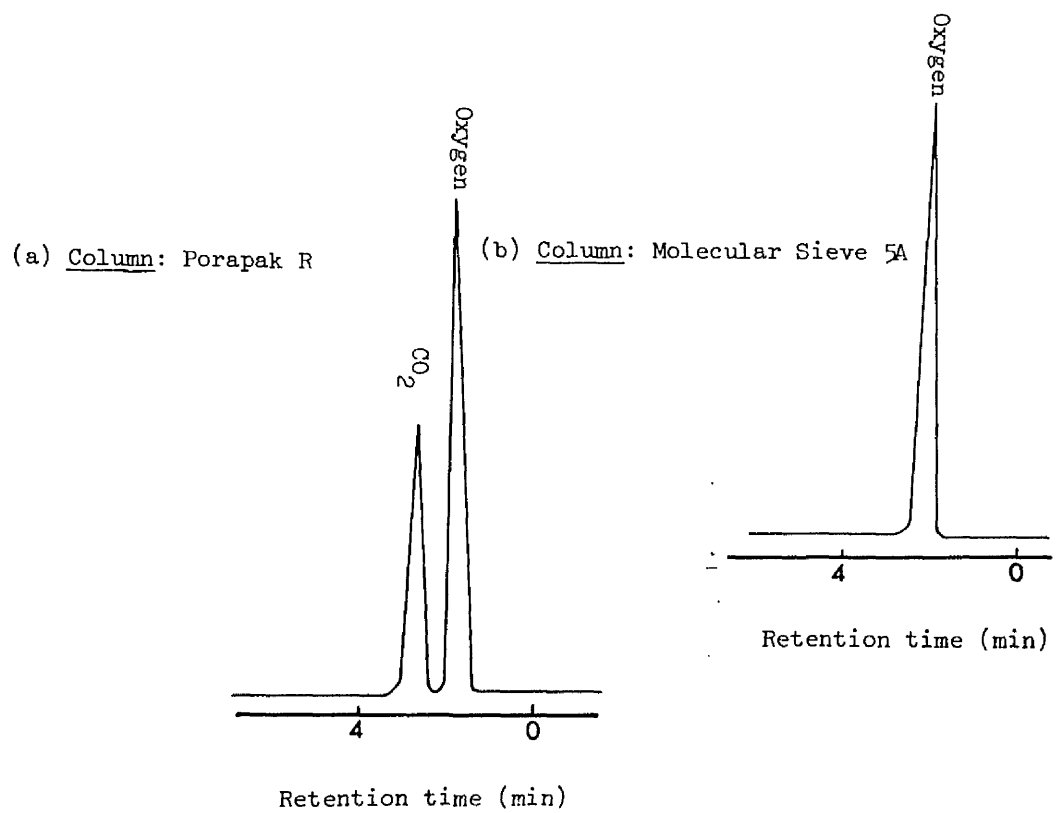


Figure 16 Typical chromatogram of gas samples from 1-week-old cultures of T.viride 1

G.L.C. conditions:

Column: Porapak R(a) and Molecular Sieve 5A(b)
Temp: 75°C
Detector: x-Section; Temp. 200° C.
Injector Temp.: 135°C
N_2 Flow. Rate: 55 mls/minute
Attenuation: 32
Carrier gas: Helium: Pressure: 38 p.s.i.
H_2 Press: 14 p.s.i.
Chart speed: 15"/hour



Text-table 3 Amounts of identified organic compounds found in pure cultures of two species of Trichoderma.

Constituents	Concn. required in air mixture to give peak heights similar to those of means of pure culture gases.							
	T.viride 1				T.longibranchiatum WBC 4576			
	Experiment Record in Appendix table No. 3			Highest record in any other investign. ml/l.air	Experiment Record in Appendix table No. 3			Highest record in any other investign. ml/l.air
	I	II	III		I	II	III	
Ethylene	trace*	0.001	trace	0.001	trace	trace	trace	< 0.001
Ammonia	-	-	-	0.005	-	-	-	0.001
Acetaldehyde	trace	trace	trace	0.001	trace	trace	trace	< 0.001
Acetone	trace	trace	trace	0.001	trace	trace	trace	< 0.001
Ethyl acetate	trace	trace	trace	trac	trace	trace	trace	trace
Ethanol	0.05	0.06	0.05	0.1	0.02	0.03	0.02	0.03
n-propanol	trace	trace	trace	trace	trace	trace	trace	trace
Iso-butanol	trace	trace	trace	trace	trace	trace	trace	trace
Methanol	trace	trace	trace	trace	trace	trace	trace	trace
Carbon dioxide	24%	27%	25%	30%	17%	16%	18%	22%
Oxygen**	7%	6%	6%	5%	9%	10%	9%	7%

* Trace implies less than 0.001 ml/litre

** lower concentration.

I.3(b) EXAMINATION OF THE EFFECTS OF KNOWN CONCENTRATIONS OF AUTHENTIC MATERIAL OF IDENTIFIED METABOLITES IN AIR ON THE GROWTH OF COLONIES OF RHIZOCTONIA SOLANI.

(i) Tests with samples of individual authentic constituents

Methods

It was decided to investigate the effects of the substances identified in more than trace concentrations in pure cultures individually. These were ammonia, acetaldehyde, acetone, ethanol and carbon dioxide; ethylene was also chosen because of its known reactivity in many biological interactions. It was appreciated that the other identified substances, and other unidentified substances might also affect the reactions but it seemed sensible to delay consideration of these possibilities till later. Single Roux bottle cultures of Rhizoctonia solani were assembled with Suba rubber caps, and air mixtures with known concentrations of authentic materials were set up in them by the procedures discussed above. A range of concentrations was chosen in each case to cover the maxima found in analyses of Trichoderma cultures.

Two experiments were set up. In the first, 3 replicate assemblies for each treatment were compared with 3 replicate assemblies set up in normal laboratory air. Colony diameters were measured and GLC analyses were made on the 1st, 7th and 14th day of incubation. In this experiment the cultures were set up initially under known concentrations of CO_2 in air, and subsequent changes in this due to replication were recorded.

In the second experiment, five replicates were set up in the same pattern; colony diameters were measured daily for 7 days, and GLC analyses were made on the 1st and 7th day. In this experiment examining the effects of CO_2 the cultures were irrigated daily with a fresh standard mixture of CO_2 and air, by the procedure described in appendix I.

Cultures were incubated, linear growth was measured and gas samples were taken and analysed as described in the General Methods. In the earlier experiments of this series, colonies were measured and gas samples were analysed on setting up, and on the 7th and 14th days of subsequent incubation. It was apparent, and expected, that metabolism of the

Rhizoctonia cultures would affect with the amounts and the activity of test substances in the later stages of incubation. This is confirmed by the earlier results. In the later tests (ethylene and ammonia) it was found that no useful information of these relatively inactive constituents was obtained by doing G.L.C. analysis on the first day only, and measuring colony diameters daily for the first seven days after assembly.

Results

The results of this series of experiments (No.1), are recorded in appendix-tables (4A to 4F), and summarised in text figures (17 to 22). The text figures show the reduction of the means of the record of diameters of R.solani colonies for each treatment as a percentage of the means for these set up with normal laboratory air. They also show the means of the concentrations of the test material, recorded during the experiments. The results of later experiments (Series No.2), are recorded in appendix-tables (5A to 5 I).

(ii) Tests with mixtures of authentic samples of identified constituents

Methods

Single Roux bottle cultures of R.solani were injected with authentic metabolites to produce mixtures containing the maximum concentrations of each found in the head spaces of the two Trichoderma species. Three replicate cultures were set up for each treatment together with 3 replicate control cultures set up with normal laboratory air. Colony diameters were measured and gas samples were taken and analysed by G.L.C. on the first, seventh and fourteenth day after assembly.

Results

The results are given in appendix table (6) and summarised in text-figure 23.

General Conclusion

No unusual secondary metabolites were found, and in these conditions both Trichoderma species produced inhibitory concentrations of several primary metabolites. Text-table (4), summarises this information:-

Text-table 4 Summary of effects of authentic metabolites on linear growth of R.solani at maximum concentrations produced by T.viride 1 and T.longibranchiatum WBC 4576 respectively on 1st and 7th day of incubation.

Organic compounds	Ethylene	Ammonia	Acetalde-hyde	Acetone	Ethanol	CO_2
ax.concn. produced by .viride 1	0.001 ml/l	<0.01 ml/l	0.001 ml/l	0.001 ml/l	0.1 ml/l	30% vol/vol
ax.concn. produced by .longibranchiatum BC 4576	0.001 ml/l	<0.01 ml/l	0.001 ml/l	0.001 ml/l	0.03 ml/l	22% vol/vol
mount of inhibition roduced by equivalent oncn. of authentic aterial 1st day after ssembly	0.001 ml/l +7%	0.01 ml/l -4%	0.001 ml/l +11%	0.001 ml/l +11%	0.1 ml/l -17% 0.03 ml/l Nil	30% vol/vol -17% 20% vol/vol -17%
mount of inhibition roduced by equivalent oncn. of authentic aterial 7th day after ssembly	+6%	+2%	+1%	-1%	-11% -2%	-41% -25%

In particular ethanol and carbon dioxide rapidly attain inhibitory levels and small changes in the concentrations of these substances may produce big changes in their effects on R.solani. It seems likely that these accumulations in older cultures may mask the effects of other substances. The production of all these primary metabolites is likely to be affected by small changes in the environment; the tests of the mixtures of ethylene, ammonia, acetaldehyde, acetone, ethanol and carbon dioxide in air show that interactions of these may lead to a higher cumulative effect than those of equivalent concentrations applied separately. There is no evidence that the other metabolites have had any appreciable effect; this does not discount the possibility of them, or other unidentified metabolites, contributing to effects in other, perhaps only slightly different, conditions.

There are not sufficiently big differences between the gas mixtures produced by the two Trichoderma species to account fully for the differences in their effects in paired petri dishes.

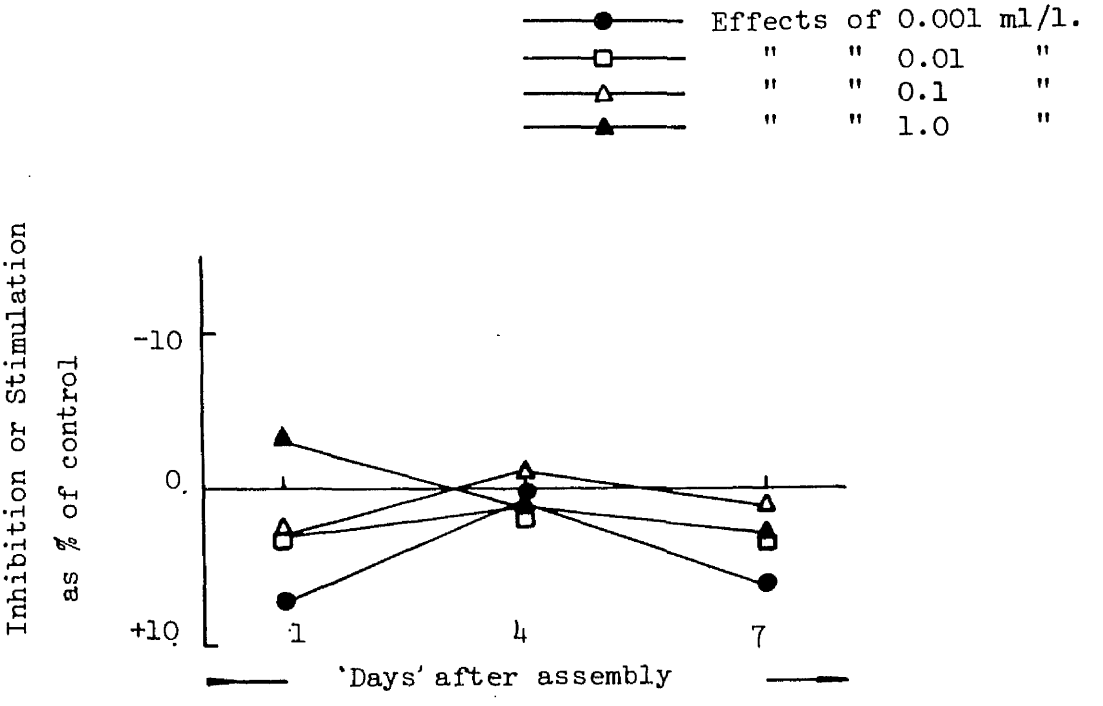


Figure 17 Effects of authentic ethylene with initial concentrations of 0.001, 0.01, 0.1 and 1.0 ml/litre air on linear growth of R. solani on 1st, 4th and 7th day after assembly.

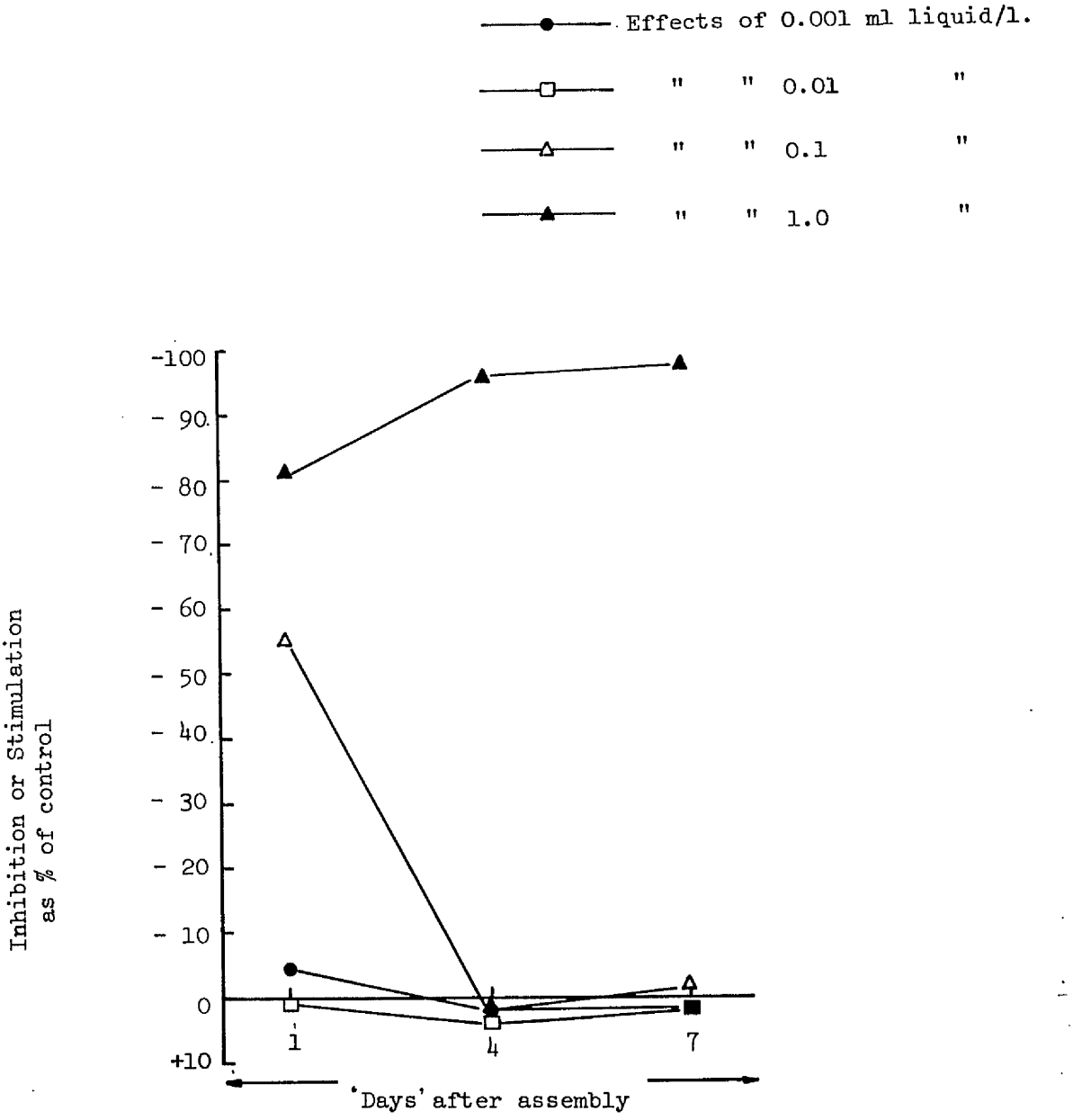


Figure 18 Effects of authentic ammonia with initial concentrations of 0.001, 0.01, 0.1 and 1.0 ml/litre air on linear growth of R.solani on 1st, 4th and 7th day after assembly.

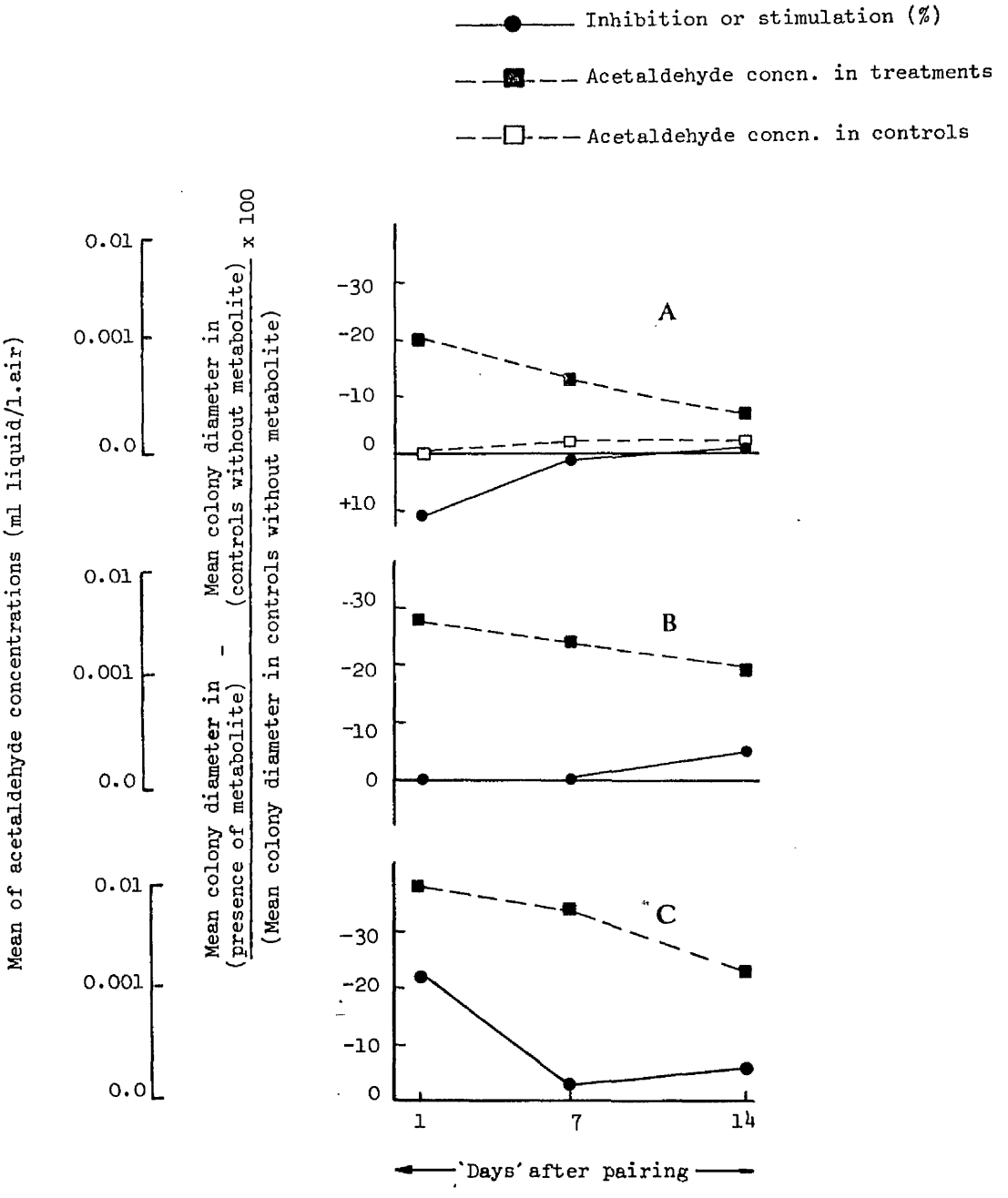


Figure 19 Effects of authentic acetaldehyde with initial concentrations of 0.001 ml/litre air 'A'; 0.005 ml/litre air 'B'; 0.01 ml/litre air 'C' on linear growth of R.solani on 1st, 7th and 14th day after assembly.

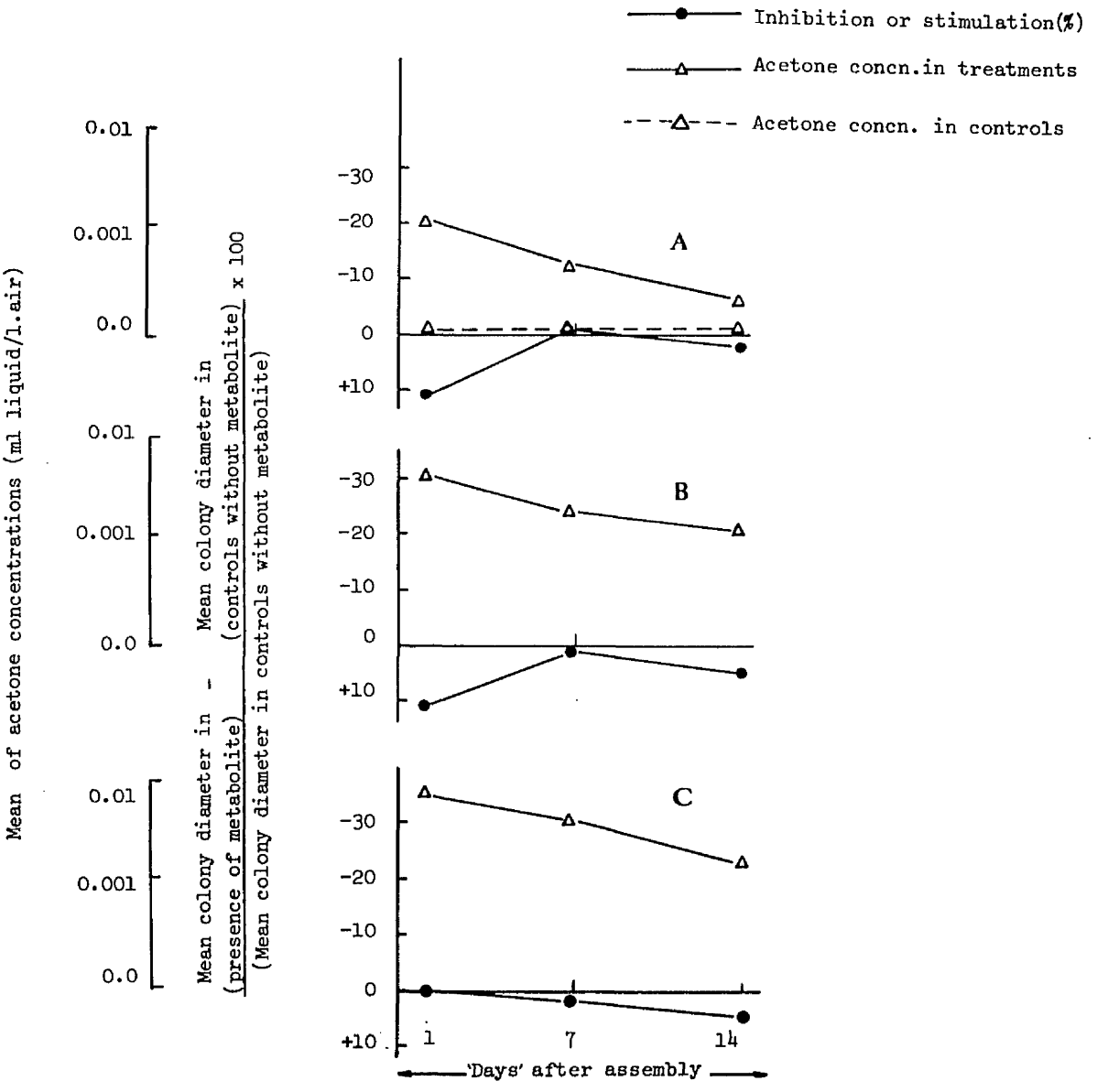


Figure 20 Effects of authentic acetone with initial concentrations of 0.001 ml/litre air 'A'; 0.005 ml/litre air 'B'; 0.01 ml/litre air 'C' on linear growth of R.solani on 1st, 7th and 14th day after assembly.

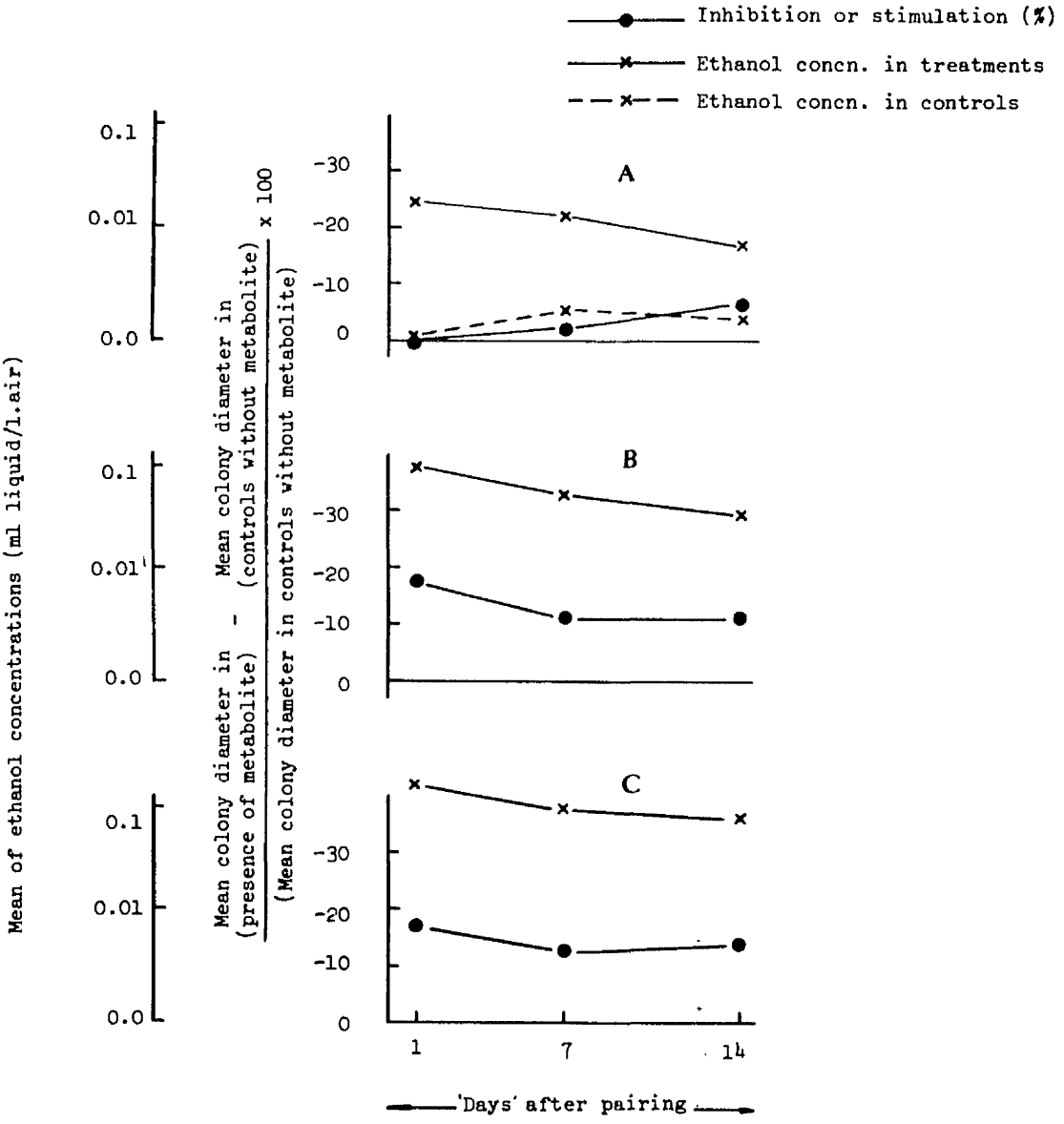


Figure 21 Effects of authentic ethanol with initial concentrations of 0.03 ml/litre air 'A'; 0.1 ml/litre air 'B'; 0.3 ml/litre air 'C' on linear growth of R.solani on 1st, 7th and 14th day after assembly.

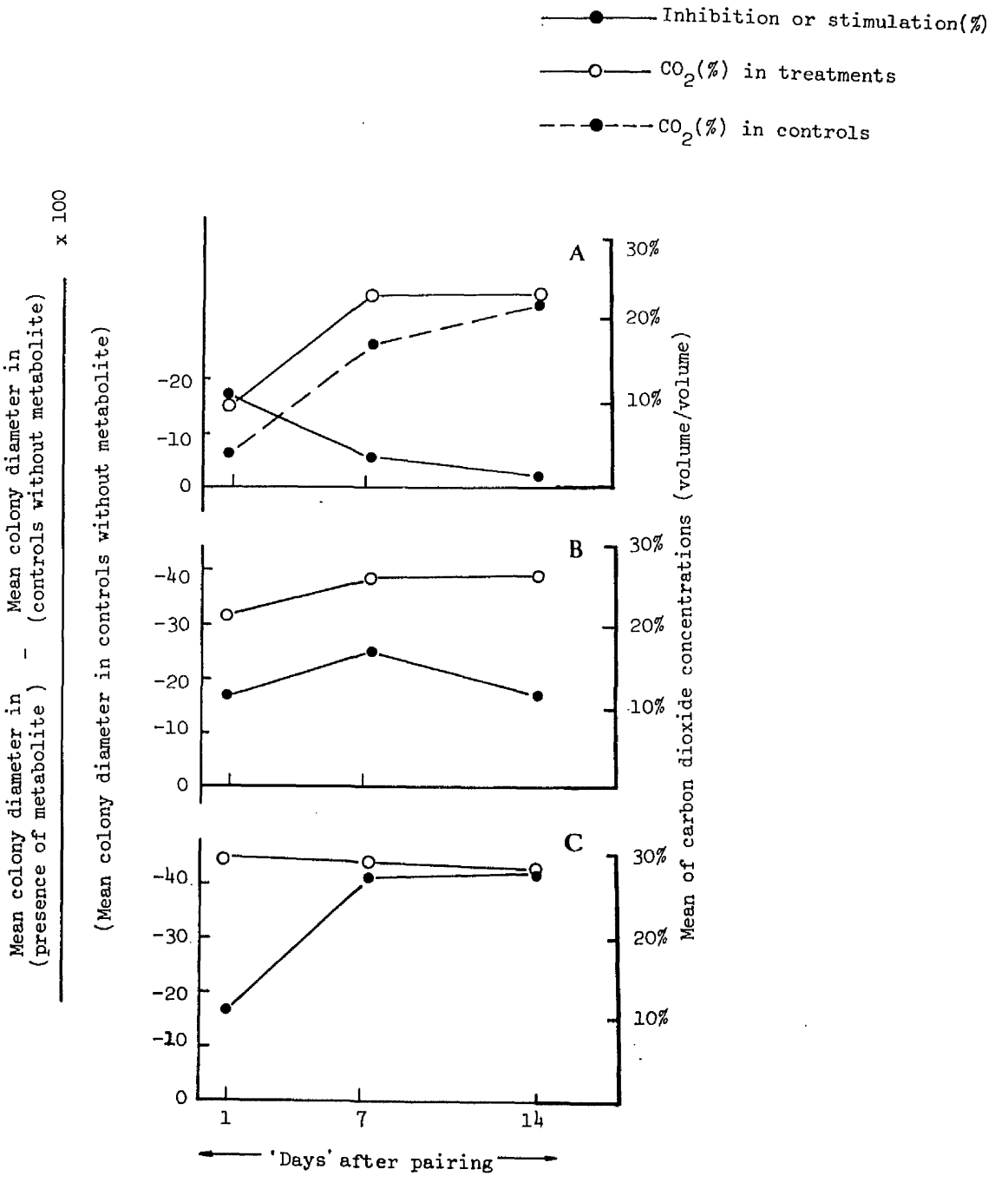


Figure 22 Effects of authentic carbon dioxide with initial concentrations of 10% v/v 'A'; 20% v/v 'B'; 30% v/v 'C' on linear growth of R.solani on 1st, 7th and 14th day after assembly

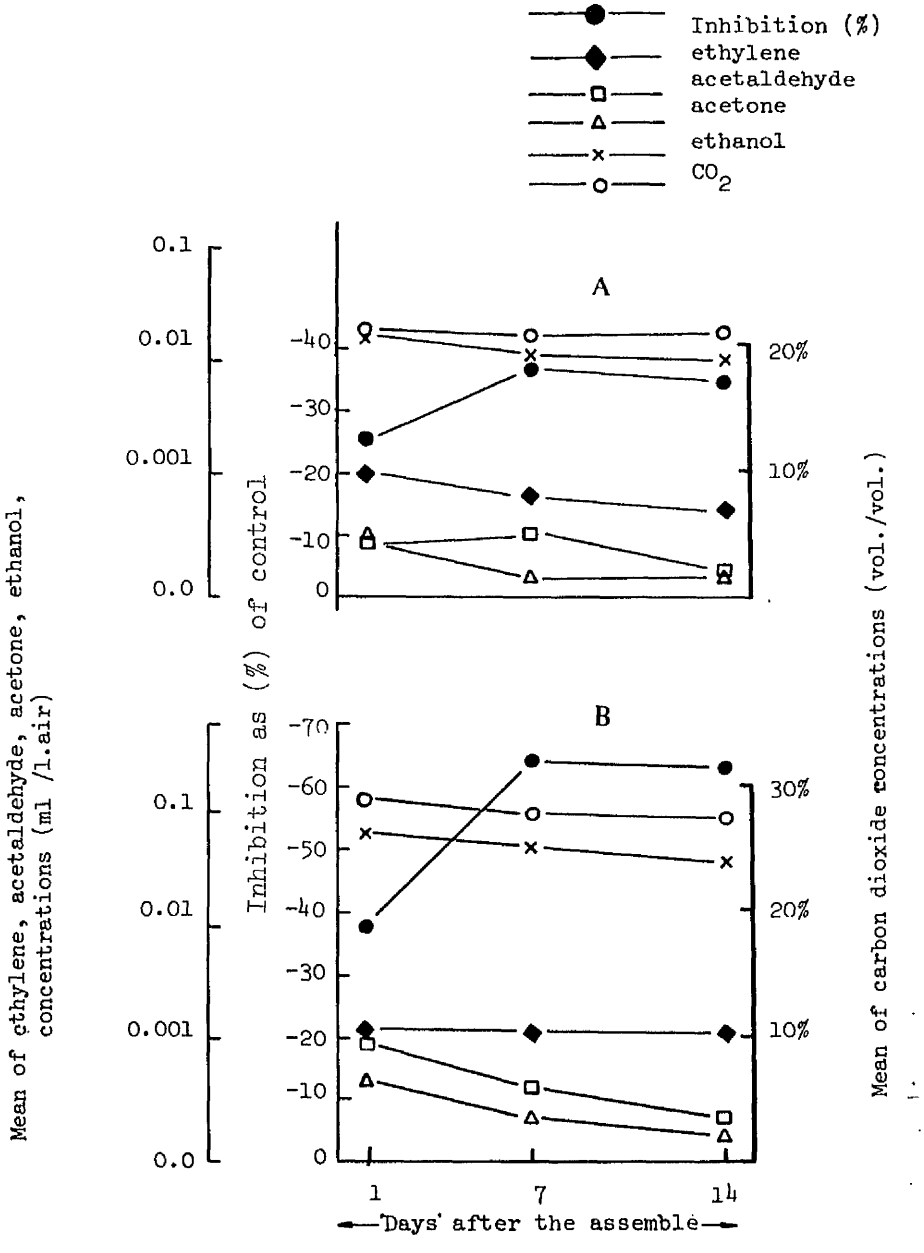


Figure 23 Comparison study of effects of mixtures of authentic metabolites with initial concentrations of 0.001 ml/litre air, ethylene, ammonia, acetaldehyde, acetone + 0.03 ml/litre air, ethanol + 20% v/v CO_2 'A'; and with 0.001 ml/litre air, ethylene + 0.005 ml/litre air, ammonia + 0.001 ml/litre air, acetaldehyde, acetone + 0.1 ml/litre air, ethanol + 30% v/v CO_2 'B' on linear growth of R.solani on 1st, 7th and 14th day after assembly.

I.3(c) ANALYSIS OF THE INTERACTIONS BETWEEN GASES FROM THE TWO REPRESENTATIVE TRICHODERMA SPECIES AND RHIZOCTONIA SOLANI IN PAIRED CULTURES

INTRODUCTION

This investigation is the appropriate complement to the pure culture studies in sections 3a and 3b. In these sections it has been noted that the amounts of carbon dioxide produced by Trichoderma cultures are big enough to account by themselves for the total amounts of inhibition seen. The differences between the amounts of this metabolite produced, or between the amounts of any other seen during that phase of the work, do not however give information to explain the difference between the activity of the two Trichoderma species. There is also evidence that the cumulative effects of metabolite mixtures may be bigger than that of the same concentrations of metabolites acting separately.

Since no other active metabolite was identified it seemed possible that the differences were due to differences in the rate at which inhibitory levels were attained in the various assemblies. The obvious way to test this would be to sample the assemblies at short intervals throughout the early stages of incubation. It was appreciated, however, that this frequent sampling would introduce unacceptably big changes in head space gases in the assemblies used. This could be overcome by using enough replicates to permit each to be discarded after sampling. It did not, however, seem sensible at this stage to design an experiment of the complexity and size needed to permit this. As an alternative experiments were set up to compare the effects of pairing Trichoderma cultures which had been permitted to build up a high concentration of their metabolites in the head space gases with those of pairing cultures which had less opportunity for such build up.

METHODS

Cultures of R.solani were paired with cultures of T.viride 1 and T.longibranchiatum WBC 4576, by the standard methods for paired assembly tests. In the first series of investigation (Series A), one experiment was done using cotton wool plugged Trichoderma cultures which were 6-day-old at the time of pairing, one with 7-day-old cultures and one with 8-day-old cultures. These ages were chosen because Dennis & Webster (1971) found that six-day-old cultures produced the biggest effects in their experiments with paired Petri dishes. Six replicate assemblies were set up for each treatment, together with three assemblies in which cultures of R.solani were paired with bottles containing the standard amount of uninoculated malt extract agar. In this series of experiments colony diameters were measured, and gas samples were taken and analysed, within 10 to 20 hours after assembly and on the 7th and 14th day of subsequent incubation.

In a second series of experiments (Series B) five replicate assemblies using Trichoderma cultures which had been put with Suba rubber caps and incubated for 7 days before pairing, were compared with five replicate control assemblies of R.solani paired with uninoculated malt agar. In this series colony diameters of R.solani were measured daily, and gas samples were taken and analysed within 24-36 hours, on the 7th and 14th day of incubation after assembly. Incubation, measurement, and G.L.C. analysis were by the standard procedures.

RESULTS

The results are recorded in Appendix-tables 7A-7F and 8A & 8B, inclusive, and summarised in text-figures 24 and 25 inclusive. The text figures record the effects as reduction of means of growth of the treated R.solani cultures as a percentage of the means of growth of the control colonies. They also show the concentrations of authentic ethanol and of CO_2 in air mixtures required to give peak heights closely similar to those produced in G.L.C. chromatograms of samples of head space gases of the experimental assemblies.

not
The amounts of ethylene were/measured in this series of experiments, for administrative reasons. No unusual secondary metabolites were seen; no other metabolites appeared on the G.L.C. traces in more than trace concentrations, of the order of tenth or less than the minimum concentration in which any of these had been shown to be active in tests with authentic material in pure culture.

The contribution of the Rhizoctonia colonies to the complete mixtures can be judged from the G.L.C. measurements of samples from the control pure cultures.

CONCLUSION

The experiments of Series "A" with the 6-day, 7-day and 8-day-old cultures of Trichoderma species were carried out independently from each other. The results seem so closely similar, that they can fairly support deductions without further formal replication. They are also closely similar in all important ways to those from the experiments with paired Petri dishes. In particular the gases from cultures of T.viride 1 have produced more inhibition than that produced by cultures of T.longibranchiatum WBC 4576;from 20% volume/volume to 30% volume/volume carbon dioxide in air was found in all assemblies on the 7th day after assembly. In pure culture tests with authentic materials, concentrations of this order produced 25% to 45% inhibition. This is of the same order as the inhibition seen in these experiments, but it is generally slightly less than that produced by gases from T.viride 1, slightly more than that produced by gases of T.longibranchiatum WBC 4576. Similarly the small differences between the concentrations found in the culture with the two Trichoderma species tend to correlate with the difference between total effects; there are more consistant differences between the amounts of ethanol produced in assemblies with 6-day and 7-day-old cultures of both Trichoderma species, only T.viride 1 producing inhibitory levels. The highese level recorded is however only equivalent to that of the 0.1 ml/litre mixture of authentic pure material which gave up to 11%

inhibition of R.solani. These differences between strains were not found using 8-day-old cultures of Trichoderma sp. It is noted, however, that in Section (3b) above an authentic mixture of 30% CO_2, 0.1 ml ethanol, 0.001 ml acetaldehyde, acetone and ethylene and 0.005 ml ammonia produced up to 60% inhibition in tests in 7 days (Text-figure 23). It is therefore concluded that the total amount of inhibition seen in these experiments could be accounted for the amounts of CO_2 and of ethanol produced.

The evidence from series "A" suggests that a difference in the rate of production of these primary metabolites by the two Trichoderma species may contribute to difference between their effects, but it is not adequate to substantiate this hypothesis fully. The results from series "B" confirm, however, that the differences between the amounts of CO_2 produced by the two Trichoderma species may result in big differences in the rates at which inhibitory levels of CO_2 build up in test assemblies. In assemblies with the sealed cultures of T.viride 1 it attains a level of 20% to 30% volume/volume of gas space within the first two days, while it is still below 20% volume/volume of gas space with T.longibranchiatum WBC 4576 at this stage. This factor, together with smaller differences in final concentrations attained in the first 7 days, could by itself account for all the differences between the recorded effects of the two species on R.solani. The differences between the ranges of ethanol concentration follow the same general pattern, although they are smaller. It seems likely that variety in the rates of formation of this compound and other primary metabolites might still contribute to the effects in some conditions.

The other work recorded in this thesis all fits this general hypothesis. In the circumstances it did not appear justifiable to spend the money or the time which would be involved in the investigation of the almost infinite variety of small environmental changes which might influence the productions of these ubiquitous substances, and so the effect which any of them might have in a particular interaction. Similarly it did not seem appropriate to carry out detailed mathematical analyses of the statistical significance of the differences and of the correlations.

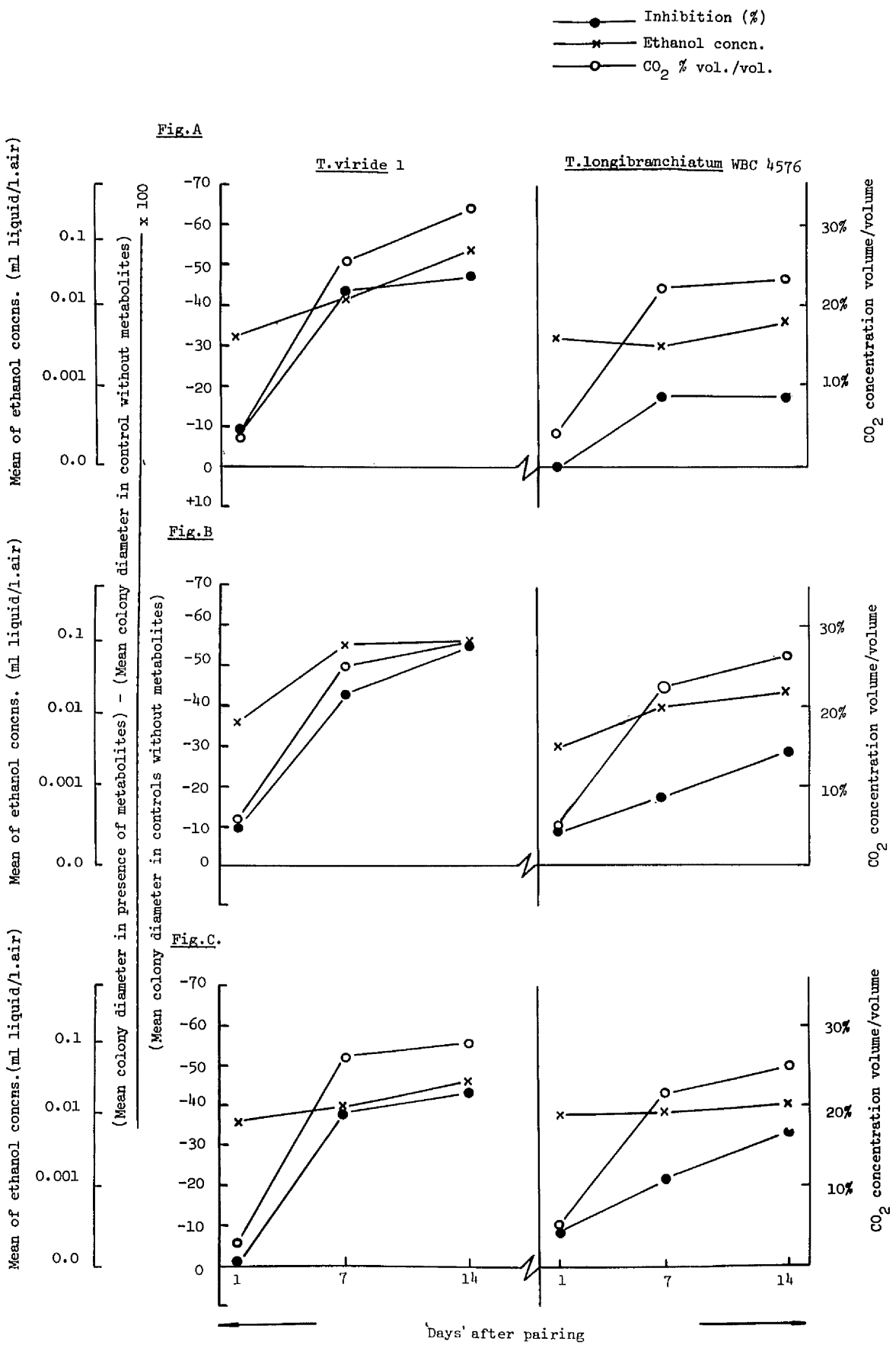


Figure 24 Comparison of inhibition of linear growth of Rhizoctonia cultures paired with Trichoderma cultures of 6-day-old (fig.A); of 7-day old (fig.B); and of 8-day-old (fig.C); and amounts of ethanol and CO_2 present in the paired assemblies (Series A).

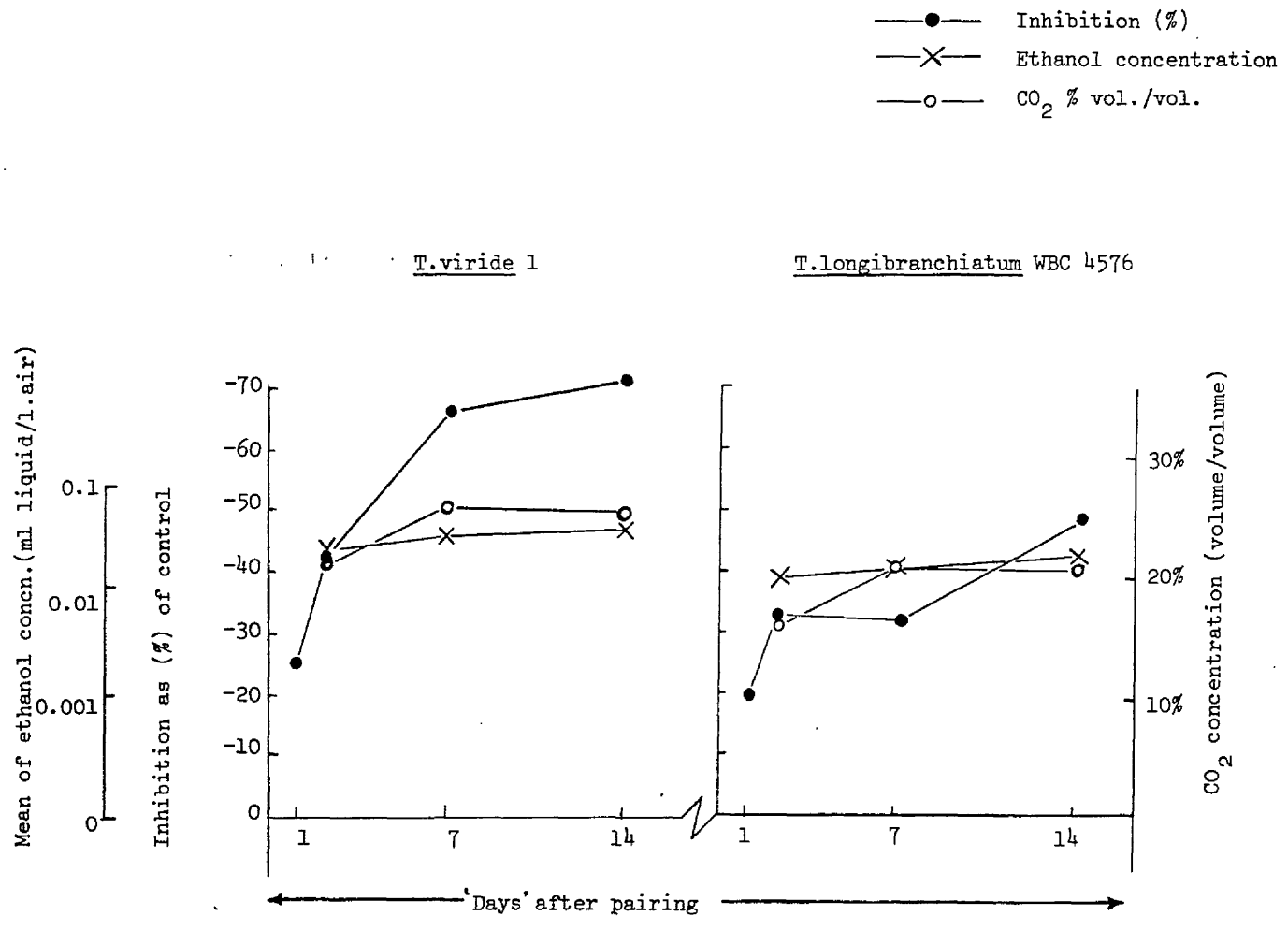


Figure 25 Comparison of inhibition of linear growth of Rhizoctonia cultures paired with Trichoderma cultures of 7-day-old; and amounts of ethanol and CO_2 present in the paired assemblies. (Series B).

I.3(d) EXAMINATION OF THE EFFECTS OF KNOWN CONCENTRATIONS OF AUTHENTIC MATERIAL OF IDENTIFIED METABOLITES IN AIR ON THE GROWTH OF COLONIES OF THE OTHER ASSAY FUNGI USED BY DENNIS & WEBSTER.

INTRODUCTION

This was an appropriate first stage of the broadening of the investigation on similar lines to that in 3(b) above.

METHODS

Measurements were made using sealed Roux bottle cultures. All the details of assembly, measurement, sampling and G.L.C. analysis followed the standard procedure as given above (Section 3.b.). Each treatment was examined in five replicate assemblies and compared with five replicate control assemblies set up in normal laboratory air. Measurements of colony diameters were made daily, and G.L.C. samples were taken and analysed within the first 24 hours after assembly and on the seventh day.

RESULTS

The results are recorded in appendix tables (5A- 5I and 9A - 9Y). Summarised in text-table (5) and in text figures (26 to 31).

DISCUSSION

There is an overall general gradation of sensitivity to these gases. Pyronema domesticum being most sensitive to the tested range and Fusarium oxysporum being least sensitive. This follows the general pattern of sensitivity to Trichoderma gases reported below. The effect of acetone and ethylene are negligible, even if the figures should be statistically significant. Hence mathematical analysis did not appear to be justified. Ethanol and carbon dioxide are generally more inhibitory, and there are big differences in the effects of acetaldehyde. The implications of these results in relation to the effects of Trichoderma gases are discussed below.

The results permit comparison of the sensitivity of the assay cultures to particular metabolite with the amounts of these metabolites found in Trichoderma culture gases. This has not been developed further in this thesis, but possibilities of ecological effects in the field are obvious.

for 7 days before pairing with assay fungi. In comparison to the effects of known concentrations of authentic metabolites.

Treatment	Test material	Range of concn. introduced	Inhibition or Stimulation as % of control measured at 2 and 7 days respectively after pairing in each experiment.											
			Pyronema domesticum[a] (day)		Rhizoctonia solani		Pythium ultimum		Fomes annosus		Mucor hiemalis		Fusarium oxysporum	
			2	7	2	7	2	7	2	7	2	7	2	7
I	T.viride 1		-95	-61	-40	-67	-33	-49	-29	-29	-17	-34	-9	-13
II	T.longibranchiatum		-45	- 5	-33	-32	-27	-11	-14	- 3	-12	- 7	-9	-10
III	Ethylene (ml /l.air)	0.001‡*	+ 2	Nil	+ 4	+ 6	+ 4	+ 1	Nil	Nil	+5	+ 1	+ 4	+ 2
		0.01	+10	Nil	+ 2	+ 3	+ 2	+ 2	Nil	+ 2	+ 2	- 1	Nil	Nil
		0.1	+ 5	Nil	+ 6	+ 1	- 2	- 1	+10	+ 8	+ 7	+ 1	Nil	Nil
		1.0	+ 8	Nil	+ 2	+ 3	Nil	+ 1	-10	+ 3	+5	+ 1	- 4	+ 1
IV	Acetaldehyde (ml liq./l.air)	0.001‡*	-39	Nil	+ 3	Nil	+19	-16	Nil	- 3	+17	+17	+ 4	Nil
		0.005	-77	Nil	+ 2	- 1	+11	-16	-17	Nil	+24	+16	- 4	Nil
		0.01	-85	Nil	-12	- 5	-25	- 7	-17	-14	-10	- 3	- 8	- 5
		0.05	-96	-98	-79	-95	-48	-19	-17	-55	-31	-32	-75	-40
V	Acetone (ml liq./l.air)	0.001‡*	+ 5	Nil	+ 7	-1	+ 8	- 1	+13	Nil	+ 7	Nil	+10	- 1
		0.005	+3	Nil	+11	+3	+ 3	Nil	Nil	Nil	+ 2	+ 2	+ 3	- 1
VI	Ethanol (ml liq./l.air)	0.03*	-16	Nil	-3	- 2	- 5	- 1	Nil	Nil	+ 7	Nil	+10	Nil
		0.1+	-24	Nil	- 6	- 2	- 5	- 4	Nil	- 3	+ 5	Nil	+ 3	- 1
		0.3	-35	Nil	- 9	- 2	- 7	- 5	-38	- 9	- 9	- 4	- 7	- 6
		1.0	-49	Nil	-13	-14	-19	-12	-38	- 6	-14	-10	-23	-14
VII	CO_2(volume/volume)	10%	-28	Nil	-30	-2	-16	Nil	-18	+ 3	-16	-14	-13	- 6
		20%*	-50	Nil	-44	-51	-30	-25	-27	-21	-36	-30	-17	-19
		30%+	-84	-60	-81	-82	-48	-43	-55	-24	-51	-49	-30	-28

(a) agar surface completely covered with the mycelium after 3-4 days.
+ = maximum concentration found in head-space gases of pure cultures of T.viride 1
* = maximum concentration found in head-space gases of pure cultures of T.longibranchiatum WBC4576

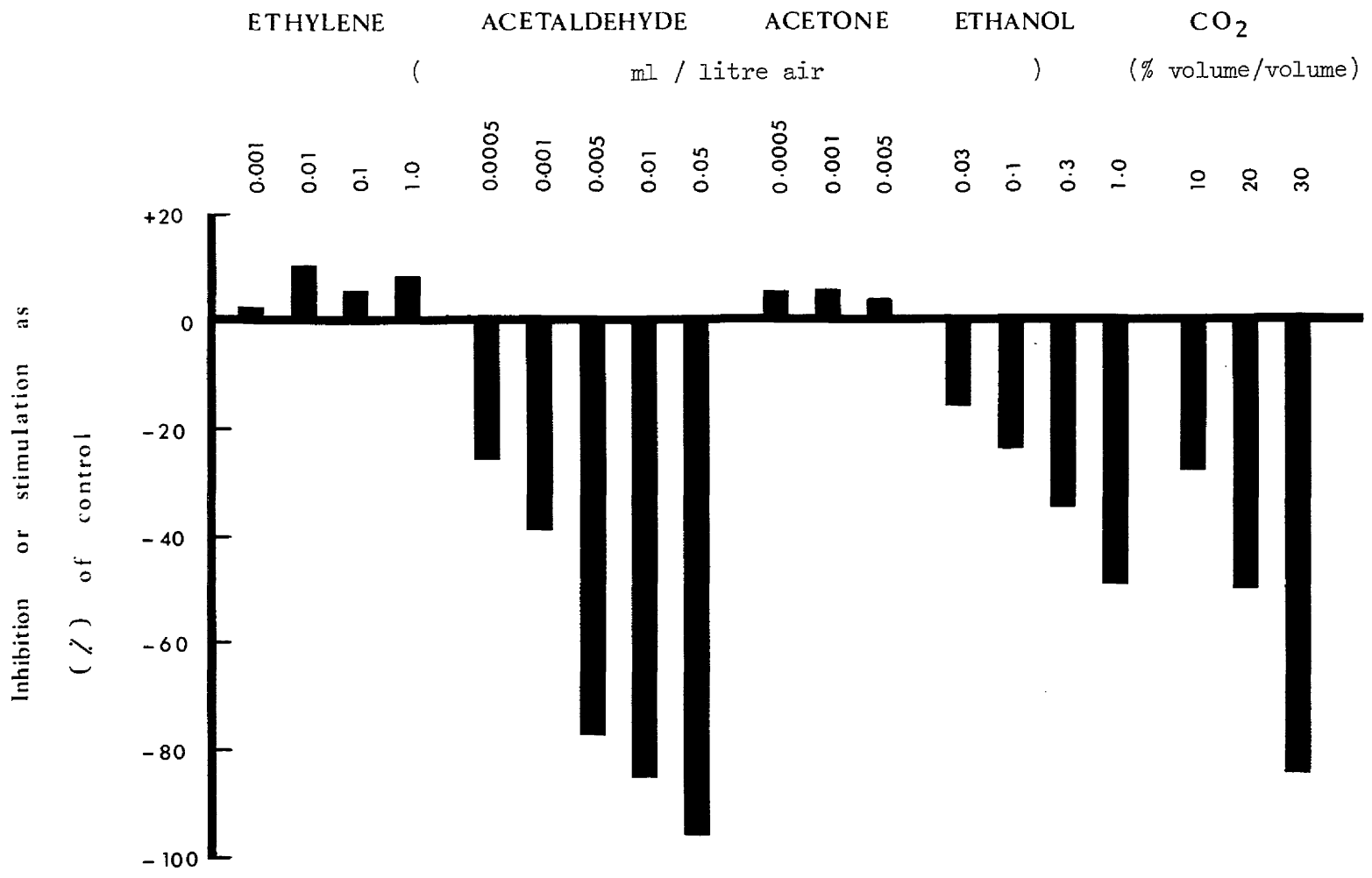


Figure 26 Effects of authentic metabolites on linear growth of Pyronema domesticum, on second day after assembly

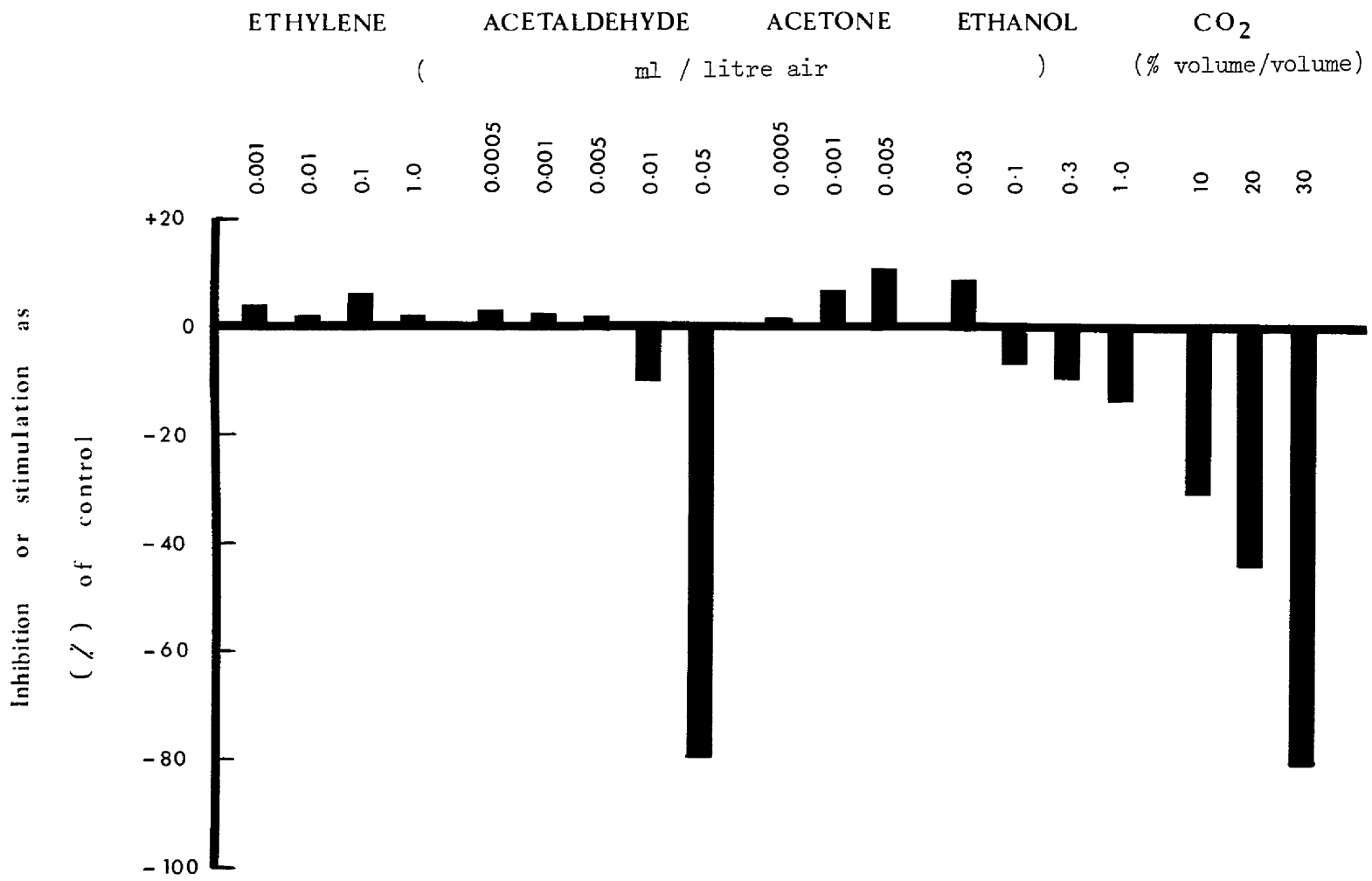


Figure 27 Effects of authentic metabolites on linear growth of Rhizoctonia solani, on second day after assembly

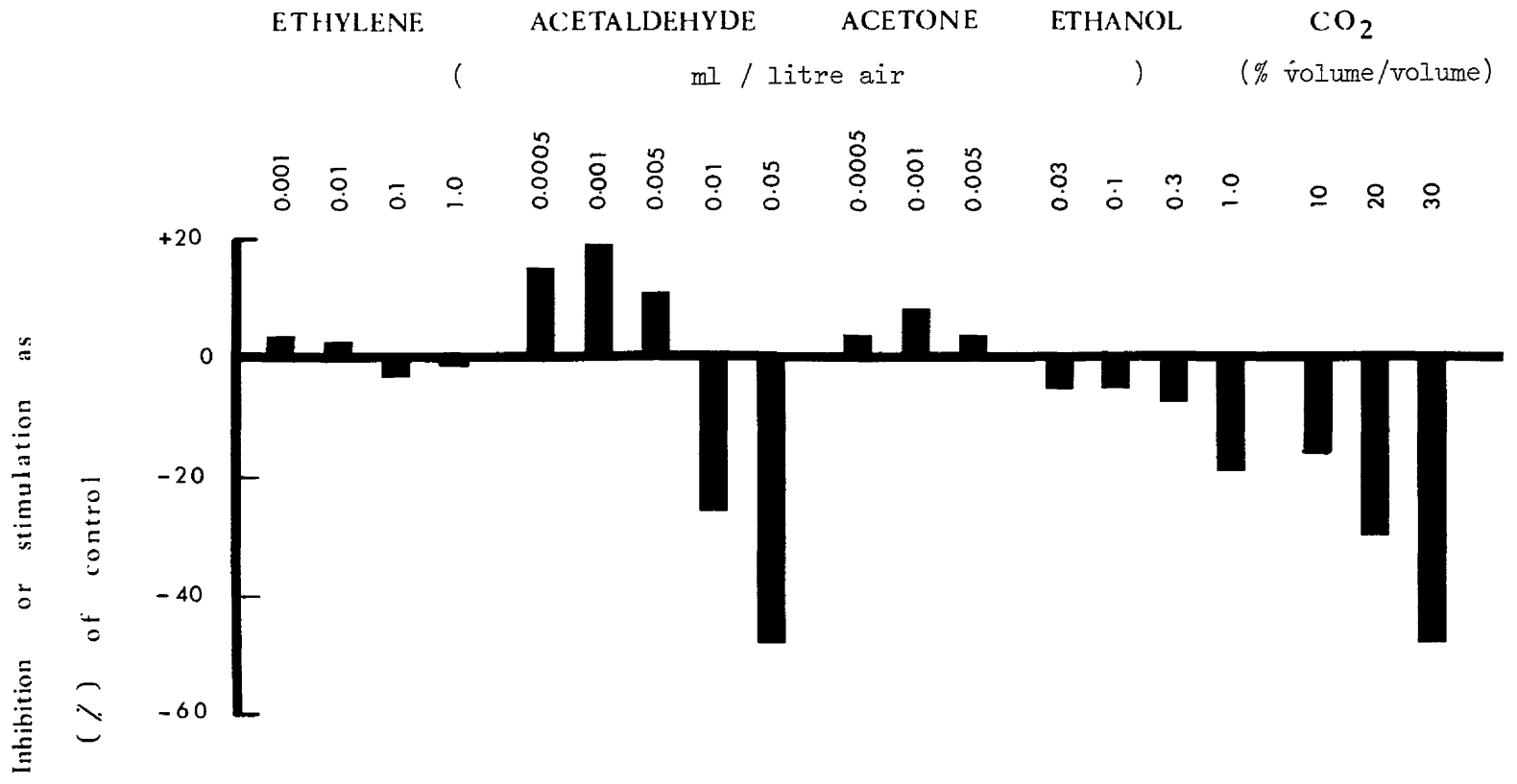


Figure 28 Effects of authentic metabolites on linear growth of Pythium ultimum, on second day after assembly

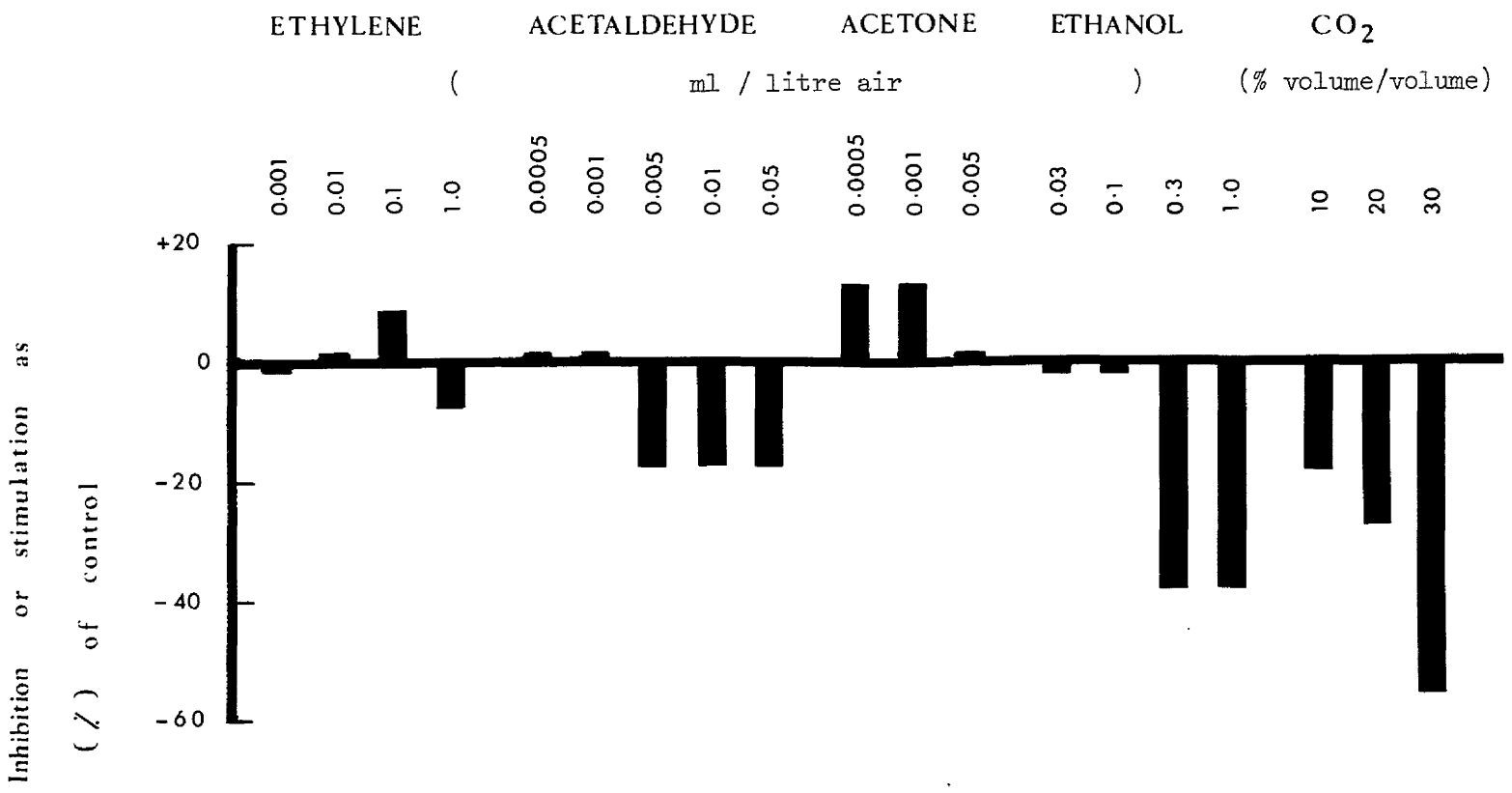


Figure 29 Effects of authentic metabolites on linear growth of Fomes annosus, on second day after assembly

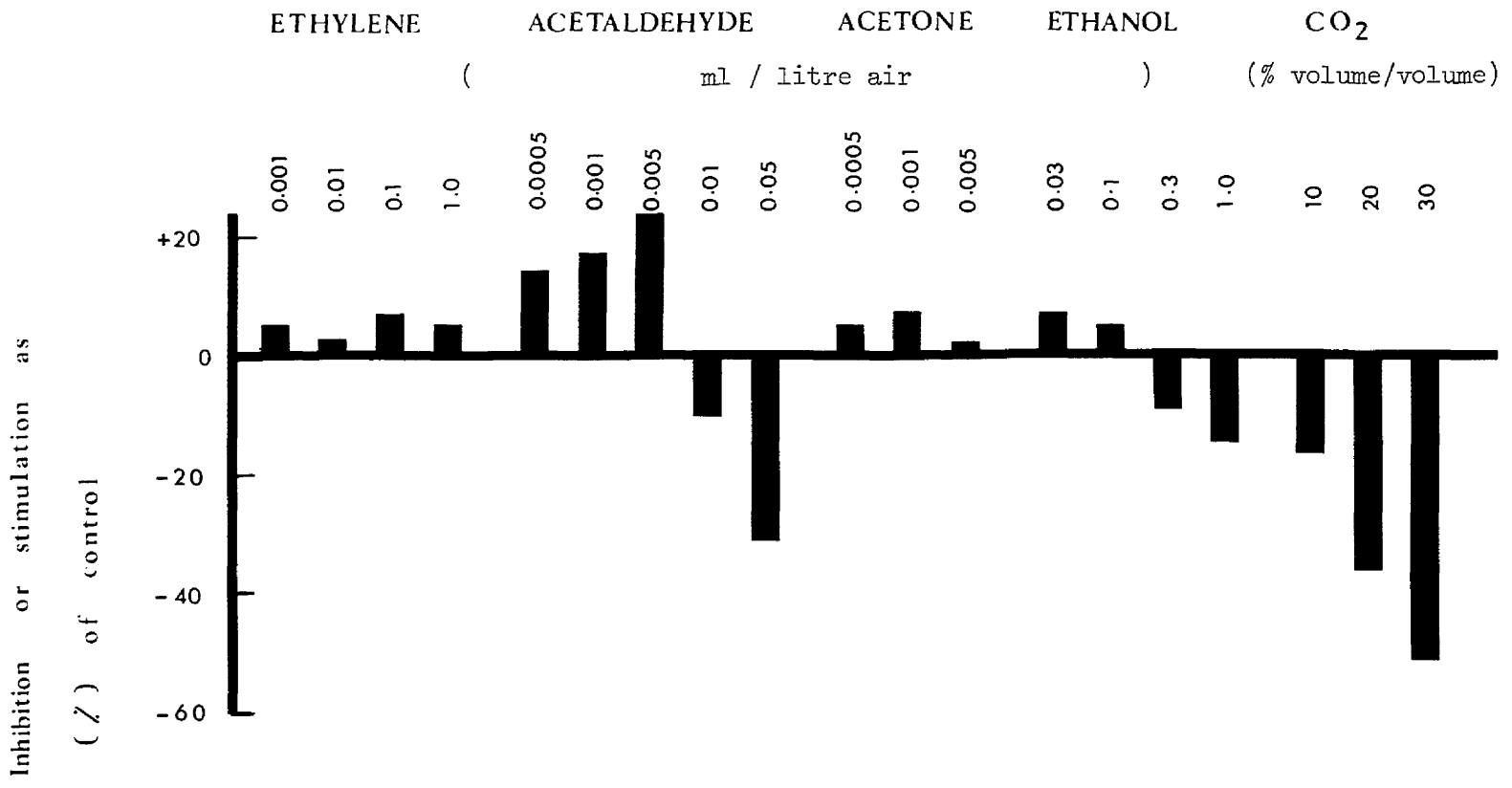


Figure 30 Effects of authentic metabolites on linear growth of Mucor hiemalis, on second day after assembly

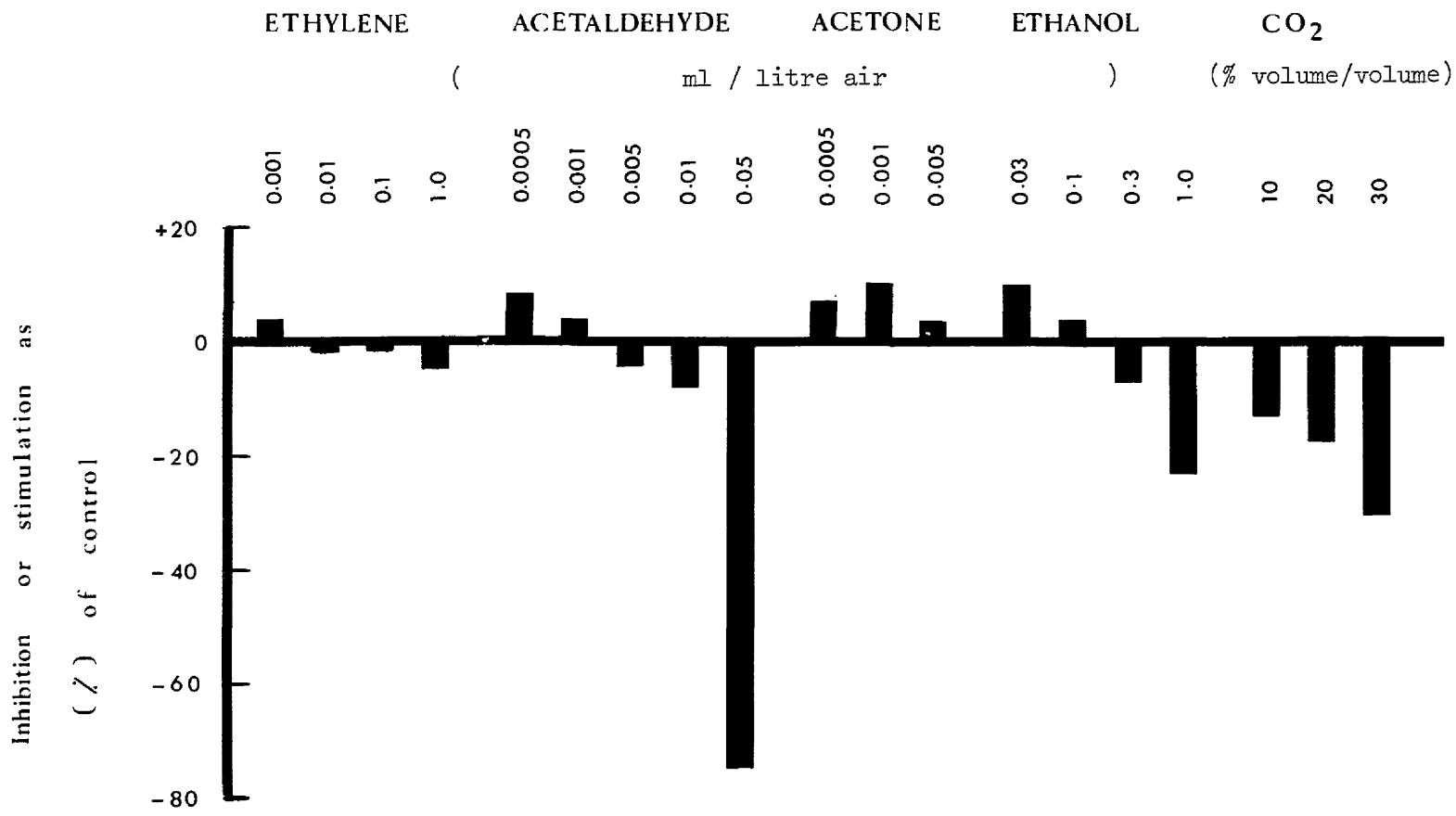


Figure 31 Effects of authentic metabolites on linear growth of Fusarium oxysporum, on second day after assembly

I.3(e) ANALYSIS OF THE INTERACTIONS BETWEEN GASES FROM THE TWO REPRESENTATIVE TRICHODERMA SPECIES AND OTHER ASSAY FUNGI IN PAIRED CULTURES.

INTRODUCTION

This is the appropriate second stage of the broadening of the study of the representative Trichoderma spp. on similar lines to 3(c) above.

METHODS.

Tests were carried out with paired Roux bottle assemblies. The Trichoderma cultures used had been incubated with Suba rubber caps for 7 days before assembly. Each treatment was examined in five replicate assemblies and compared with 5 replicate controls in which the assay fungus was paired with bottles containing uninoculated 2% malt agar. Colony diameters were measured daily and samples of head space gases were taken and analysed on the second and seventh day after pairing. All other experimental details were as described in the General methods.

RESULTS.

The results are recorded in appendix tables (10 A to 10 E) and summarised in text figures (32 and 33). Results with R.solani are recorded in appendix tables (8A - 8B).

CONCLUSION.

From figures (32) and (33) it is concluded that:-

Acetaldehyde:

Concentrations of authentic material equal to the maximum found in the Trichoderma culture gases inhibited the growth of Pyronema domesticum to about half the extent of that produced by the pure culture gases. This was so for both the strains of Trichoderma at both the stages of incubation which were measured. Hence it seems likely that this could contribute appreciably to the effects of the total culture gases on this species. These concentrations produced slight stimulations of the growth of the other assay fungi in pure culture. Their small effects are masked by those of the inhibitory products in tests with complete culture gases.

Acetone:

The concentrations found in these tests are unlikely to have any significant effects on the growth of the assay fungi.

Ethanol:

Similarly the concentrations found in these tests could contribute significantly to the effects of the complete culture gases on Pyronema domesticum, but they are unlikely to have contributed in more than a minor way to the other effects seen.

Carbon dioxide:

After 2 days incubation the assemblies with T.viride 1 had a substantially higher concentration of CO_2 than that in assemblies with T.longibranchiatum WBC 4576. At 7 days the difference between the two species were slight and less consistent. The concentration of this gas alone could account for all the inhibition produced by the Trichoderma culture gases, the differences in rate of build up on inhibitory concentrations could account for the differences between the effects of the two species.

As before, however, it is emphasised that these results are for defined conditions; it seems likely that slight changes in these conditions could affect the proportions of these ubiquitous primary metabolites in culture gases, and to affect their contribution to the effects of these gases on other organisms. It is also emphasised that other unidentified metabolites may be present which may affect these interactions and which might affect them in slightly different conditions.

FIGURE 32 A EFFECTS OF T. viride 1 (LEFT) AND T. longibranchiatum WBC 4576 (RIGHT) ON LINEAR GROWTH OF ASSAY FUNGI ON THE SECOND DAY AFTER PAIRING

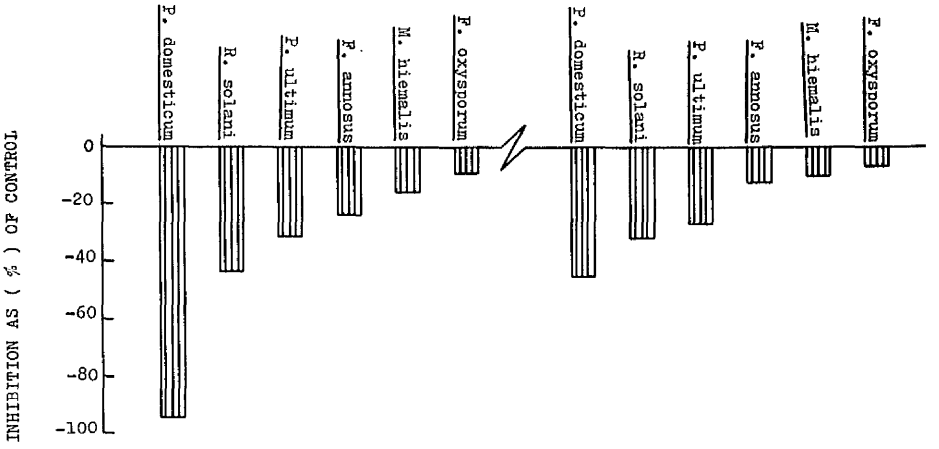


FIGURE 32 B RANGE AND MEAN OF AMOUNTS OF IDENTIFIED GASEOUS METABOLITES FOUND IN TEST ASSEMBLIES ON THE SECOND DAY AFTER PAIRING

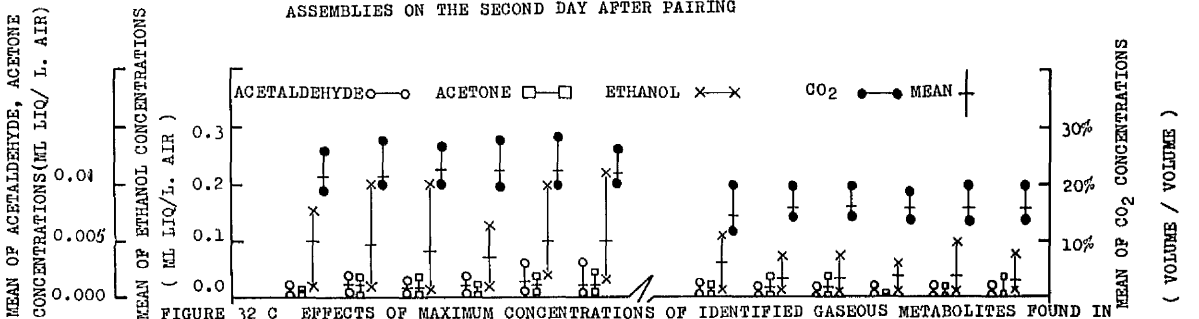
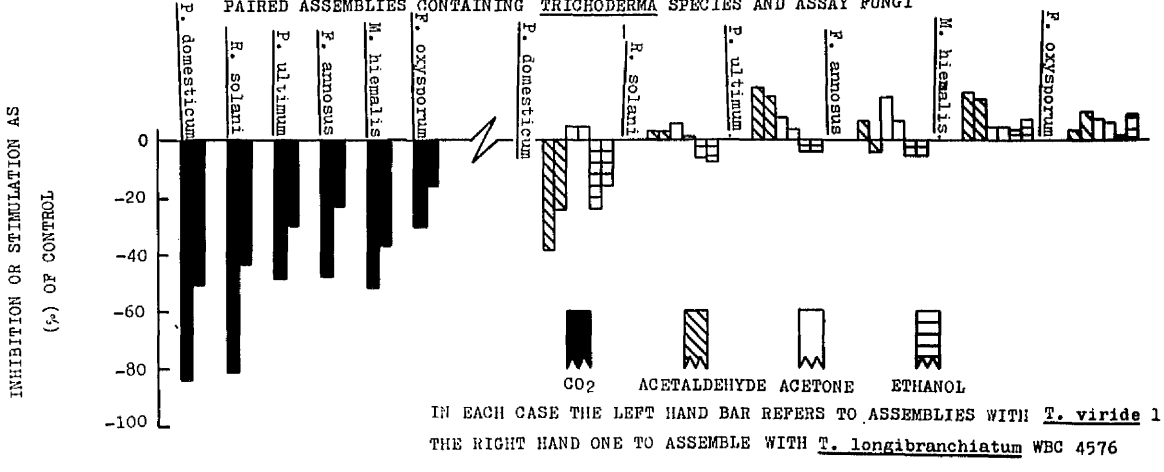


FIGURE 32 C EFFECTS OF MAXIMUM CONCENTRATIONS OF IDENTIFIED GASEOUS METABOLITES FOUND IN PAIRED ASSEMBLIES CONTAINING TRICHODERMA SPECIES AND ASSAY FUNGI



IN EACH CASE THE LEFT HAND BAR REFERS TO ASSEMBLIES WITH T. viride 1, THE RIGHT HAND ONE TO ASSEMBLE WITH T. longibranchiatum WBC 4576

FIFURE 33 A EFFECTS OF T. viride 1 (LEFT) AND T. longibranchiatum WBC 4576 (RIGHT), ON LINEAR GROWTH OF ASSAY FUNGI ON THE SEVENTH DAY AFTER PAIRING

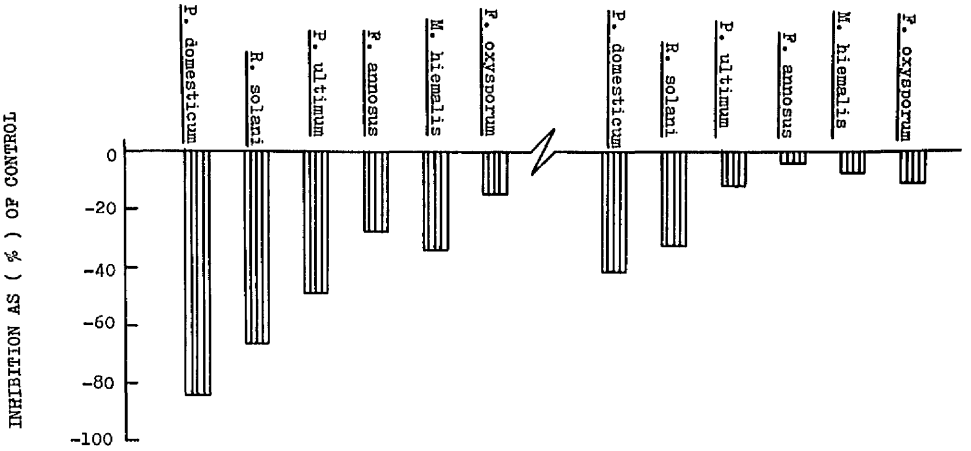


FIGURE 33 B RANGE AND AMOUNTS OF IDENTIFIED GASEOUS METABOLITES FOUND IN TEST ASSEMBLIES ON THE SEVENTH DAY AFTER PAIRING

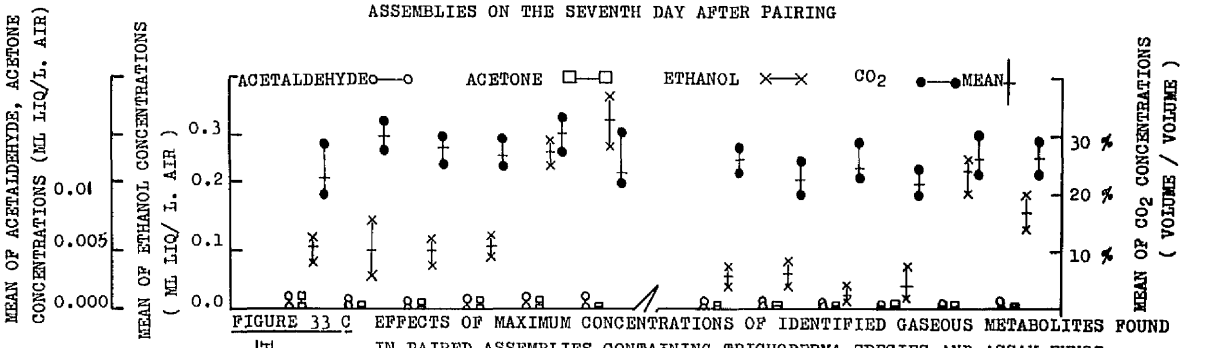
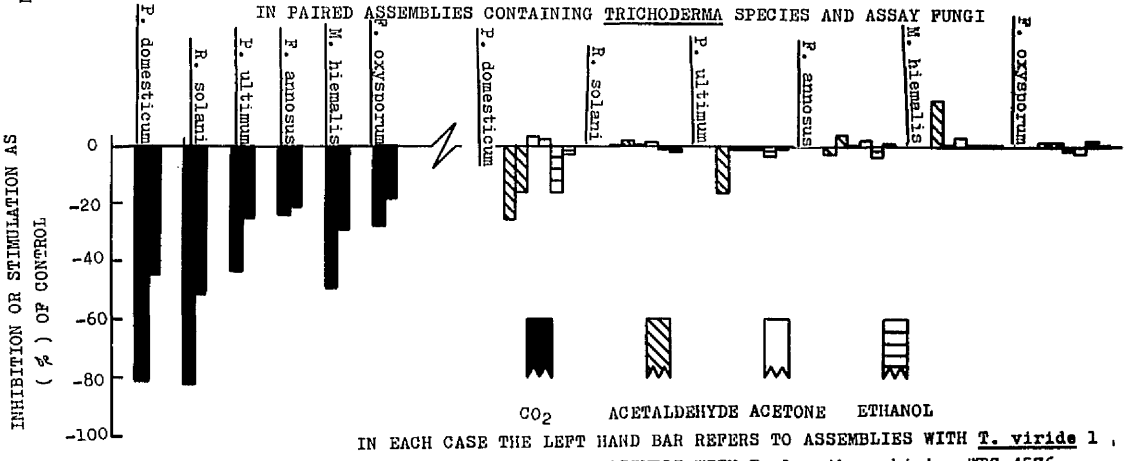


FIGURE 33 C EFFECTS OF MAXIMUM CONCENTRATIONS OF IDENTIFIED GASEOUS METABOLITES FOUND IN PAIRED ASSEMBLIES CONTAINING TRICHODERMA SPECIES AND ASSAY FUNGI



IN EACH CASE THE LEFT HAND BAR REFERS TO ASSEMBLIES WITH T. viride 1 , THE RIGHT HAND ONE TO ASSEMBLE WITH T. longibranchiatum WBC 4576

PART I.4

QUANTITATIVE EXAMINATION OF THE ACTIVITY OF OTHER SPECIES OF *TRICHODERMA*

I.4 QUANTITATIVE EXAMINATION OF THE ACTIVITY OF OTHER SPECIES OF TRICHODERMA

This is reported in two phases:-

(a) a detailed examination of the effects of two further species which they (Dennis and Webster) found to inhibit R.solani, and two further species which they found to have no measured effect,

(b) a G.L.C. analysis of head-space gases from all the other available strains of Trichoderma in standard culture conditions and comparison of their content with their recorded activity.

(a) ANALYSIS OF THE INTERACTIONS BETWEEN GASES FROM TWO FURTHER SPECIES WHICH THEY FOUND TO INHIBIT R.SOLANI AND TWO FURTHER SPECIES WHICH THEY FOUND TO HAVE NO MEASURED EFFECT IN PAIRED CULTURES.

METHODS

Trichoderma viride 14 and Trichoderma polysporum 2 were chosen as representative active strains; Trichoderma pseudokoningii A/196-1 and Trichoderma polysporum 74 were chosen as inactive ones. The growth of R.solani cultures paired with 7-day-old cultures of each strain were compared with those of cultures paired with uninoculated malt agar in the paired Roux bottles. Colony diameters were measured daily and samples of head space gases were taken and analysed on the second and seventh day after pairing. All other standard experimental procedure is as described in General Methods.

RESULTS

The results are given in appendix table 11 and summarised in text-figure (34).

CONCLUSION

The results fit and strongly support the hypothesis that the inhibition of R.solani by these four strains in these conditions can be accounted for by the production of gaseous primary metabolites, particularly of CO_2 and that differences in the amounts of their activity can be accounted for by difference in the rate of production of these metabolites.

FIGURE 34 A EFFECTS OF TRICHODERMA, ACTIVE AND NON-ACTIVE SPECIES ON LINEAR GROWTH OF R. solani ON THE SECOND DAY (LEFT) AND SEVENTH DAY (RIGHT) AFTER PAIRING

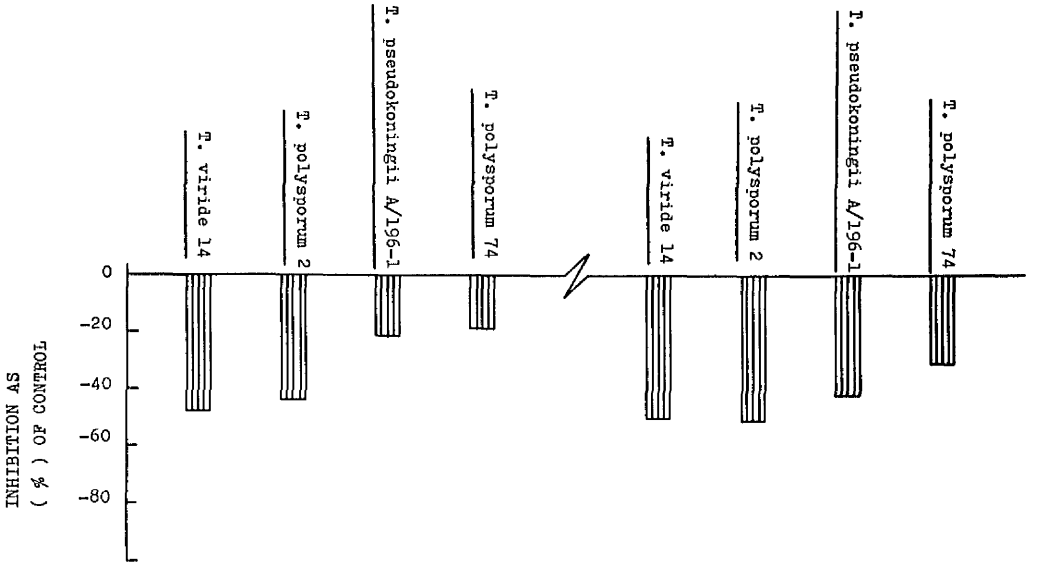


FIGURE 34 B RANGE AND MEAN OF AMOUNTS OF IDENTIFIED GASEOUS METABOLITES FOUND IN TEST ASSEMBLIES ON SECOND AND SEVENTH DAY AFTER PAIRING

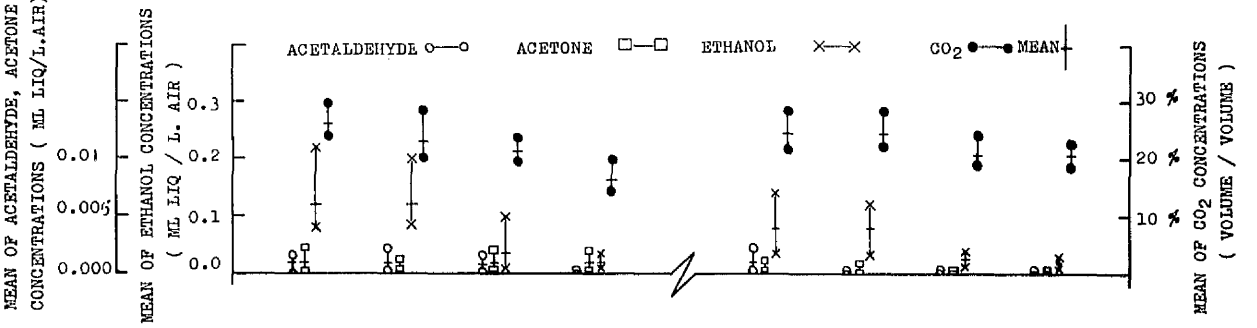
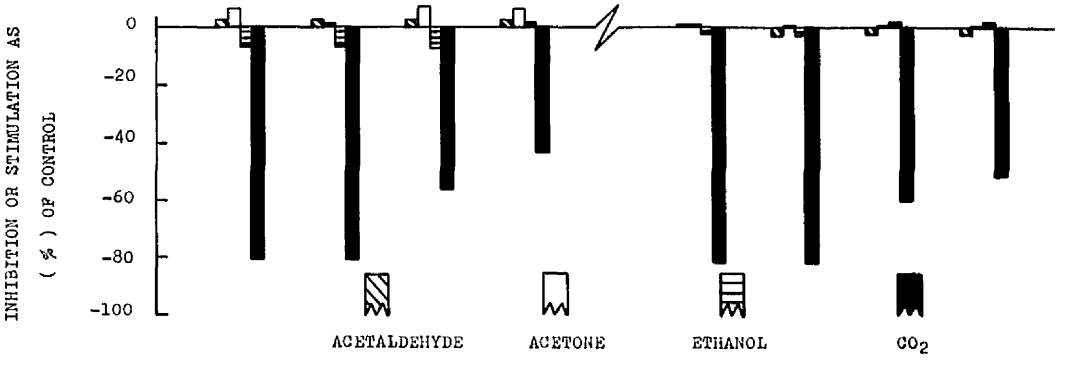


FIGURE 34 C EFFECTS OF MAXIMUM CONCENTRATIONS OF IDENTIFIED GASEOUS METABOLITES FOUND IN PAIRED ASSEMBLIES CONTAINING TRICHODERMA SPECIES AND R. solani



(b) G.L.C. ANALYSIS OF HEAD SPACE GASES FROM ALL THE OTHER AVAILABLE STRAINS OF TRICHODERMA IN STANDARD CULTURE CONDITIONS, AND COMPARISON OF THEIR CONTENT WITH THEIR RECORDED ACTIVITY.

METHODS

Three replicate cultures of each of the tested strains were set up on malt agar in Roux bottles with Suba rubber caps; samples were taken and analysed by standard G.L.C. techniques within the first 24 hours of assembly, and after 7 and 14 days of incubation. A second experiment was set up in precisely the same way except that the Roux bottles were fitted with cotton wool plugs.

RESULTS

The results in sealed bottles are recorded in appendix table (12A) and summarised in the text-figure (35). The results of those incubated in cotton wool plugged bottles are given in appendix table (12B), and summarised in text-figure (36).

CONCLUSION

This survey has not revealed the presence of any metabolite other than the expected primary ones in the expected range of concentrations.

There are substantial differences in the amounts produced by the different strains; the difference between the record from sealed culture and from these fitted with cotton wool plugs are likely to relate to the rate of formation in the first few days of incubation.

Dennis & Webster do not give enough information of experimental variation in their work to permit detailed comparison of the results but there is a substantial amount of agreement between the relative activity which they report in their Table 1, and the difference in amounts of CO_2 produced by these strains.

This general fit, and the fact that no evidence of any other reactions has been revealed, suggest that further examination would not be likely to be profitable.

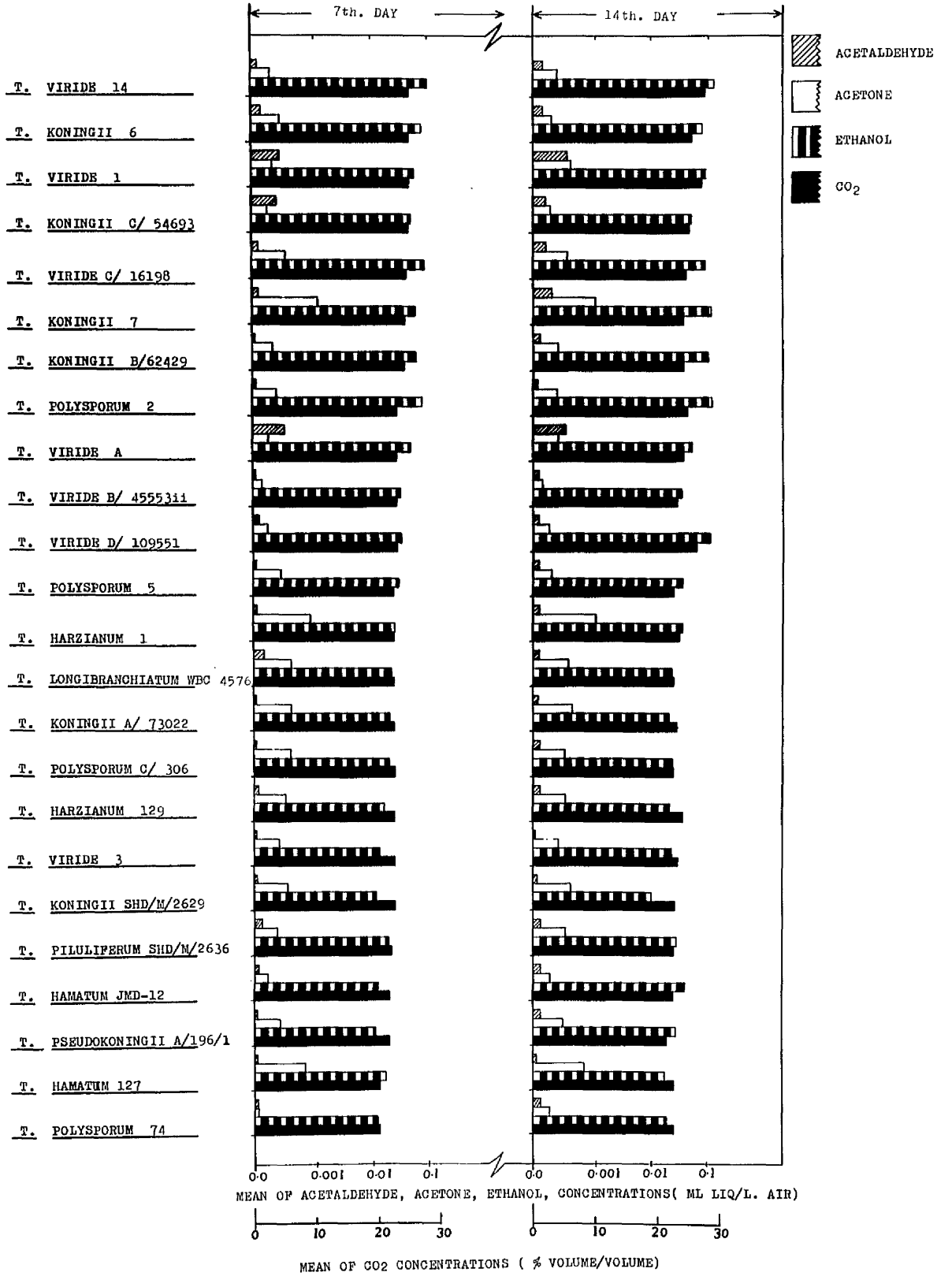


FIGURE 35 THE AVERAGE PRODUCTION OF VOLATILE METABOLITES BY TRICHODERMA STRAINS INCUBATED IN BOTTLES PLUGGED WITH SUBA RUBBER CAP. STRAINS ARE ARRANGED DUE TO AMOUNTS OF CO2 PRODUCED BY EACH ON SEVENTH DAY AFTER INOCULATION

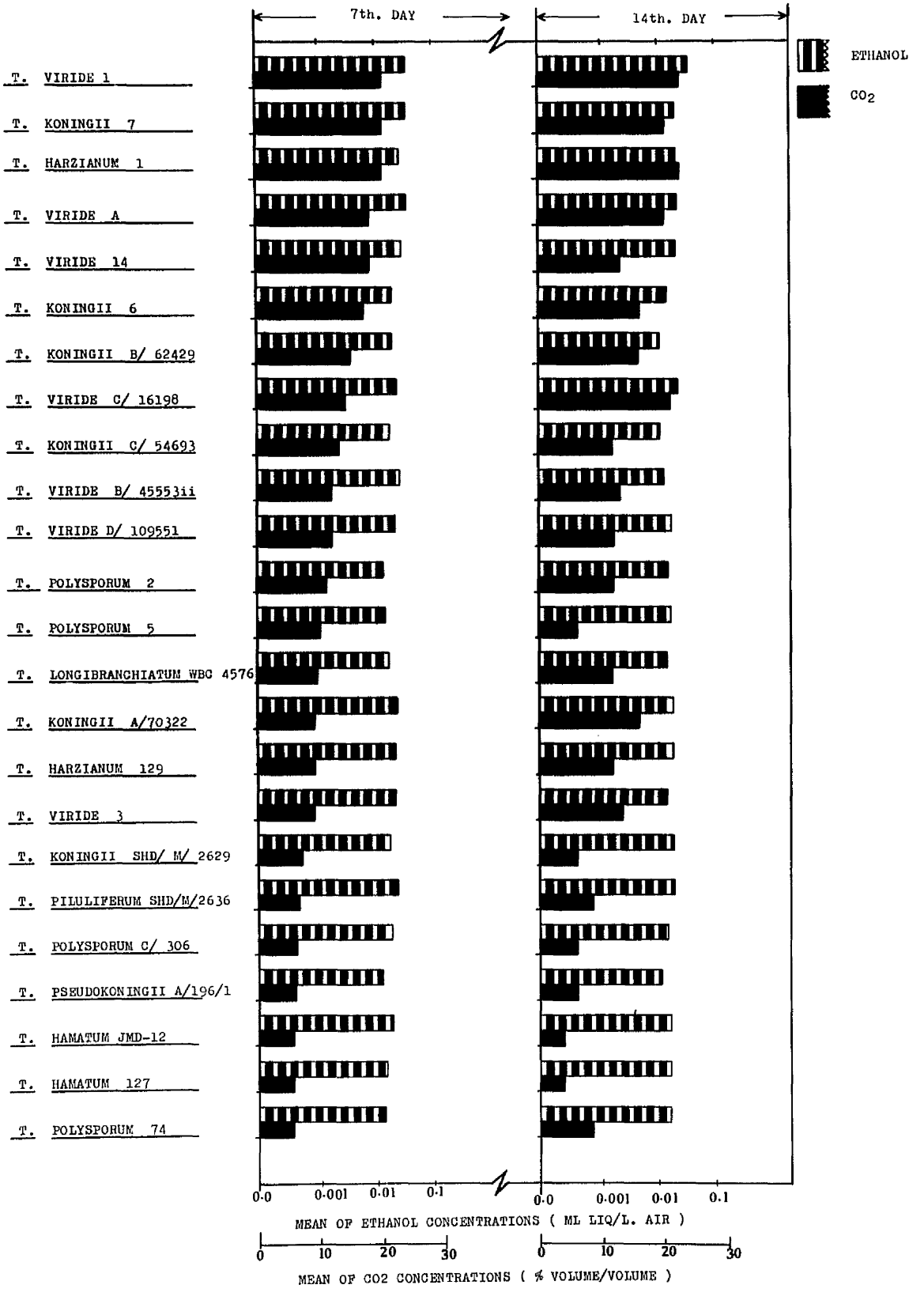


FIGURE 36 THE AVERAGE PRODUCTION OF PRIMARY METABOLITES (ETHANOL AND CARBON DIOXIDE) BY TRICHODERMA STRAINS INCUBATED IN COTTON-WOOL PLUGGED BOTTLES. STRAINS ARE ARRANGED DUE TO AMOUNTS OF CO2 PRODUCED BY EACH ON SEVENTH DAY AFTER INOCULATION

PART II

AN INVESTIGATION OF THE DIFFERENCES BETWEEN THE BIOLOGICAL EFFECTS OF CULTURE GASES FROM THE REPRESENTATIVE SPECIES OF *TRICHODERMA* ON *ERWINIA* spp.

PART II. 1.

LITERATURE REVIEW

LITERATURE REVIEW

Approximately 200 species of plant pathogenic bacteria have been recognized. Generally they occur in only five families, in three orders, and they are represented by six genera:
Pseudomonas (90 species), Xanthomonas (60), Erwinia and (Pectobacterium)(17), Corynebacterium (11), Agrobacterium (7), and Streptomyces (2) (Buddenhagen, 1965; Crosse, 1968; Starr & Chatterjee, 1972).

Erwinia Winslow et al., is a particularly interesting genus of bacterial phyto-pathogens which are widely distributed throughout the globe, causing rots, blights and wilts in a variety of crop plants. On the basis of its disease-producing ability in plants Bergey et al. (1939), gives the group tribal ranking, Erwineae, in the family Enterobacteriaceae. Other major characters of the group are:-
short rods peritrichously flagellated, Gram-negative, do not form spores, their individual cells may contain indigoidine, facultative anaerobes, ferment many carbohydrates and glycerol with the formation of acid only, seldom acid and gas. Their shared sensitivity to a group of bacteriophages and to bacteriocin (anti-Erwinia sera) suggests a common relationship. There are however big differences between some of the bacteria which can be included in the group. In particular Waldee (1945) has proposed that the strongly pectolytic strain which cause soft rot diseases should be separated into a new genus Pectobacterium. These bacterial soft-rots occur most commonly on vegetables and some annual ornamentals that have fleshy storage tissues. (Elrod, 1942; Abo-El-Dahab & El-Goorani, 1964; Chatterjee & Gibbins, 1971; White & Starr, 1971; Starr & Chatterjee, 1972; Erskine, 1973; Shinde & Lukezic, 1974).

They cause serious diseases of crops in the field, in transit and especially in storage, resulting in greater total loss of production than any other bacterial disease (Agrios 1969). When the soft-rot bacteria enter wounds or are otherwise brought in contact with parenchymatous cells (Adams, 1975), they feed and multiply at first on the liquid released by the broken cells on the wound surface or on liquid in the intercellular spaces.

Inoculation is followed by rapid multiplication of the bacteria which produce increasing amounts of pectolytic (a pectin-methyl-esterase, pectin-trans-eliminase, and a poly methylgalacturonase) and, in lesser quantity, cellulolytic enzymes (Wood, 1951, 1955 and 1960; Friedman & Ceponis, 1959; Walton & Cappellini, 1962). The pectolytic enzymes breakdown the pectic substances of the middle lamella and of the cell wall and cause maceration of the tissues. The cellulolytic enzymes cause partial breakdown and softening of the cellulose of the cell walls. As a result of the action of these and other enzymes water from the protoplasts diffuses into the intercellular spaces; the cells plasmolyze, collapse and die (Kraght & Starr, 1953; Starr & Moran, 1962). Acid production by E.carotovora in host plant is another factor in virulence (Friedman and Ceponis, 1964).

The most important bacterium on many fleshy plants and vegetables is Bacillus (Pectobacterium) carotovora (Jones L.R.) which was first described by Jones in 1900. Erwinia (Pectobacterium) atroseptica (van Hall), has been described as the organism commonly associated with the black-leg disease of potato (Pérombelon, 1972, 1975) and Erwinia (Pectobacterium) aroideae (Townsend), with the slimy decay of various tropical and sub-tropical crops, or crops grown in green houses, such as tomatoes. Graham & Dowson (1960) reported that these organisms have been differentiated from E.carotovora on the basis of their capacity to utilize various sugars. Because of their morphological similarity therefore, same authors pointed out that their is some question as to whether these three bacteria should be designated as different species or variants of a single species which is the Pectobacterium carotovorum. This species is divided into four sub-types: P.carotovorum itself (or, more strictly , P.carotovorum var. carotovorum), P.carotovorum var. atrosepticum, P.carotovorum var. aroideae and P.carotovorum var. chrysanthemi. In India, Hingorani & Addy (1953) reached essentially the same conclusions, they recognized two species, Erwinia carotovora and E.atroseptica and considered E.aroideae as a non-gas forming strain of E.carotovora. It has been found also that, E.carotovora and E.aroideae produce acid on ethanol agar, whereas E.atroseptica does not (Massey, 1924; Burkholder & Smith, 1949; Dowson, 1957; Malcolmson, 1959). Echandi et al, (1957), found that E.aroideae produced H_2S abundantly whereas those of

E.carotovora produced little or none. They regarded E.aroideae as a valid member of that species because of its production of hydrogen sulphide, its strong growth at 40°C, and its complete lack of gas forming ability on carbohydrate substrates. Studies on the biochemical reactions used for separation of soft-rot coliforms are carried out by many investigators and reviewed by Graham (1964, 1972).

The characters of the soft-rot bacteria (Pectobacterium, Waldee) and their difference from Erwinia amylovora (Burrill) Winslow et al., and Erwinia tracheiphila, as described by several investigators are summarised in the following table:-

Pectobacterium spp.	E.amylovora	E.tracheiphila
1.Facultative saprophyte.	1.Strict to living plants	1. Same as amylovora.
2.Produce pectolytic enzymes which degrade middle lamella of hosts.	2.Dessicate infected tissue (blights) of orchard trees.	2. Cause wilting of cucurbits and clogging vascular tract.
3.The min.,optimum and max. temp. for their growth in culture are 2°C, 25°C, 37°C, respectively.	3.Optimum temperature for their growth in culture is 25-26°C.	3. Same as amylovora.
4.Grew well in the depth of shake culture as well as at the surface.	4.Grew most profusely at the surface of the shake culture.	4. Grew mostly at the surface only.
5.Cells are rod-shaped 2.25 µ long x 0.75 µ diam.	5.Cells are rod-shaped 1.5 µ long x 1 µ wide, longer form of up to 35 µ have been observed in cultures.	5. Cells are rod-shaped 1.75 µ long x 0.6 µ dia. In young (4-5-day-old) cultures, many of the bacteria appear coccoid, with diam. of 1.5 µ.
6.Occurs singly or in chains and at temp. of 27°C. or higher form much longer rods and also filaments.	6.Occurs singly but pairs or chains of 3 to 4 bacteria also exist.	6. Occurs singly, longer chains may be present.
7.Each rod has 2 to 6 peritrichous flagella.	7.The bacterium possesses flagella over its entire surface.	7. Each cell has 4-8 peritrichous flagella.

These characters and some others have been reported by many investigators (Wood, 1951, 1960; Gregg, 1952; Graham, 1958 (a), 1964, 1972; Crosse, 1959; Burkholder, 1960; Billing et al., 1961; Billing & Baker, 1963; Voros & Goodman, 1965; Nelson & Dickey, 1970; Shinde & Lukezic, 1974; Schroth et al., 1974; Stanghellini & Meneley, 1975).

Most studies on these phytopathogenic bacteria in the soil have been prompted by the desire to control disease rather than to elucidate the ecology of the causal organisms (Crosse, 1968).

The work which has been done on these shows that there is a big difference between the survival of the "soft-rot" species and that of the others. Many workers have isolated phytopathogenic bacteria from soil using a range of selective media containing pectin, sodium polypectate or crystal violet pectates (Echandi et al., 1957; Miller & Schroth, 1972; Guppels & Kelman, 1974; Webb, 1974).

Kerr (1953) and Graham (1958a) isolated them from a variety of scottish soils using a direct plating technique. It has been suggested however, that these isolations are probably from fragments of plant or animal debris in the soil (Leach, 1930; Tamimi & Banfield, 1969). Voronkevich (1960, 1973) concluded more positively that the soft-rot species persist in decaying plant residues, but that they are unable to survive once these residues have been fully decomposed.

The other species of Erwinia have been found to decline more rapidly in soil. This is to be expected from their greater dependence on burry host tissue.

E.amylovora has been found to decline rapidly to undetectable levels in five weeks in unsterilized soil and in 8 weeks in sterilized soil in the laboratory. The results showed that loam and clay were more favourable to the persistence of the organism than sand, because the last type contained very little organic matter (Ark, 1932). By using the above methods of direct plating, and inoculation onto sensitive fruits, Thomas & Parker (1933) found that some species failed to overwinter in natural soils. These and other similar studies are reviewed by Schroth et al. (1974).

E.tracheiphila, the cucurbit wilting pathogen does not survive for long in the soil, but overwinters within the body of cucumber beetles which transmit to succeeding crops (Rand & Cash, 1920; Burkholder, 1960). These bacteria however, vanish very rapidly in dry soil but they persist for longer in water logged soils (Leach, 1940).

It has been suggested that survival of these "short living" bacteria may be affected by interactions with the other soil flora. Lee (1920) found that they may remain viable almost indefinitely in sterile soils, and some increased in number. This could also explain their better survival in soils with low moisture contents, or in soils held at low temperature, than in moistened warm soils (Graham, 1953; Nelson & Semenuik, 1963). For these reasons, their decline in soils has been attributed to the activities of antagonistic micro-organisms. Patrick (1954) isolated a great number of antagonists from virgin soils and claimed that there was some correlation between the sensitivity of the phytopathogenic bacteria to these organisms and their ability to survive in the soil. The author found that E.tracheiphila was the most susceptible to the antagonistic action of the soil microflora. He noted that Erwinia atroseptica and E.carotovora have very different antibiotic spectra from Erwinia amylovora and the E.tracheiphila. Two years later, Pridham et al. (1956) found that E.carotovora and E.aroideae were the most tolerant to Streptomyces metabolites.

There have been few studies of the effects of authentic volatile metabolites on Erwinia species; though there are many recorded the study of the effects of CO_2 in particular and soil bacteria in general e.g. Gray & Wallace, 1957; Stotzky & Goos, 1965, 1966; Wells, 1974; Nielson, 1964, however, concluded that exposure to up to 20% of CO_2 had no effect on the rate of decay of potato tubers infected with E. aroideae , E. atroseptica or E. carotovora.

PART II.2

EXAMINATION OF THE EFFECTS ON MEASURABLE VISIBLE GROWTH IN AGAR CULTURE

II.2. EXAMINATION OF THE EFFECTS ON MEASURABLE VISIBLE GROWTH IN AGAR CULTURE .

A brief preliminary investigation was made by measuring gross macroscopic effects on "streak inoculae" in agar surfaces. It was appreciated that this crude technique could reveal only gross inhibitory effects; if, however, the responses were sufficiently sensitive it would be a simple and quick method and it would permit direct comparison with the work on fungi. It seemed sensible to investigate this possibility before developing more elaborate work.

MATERIALS & METHODS

Two ml of an aqueous suspension of cells from a 4-to 5-day old culture of the bacterial species being examined was spread evenly over the surface of a Petri dish plate of Bouillon Agar and left on the laboratory bench for a few minutes until the free liquid had been absorbed. The agar was then sliced to give blocks meaning of (35 mm long x 15 mm wide x 3 mm deep), and three reference lines were drawn with a sterile inoculating loop on the inoculated surface. The same suspension was used to inoculate all the blocks used in any test. The blocks were then placed on a glass strip and inserted into Roux bottles (Figure 3). The Roux bottles contained 0.5-1 ml of deionized water, supplement to keep the atmosphere at 100% R.H. and to prevent drying out the gas. They were then attached to 7-day-old Trichoderma cultures on 2% malt agar in the normal way for paired assemblies (Section I.3.c.), or fitted with serum caps through which known amounts of test materials were introduced by the standard technique for tests of authentic metabolites. Three inoculated agar blocks were set up in each of these Roux bottles for each treatment; subsequent growth on the reference lines was compared with that on the same number and arrangement of replicate blocks in control assemblies containing air or paired with Roux bottles containing uninoculated 2% malt agar (Figure 2). After examination scrapings from the inoculated surface on which no growth was visible were transferred to slopes of Bouillon agar; and any growth on these slopes during 7 days further incubation in normal air was recorded. All cultures were incubated at 24°C in the dark.

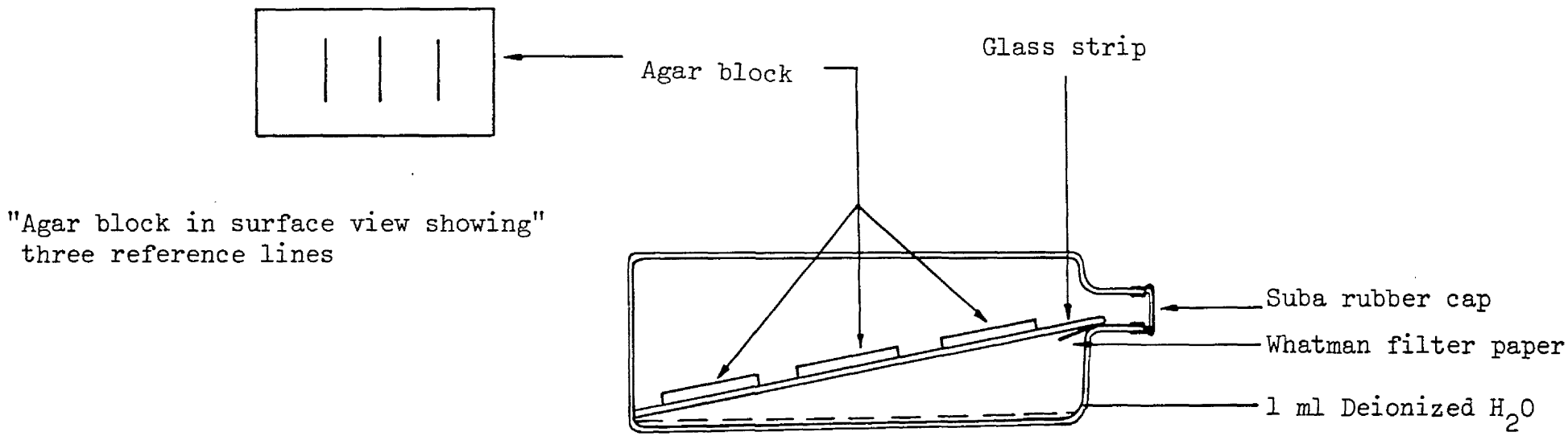


Figure 37 Roux bottle in section showing arrangement of glass strip and agar blocks used for bacterial tests.

RESULTS

The results are recorded in appendix tables 14A to 14 O. In these growth is recorded by eye on a scale from 0 = no visible growth to 3 = growth equal to the maximum produced in control cultures at the end of the experiment. All cultures were incubated and examined daily for 7 days. No changes were seen in any culture after the 14th day. The growth in the subcultures made from the scrapings taken from uninoculated surfaces on the 7th day is shown in the final column.

DISCUSSION AND CONCLUSION

The tests with the Trichoderma cultures (Appendix 13) did not reveal any effects on bacterial growth. They do not, however, show that no such effects were present; in particular they do not measure any qualitative effects which may have developed. It was appreciated that some obvious refinements could make the technique more informative; e.g. more rigorously controlled and less dense inoculae might result in visible differences in the density of colonies after incubations in different treatments. An economic way to explore this possibility seemed to determine the degree to which concentrations of metabolites known to be commonly present above Trichoderma cultures approached the inhibitory level required to prevent visible colony development in these colonies.

The results of these tests summarised in text table 6 suggest that the concentrations required for bacteriostasis are substantially higher than those found in Trichoderma cultures. In appendix tables 14A to 14 O the last column indicates the viability of bacteria after subculturing to fresh media in air. The results indicate that the bactericidal concentrations are likely to be very much higher than those recorded in text table 6.

It was concluded that further development of this technique is unlikely to be a profitable use of effort.

Text-table 6 Comparison of minimum concentrations of authentic identified metabolites and maximum concentration found above Trichoderma cultures required to inhibit viable growth of assay species of Erwinia.

Metabolites tested	Minimum concentrations required to inhibit viablr growth of bacterial colonies				
	E.amylovora	E.aroideae	E.atroseptica	E. carotovora	E.tracheiphila
ethylene [0.001] [a]	> 10.0 ml /l.air	> 10.0 ml /l.air	>10.0 ml /l.air	>10.0 ml /l.air	>10.0 ml /l.air
ammonia [<0.01]	1.0 ml liq/l.air	1.0 ml liq/l.air	1.0 ml liq/l.air	1.0 ml liq/l.air	1.0 ml liq/l.air
acetaldehyde [0.001]	0.01 ml liq/l.air	0.01 ml liq/l.air	0.005 ml liq/l.air	0.005 ml liq/l.air	0.005 ml liq/l.air
acetone [0.001]	0.5 ml liq/l.air	0.5 ml liq/l.air	0.25 ml liq/l.air	0.5 ml liq/l.air	0.25 ml liq/l.air
ethanol [0.1]	0.3 ml liq/l.air	0.6 ml liq/l.air	0.3 ml liq/l.air	0.3 ml liq/l.air	0.3 ml liq/l.air
CO_2 [30%]	> 30% V.V.	> 30% V.V.	> 30% V.V.	> 30% V.V.	> 30% V.V.

(a) = maximum concentrations found in head-space gases of pure cultures of Trichoderma

PART II.3

EXAMINATION OF THE EFFECTS ON GROWTH AND SURVIVAL IN SOIL

II.3 EXAMINATION OF THE EFFECTS ON GROWTH AND SURVIVAL IN SOIL.

INTRODUCTION

From the author's previous work with these bacteria and from consideration of the reports in the literature review, it seemed appropriate to investigate the effects of *Trichoderma* volatiles on representatives of the two ecological types in the genus. *E.tracheiphila* and *E.aroideae* were chosen as convenient representative of each.

It was also apparent that the interactions would be likely to be affected substantially by the medium on which the bacteria were grown. A comprehensive investigation of all the interaction would not be possible with the time and labour available. Furthermore, it seemed sensible to start with a limited investigation to see whether new information of significant general interest was likely to be involved.

From previous work it seemed more likely that any effects which we could identify would be due to the liberation of common primary metabolites. This determination would be a useful step in the development of knowledge, but its nature would not be a good justification for major spending of public money.

Hence a simple investigation was carried out on the same general lines as were used for the above work with assay fungi.

METHODS

The following media were used:-

(a) a mixed garden loam. This was collected as a single sample from aerated plot in the University ground at Garscube Estate, Glasgow, N.W. The wider sample used in the investigation was collected at one time, air dried, mixed thoroughly and passed through a No.8 sieve, and stored in an unsealed plastic bag in the laboratory until required.

(b) a fine grain clay, supplied by the Glasgow School of Arts.

(c) an acid washed sand, supplied by Messrs. Hopkins & Williams.

(d) Bouillon agar. As discussed in the General Methods, 1.a.

The soil cultures were prepared by placing 50g of air dried soil in a Roux bottle, and adding the maximum amount of deionized water to saturate the soil without leaving free water on the surface. This was of the order of 20 ml. Agar cultures were prepared by adding 70 ml of Bouillon agar to a Roux bottle. The bottles were plugged and autoclaved at 120°C for one hour.

Five ml of an aqueous suspension of cells from a 4-to 5-day-old culture of Erwinia species under test was added to each cooled flask and the contents of the bottle with soil were shaken to distribute the inoculae as evenly as possible through the medium. The same suspension was used for each flask in any one test.

The effects of culture gases were examined by joining these soil or agar cultures with 7-day-old cultures of Trichoderma species on malt agar in standard paired assemblies.

The effects of authentic metabolites were examined by allowing known amounts to volatilize in the bottle by the standard single Roux bottle tests (cf. General Methods, 1.b.).

Test cultures and controls were set up in triplicate in each case and they were incubated in the dark in an air conditioned room at 24°C, 70-80% R.H. 5 ml samples of head space gases were taken by gas syringe on the day of assembly and on the 7th day, and analysed by the standard G.L.C. technique.

After incubation the number of viable bacterial cells in each culture was estimated by shaking 1 gram of the culture medium in 9 ml of deionized water, making serial dilution of the suspension in water and plating out samples of appropriate dilution on Bouillon agar plates. The number of colonies on the plates were counted after they had been incubated at 24°C for 48 hours in the dark.

RESULTS

The results of the replicate investigations of the effects of complete culture gases on bacteria are given in appendix tables (15A to 15R). The results of the replicate investigations of the effects of air/gas mixtures of authentic metabolites, in the range of concentrations at which they are found in culture gases, are given in appendix tables (16A to 16H).

The results are summarised in text table (7). The critical comparisons of these effects are made in text figures (38 and 39) and text tables (8 and 9). Appendix tables 17A and 17B give the results of an investigation of the changes in concentration of known mixtures of authentic acetaldehyde and CO_2 during storage above sterile soils and agar. These are summarised in text figures 40 and 41.

DISCUSSION AND CONCLUSION

The results in all replicates are so closely similar in all important respects that the correlations are obviously significant without further mathematical analysis. The inconsistency in some results with Expts. III do not seem to be sufficiently large to affect this decision.

From text table (7), tests I and II the recorded inhibition of both bacterial species is greater in assemblies with T.viride 1 than in those with T.longibranchiatum WBC 4576. This is consistently so for all tests and media used. The differences in amounts of CO_2 produced by these species could account for this difference (text figures 38 and 39). Differences in the amounts of ethanol produced may also contribute appreciably to the effects against E.aroideae but not to those against E.tracheiphila; none of the other metabolites examined seem likely to have contributed appreciably to any inhibition of either species. It is likely, however, that quite small differences in environmental conditions might result in some of them playing a higher part in some interactions.

Similarly the differences in sensitivity of the two bacterial species to carbon dioxide could account for differences in response to Trichoderma culture gases on all the media tested (text table 8).

From text table (9) it appears that many more viable cells were recovered from cultures on loam than on clay or sand and many more were obtained from agar cultures than from loam. In many cases the smaller

viable population correlates with the lower amount of inhibition with the presence of the Trichoderma culture gases and of the authentic CO_2/air mixtures. This was not sufficiently consistent, so to be unequivocal evidence of the population in the less favourable growth media being less sensitive to the inhibitory factor or factors. In particular the comparison of record of inhibition of E.tracheiphila by 30% CO_2 and the very high numbers of surviving cells in the controls does not fit this hypothesis. It may be, however, that this is an optimum nutritive condition for inhibition and that the loam soil provides an environment nearer to this than that provided by the other soils and by the agar.

From text figures 40 and 41 it appears that there is a difference in the amount of sorption of the metabolites by the different soils and agar. The greatest sorption was shown by clay and agar, in which least inhibition was measured. This relationship is not consistently similar to the other medias, however. There is apparently more sorption by loam than by sand, but more inhibition by loam than sand. It may be, however, that there is an interaction here between reduction of gas concentration and increase in inhibitive level of the loam.

Table 1 Summary of results of percentage reduction or stimulation in numbers of bacterial cells of E.tracheiphila and E.aroideae respectively from different types of soils and agar layers exposed to known concentrations of authentic gases, and to 7-day-old Trichoderma cultures for 7 days after assembly.

Treatment	Test material	Range of concn. introduced	Erwinia tracheiphila												Erwinia aroideae									
			Agar Expt. No.			Loam Expt. No.			Clay Expt. No.			Sand Expt. No.			Agar Expt. No.			Loam Expt. No.			Clay Expt. No.		Sand Expt. No.	
			I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	I	II
I	T.viride 1		-10	-38	-47	-45	-65	-49	-16	-33	-20	-21	-34	-24	-8	-31	-23	-25	-28	-31	-8	-14	-17	-14
II	T.longibranchiatum WBC 4576		-6	-16	-	-35	-29	-	-8	-16	-	-18	-20	-	-7	-11	-	-14	-18	-	-2	-7	-9	-9
III	Acetaldehyde (ml liquid/l.air)	0.001‡	+2	-5	-7	-2	-5	-7	-1	-1	-4	-2	+2	-3	+2	-2	-1	-4	-2	-3	+2	-2	-4	-2
		0.005	-1	-13	-13	-10	-15	-20	-3	-8	-12	-6	-4	-9	-9	-2	-3	-9	-4	-14	-2	-3	-4	-3
		0.01	-30	-23	-14	-29	-45	-45	-19	-27	-13	-12	-7	-14	-14	-8	-9	-28	-21	-28	-11	-8	-9	-7
		0.05	-100	-100	-77	-100	-100	-100	-100	-100	-100	-100	-100	-100	-80	-78	-90	-100	-80	-100	-88	-62	-100	-91
IV	Acetone (ml liquid/l.air)	0.001‡	+2	+2	+4	+2	-1	-2	-4	-1	+3	-3	-3	-5	+3	+3	+1	-1	+2	-3	-1	+3	-1	-1
		0.05	-2	-1	-3	-5	-6	-9	-4	-4	-2	-3	-6	-14	-2	-5	-3	-3	-1	-9	-3	-2	-2	-2
		0.1	-14	-19	-22	-26	-30	-33	-5	-9	-12	-26	-33	-29	-2	-6	-12	-5	-8	-17	-6	-4	-6	-16
V	Ethanol (ml liquid/l.air)	0.03 *	+3	+1	-1	-2	-8	-7	+1	-3	-1	+1	-1	-3	-3	-1	-2	-2	-1	-2	+3	-2	-3	-4
		0.1 +	-2	-3	-1	-4	-12	-19	-2	-9	-5	-2	-9	-12	-6	-3	-6	-14	-11	-19	-3	-3	-7	-11
		0.3	-28	-43	-35	-36	-56	-43	-20	-24	-18	-20	-32	-40	-11	-13	-18	-25	-29	-33	-12	-8	-11	-20
		0.6	-44	-61	-80	-58	-85	-65	-44	-41	-48	-68	-77	-82	-29	-36	-40	-59	-61	-73	-32	-38	-38	-52
VI	CO_2 (Volume/volume)	20% *	-9	-20	-22	-30	-42	-38	-18	-18	-9	-14	-22	-30	-9	-15	-2	-12	-21	-17	-9	-7	-6	-8
		30% +	-16	-40	-51	-44	-65	-56	-20	-24	-25	-22	-35	-40	-12	-28	-10	-18	-34	-31	-12	-18	-16	-20

+ = maximum concentration found in head-space gases of pure cultures of T.viride 1

* = maximum concentration found in head-space gases of pure cultures of T.longibranchiatum WBC 4576

Figure 38 A. Effects of T.viride 1 (left) and T.longibranchiatum WBC 4576 (right) on growth and survival of Erwinia tracheiphila on 7th day after pairing.

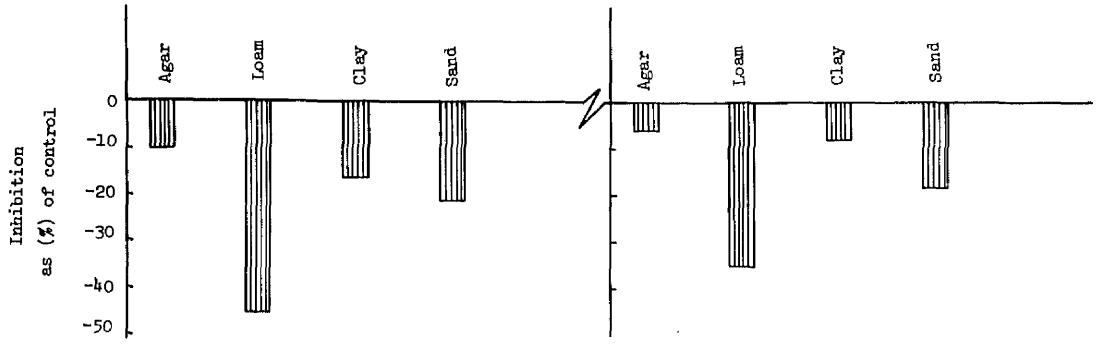


Figure 38 B. Range and mean of amounts of identified gaseous metabolites (acetaldehyde, acetone, ethanol and CO_2) found in test assemblies on the 7th day after pairing. + = Mean.

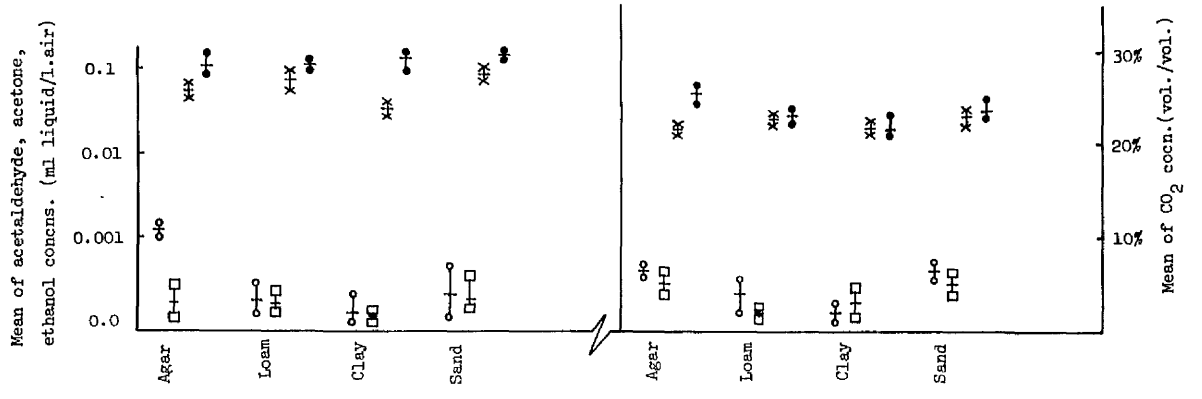


Figure 38 C. Effects of maximum concentrations of identified gaseous metabolites found in T.viride 1 (left) and T.longibranchiatum WBC 4576 (right) - paired assemblies on E. tracheiphila.

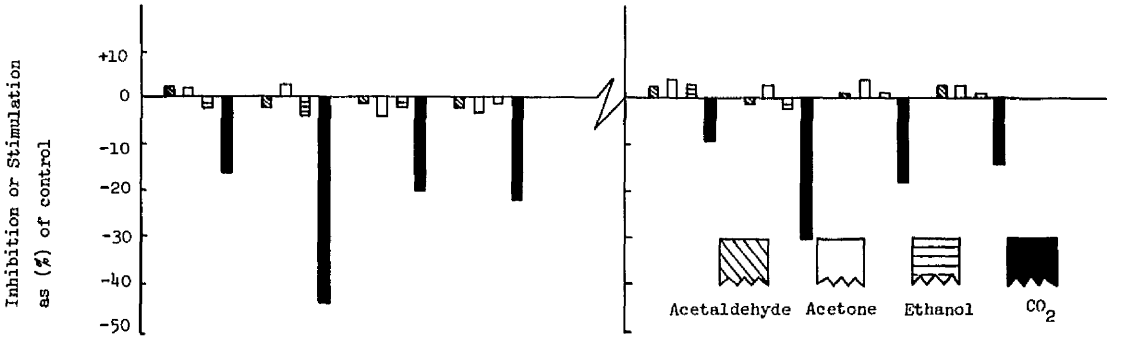


Figure 39 A. Effects of T. viride 1 (left) and T.longibranchiatum WBC 4576 (right) on growth and survival of Erwinia aroideae on 7th day after pairing

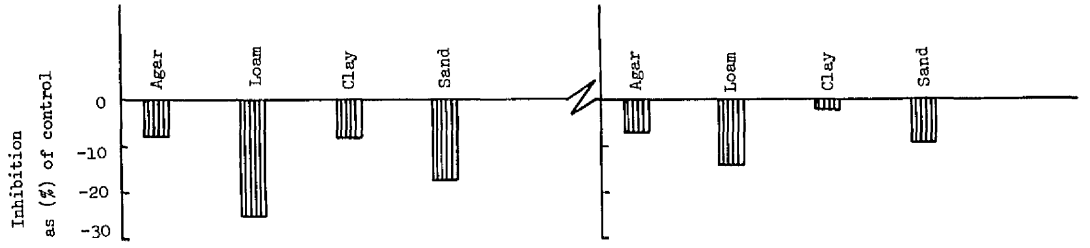


Figure 39 B. Range and mean of amounts of identified gaseous metabolites (acetaldehyde, acetone, ethanol and CO_2) found in test assemblies on the 7th day after pairing. + = Means.

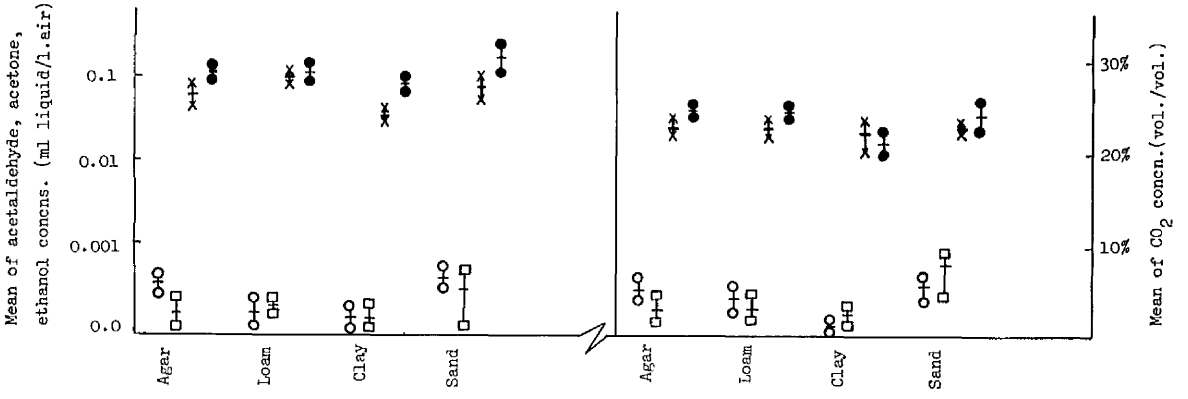


Figure 39 C. Effects of maximum concentrations of identified gaseous metabolites found in T.viride 1 (left) and T.longibranchiatum WBC 4576 (right)-paired assemblies on E.aroideae.

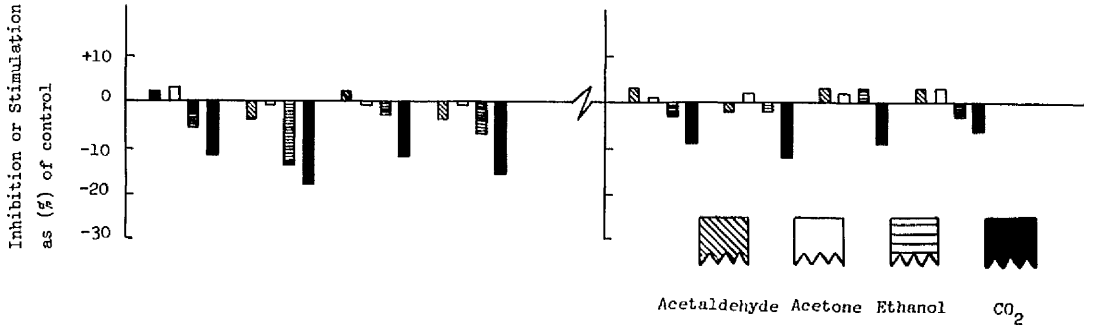


Table 8 Difference between measurements of inhibition of growth of *E.tracheiphila* and *E. aroideae*, when exposed to culture gases.

Treatment	(Mean of E.tracheiphila) − (Mean of E.aroideae)[a] of measurement									
	Agar Expt. No.			Loam Expt. No.			Clay Expt. No.		Sand Expt. No.	
	I	II	III	I	II	III	I	II	I	II
Complete gas from *T.viride* 1	2	7	23	20	34	21	8	19	4	20
Complete gas from *T.longibranchiatum* WBC 4576	+ 1	5	-	21	11	-	6	9	9	11
20% vol/vol CO_2	0	5	20	18	21	21	9	11	8	14
30% vol/vol CO_2	4	12	41	26	31	25	8	6	6	15

(a) the significance of the correlation is evident without formal mathematical analysis.

Table 9 Summary of effects of medium types on the percentage [illegible] in numbers of bacterial cells, exposed to complete gas mixtures from Trichoderma cultures in a typical experiment.

Treatment		E. tracheiphila +				E. aroideae +			
		Agar	Loam	Clay	Sand	Agar	Loam	Clay	Sand
paired with T.viride 1	I	391×10^8	215×10^6	63×10^6	48×10^6	410×10^8	146×10^6	43×10^6	18×10^6
Growth in control	II	455×10^8	537×10^8	419×10^6	620×10^6	339×10^8	475×10^6	71×10^6	36×10^6
	III	386×10^8	383×10^8	117×10^6	134×10^6	186×10^8	619×10^6	-	-
Inhibition (%)	I	10	45	16	21	8	25	8	17
	II	38	65	33	34	31	28	14	14
	III	47	49	20	24	23	31	-	-
paired with T.longibranchiatum.	I	273×10^8	187×10^6	50×10^6	35×10^6	278×10^8	291×10^6	45×10^6	23×10^6
Growth in control	II	437×10^8	390×10^8	50×10^6	46×10^6	391×10^8	290×10^6	101×10^6	35×10^6
Inhibition (%)	I	6	35	8	18	7	14	2	9
	II	16	29	16	20	11	18	7	9
Exposed to 30% CO_2									
Growth in control of 3 experiments	I	400×10^8	258×10^6	277×10^6	182×10^6	376×10^8	349×10^6	206×10^6	104×10^6
	II	460×10^8	335×10^6	261×10^6	156×10^6	444×10^8	440×10^6	198×10^6	101×10^6
	III	274×10^8	468×10^6	396×10^6	111×10^6	493×10^8	394×10^6	-	-
Inhibition (%)	I	16	44	20	22	12	18	12	16
	II	40	65	24	35	28	34	18	20
	III	51	56	25	40	10	31	-	-

+ number of colonies recorded for gram of soil.

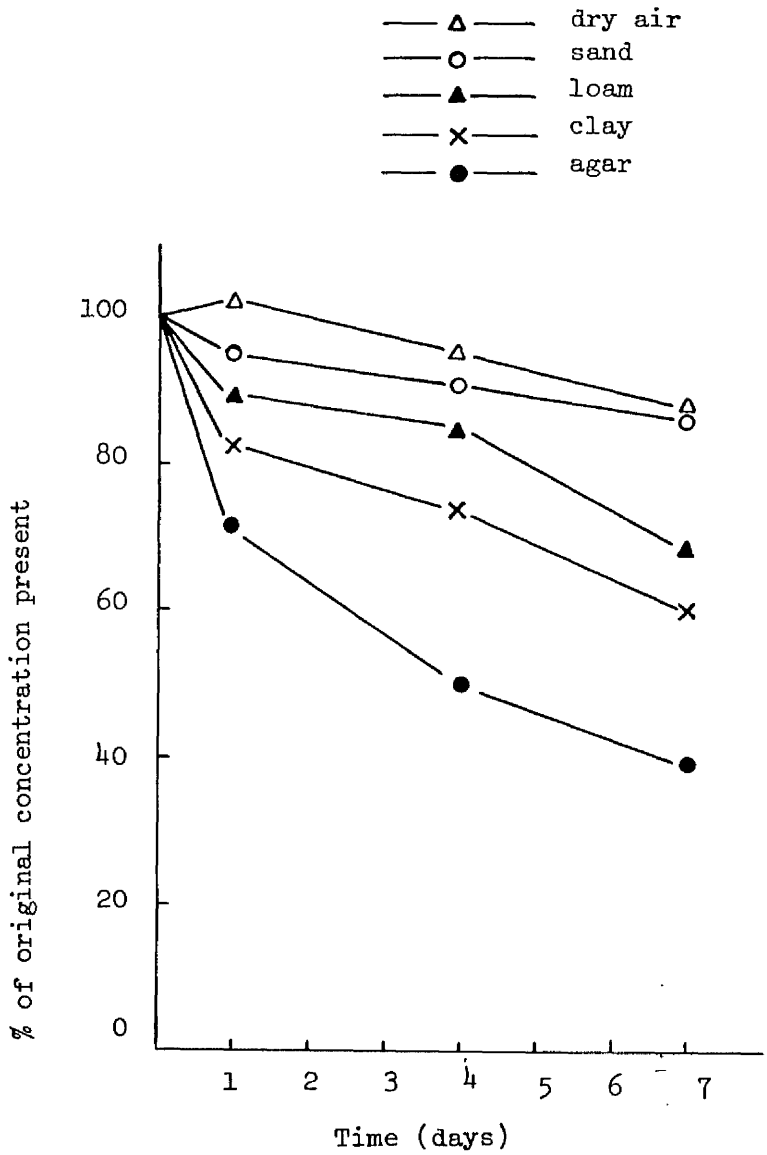


Figure 40 Changes in concentration of volatiles of authentic acetaldehyde (0.01 ml liquid/litre air) in test assemblies of agar, loam, clay, and sand compared to dry air; expressed as % of concentration of each, present immediately after the assemble.

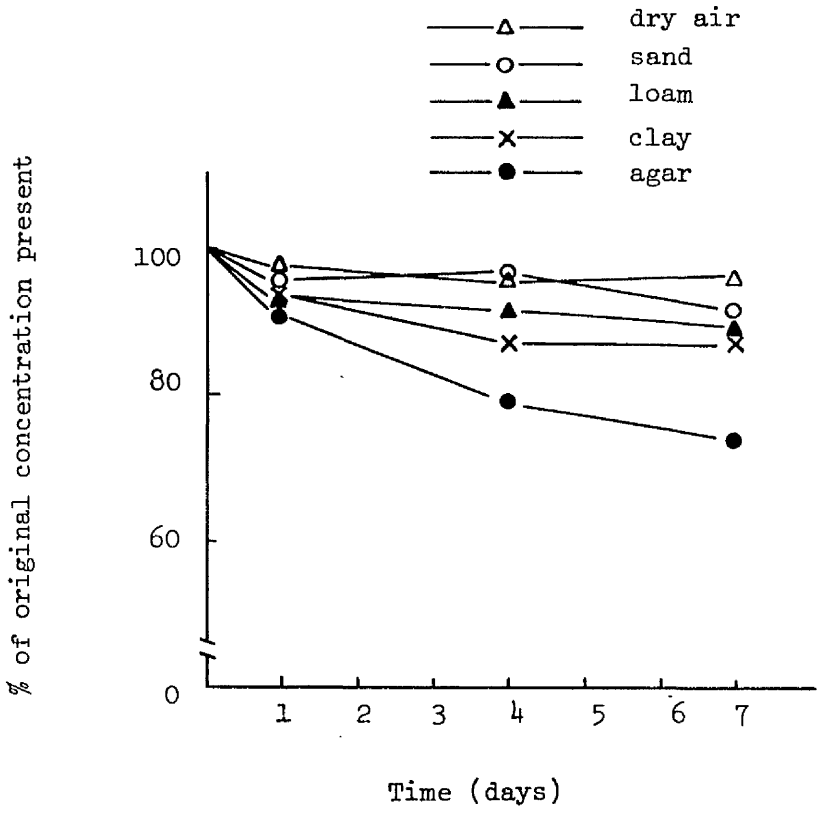


Figure 41 Changes in concentration of volatiles of authentic CO_2 (30% vol./vol.) in test assemblies of agar, loam, clay and sand compared to dry air; expressed as % of concentration of each, present immediately after the assemble.

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APPENDIX I

THE DEVELOPMENT OF A SIMPLE APPARATUS USED TO AERATE CULTURES WITH KNOWN CONCENTRATIONS OF CO_2/ AIR MIXTURES

THE DEVELOPMENT OF A SIMPLE APPARATUS USED TO AERATE CULTURES WITH KNOWN CONCENTRATIONS OF CO_2/AIR MIXTURES.

INTRODUCTION:

From previous work in this department and earlier experiments in this investigation it seemed likely that carbon dioxide would be found to play a big part in the interactions. It also seemed likely that the investigations would involve some determination of the rate at which the concentration in an assembly build up to an inhibitory level. The identification of this inhibitory level involves exposure to a series of consistent concentrations; this would not be possible in the simple sealed assemblies, as the build up to CO_2 through respiration of the assay cells will produce a continuously increasing concentration during the test. Hence it seemed necessary to develop a reflooding system. The same problem would, of course, have been involved if any of the other primary metabolites were shown to be produced in any significant inhibitory concentration.

The apparatus which was developed is shown in appendix figure (1). The development of this apparatus involved the following investigations, using 20% CO_2 volume/volume of air space mixture throughout.

(a) Uniformity of movement of gases through manifold outlets from the apparatus.

In the early version of the apparatus the outlet of Container 'A' was fitted with a manifold from which it was hoped to aerate several Roux bottles simultaneously.

To test the uniformity of movement of gas mixture through the outlets from this manifold a 20% CO_2/air mixture was made in the container, a single Roux bottle was then attached to each of five outlets of the manifold, the appropriate taps were opened and five litres of the mixture was displaced through the Roux bottle. The bottles were then sealed and the amount of CO_2 in each was measured by 3 replicate G.L.C. analysis. This experiment was repeated four times. The results are given in table (1).

Empirical observation of the results shown that there are unacceptably big differences between the amounts of CO_2 introduced into the bottles. There is no indication of any consistent relationship between the position of a bottle on the manifold used and the relative amount of CO_2 delivered into it in replicate experiments. The differences between readings for bottles are obviously very much bigger than the small differences found in G.L.C. measurements of replicate samples from bottles.

In succeeding experiments gas mixtures were made up similarly and one litre batches were passed successively into single bottles attached to the manifold. The bottles were sealed and their CO_2 contents were determined as above. The results are given in table (2). Empirical observation shows that the differences between amounts of CO_2 introduced into the bottles was substantially reduced, but that the differences between them was still unacceptable high. Two possible causes of this irregularity were then examined.

(i) The uniformity of the mixture of gases prepared in the container.

Gas mixtures were made by partially evacuating the container and returning it to atmospheric pressure by the addition of authentic CO_2 (British Oxygen Company). Thorough mixing of the contents was then effected by rotating the paddle at 700 r.p.m. for approximately 30 minutes. Successive 1 litre batches of mixture were then displaced into a Roux bottle attached successively to the outlet by the introduction of measured volumes of water from reservoir 'B'. Three replicate samples were taken for each bottle for G.L.C. analysis.

Table (3) give the results of G.L.C. analyses of successive samples from the stirred container, and compares them with analysis of an unstirred container (Winchester). In succeeding experiments gas mixtures were made up similarly, using same container (c.f. table 4). From the empirical observation it was concluded that stirring procedure is a useful increase in homogeneity of the mixture in the time available. It was also noted that the concentration of CO_2 in the mixture did not fall off or increase consistently with the addition of successive fresh volumes of water to the container. This indicates that this simple method of

displacing the mixture is a satisfactory one for our purposes; the concentration of CO_2 is not noticeably affected by its differential solubility in the successive amounts of water.

(ii) The amount of fresh mixture required to replace the air contained in Roux bottle by the system used.

The contribution of this factor to the experimental variation found was assessed by passing one, two and three litre volumes of a standard mixture into a series of Roux bottles attached singly and successively to the container.

The results are recorded in table (5). Empirical study shows that, as was to be expected, there is only erratic and partial replacement by reflooding with 1 litre. Three litres produced a satisfactorily consistent replacement mixture.

(b) Effect of successive aeration on the build up of CO_2 in a container.

It is apparent from (ii) above that 100% replacement of gases will not be produced by this system. The effect of the presence of the successively residual CO_2 in building up the final concentration after successive replacements was tested by aerating a single Roux bottle a number of times and measuring the changes in CO_2 concentration produced.

The results are given in table (6). Empirical observation shows that this factor is unlikely to make more than a trivial concentration to experimental error.

CONCLUSION

From these investigations following procedure was evolved.

(a) The mixture was made up and stirred for 30 minutes at 700 r.p.m.

(b) Bottles were aerated individually from a single outlet.

(c) 3 litres of mixture was passed through each bottle at each aeration.

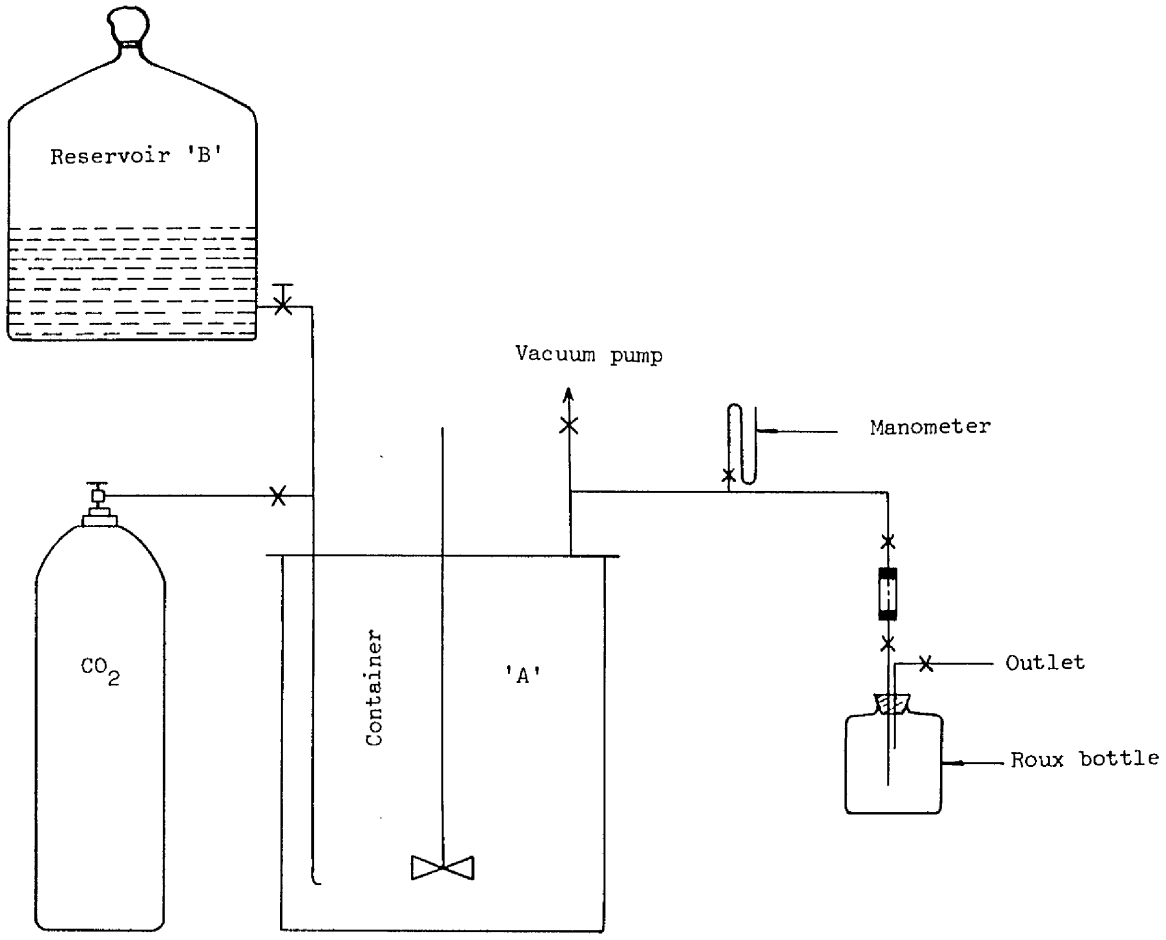

Figure 1 Diagram of apparatus used to aerate Roux bottles with standard CO_2/air mixture (X = tap).

Table 1 Heights of G.L.C. peaks (mm) produced by CO_2 in samples of gases taken from five Roux bottles aerated simultaneously from a manifold in the standard apparatus. One litre CO_2/air displaced by a litre water.

le	G.L.C. sampling no.	EXPERIMENT NUMBER I	II	III	IV
	1	19	17	15	18
	2	18	15	14	15
	3	19	15	14	15
ıs		19	16	14	16
	1	19	18	10	15
	2	18	18	12	14
	3	18	19	12	15
ıs		18	18	11	15
	1	17	8	8	22
	2	17	8	8	20
	3	15	9	9	20
ıs		16	8	8	21
	1	14	10	20	9
	2	14	10	22	9
	3	14	11	22	8
ıs		14	10	21	9
	1	11	12	11	9
	2	10	9	8	9
	3	12	9	10	8
ıs		11	10	10	9
and Range		15.66 +3.34 −5.66	12.53 +6.47 −4.53	13.00 +9 −5	13.73 +8.27 −5.74
g^{-2}		9.238	16.267	23.714	23.352

Table 2 G.L.C. peak heights (mm) produced by CO_2 samples of gases taken from five Roux bottles aerated singly from a manifold in the standard apparatus. One litre CO_2/air displaced by a litre water.

Roux bottle no.	G.L.C. sampling no.	EXPERIMENT NUMBER I	II	III	IV
I	1	13	18	17	12
	2	13	18	17	12
	3	14	19	17	12
Means		13	18	17	12
II	1	22	26	18	21
	2	20	25	18	20
	3	19	25	18	20
Means		20	25	18	20
III	1	20	24	29	20
	2	21	24	28	20
	3	21	24	28	20
Means		21	24	28	20
IV	1	21	20	18	18
	2	23	21	18	19
	3	20	20	17	19
Means		21	20	18	19
V	1	20	20	17	18
	2	21	19	17	18
	3	20	19	16	18
Means		20	19	17	18
Mean and Range		19.20 +3.80 -6.20	21.53 +4.47 -3.53	19.53 +9.47 -3.53	17.80 +2.20 -5.80
σ^2		8.24	8.12	21.12	2.74

Table 3 Test the consistency of CO_2 concentrations at different levels of the main container whereby the paddle had and not been operated at each experiment. One litre CO_2/air also displaced by 1·1. H20 on each replicate treatment.

Aeration no.	G.L.C. sampling no.	Unstirred Winchester Experiment Number I	II	III	Stirred container Experiment Number I	II	III
	1	18	18	17	19	20	20
I	2	18	18	16	19	20	19
	3	19	18	16	19	20	19
Means		18	18	16	19	20	19
	1	15	14	16	18	20	19
II	2	15	14	16	18	20	19
	3	16	14	16	18	20	19
Means		15	14	16	18	20	19
	1	16	15	14	18	20	20
III	2	18	15	13	19	20	20
	3	19	14	14	19	20	20
Means		18	15	14	19	20	20
	1	14	13	18	20	20	20
IV	2	13	14	18	20	20	20
	3	14	12	18	20	20	20
Means		14	13	18	20	20	20
	1	16	13	12	20	20	20
V	2	15	14	13	20	20	20
	3	15	13	12	20	20	20
Means		15	13	12	20	20	20
	1	18	12	11	20	20	20
VI	2	18	12	12	20	20	19
	3	18	12	12	20	20	19
Means		18	12	12	20	20	19
	1	13	18	19	18	20	20
VII	2	14	18	19	19	20	20
	3	13	18	19	19	20	20
Means		13	18	19	19	20	20
	1	14	15	12	18	20	20
VIII	2	13	13	12	18	20	20
	3	12	13	12	18	20	20
Means		13	14	12	18	20	20
	1	18	12	10	18	20	20
IX	2	16	12	12	18	20	20
	3	14	13	11	18	20	20
Means		16	12	11	18	20	20
	1	14	11	16	18	20	20
X	2	13	12	16	18	20	20
	3	15	12	16	18	20	20
Means		14	12	16	18	20	20
Mean and range		15.46 $^{+3.54}_{-3.46}$	14.10 $^{+3.90}_{-3.10}$	14.60 $^{+4.40}_{-3.60}$	18.83 $^{+1.17}_{-0.83}$	20 $^{+0.0}_{-0.0}$	19.76 $^{+0.24}_{-0.76}$
σ^2		4.39	4.92	7.62	0.76	0.0	0.18

Table 4 Test the consistency of CO_2 concentrations at different levels of the main container whereby the paddle had and not been operated. One litre CO_2/air displaced by 1 litre H2O on each aeration replicate.

oux ottle eration o.	G.L.C. sampling from each bottle	Unstirred container Experiments I	Unstirred container Experiments II	Stirred container Experiments I	Stirred container Experiments II
	1	19	26	20	22
I	2	18	24	21	21
	3	18	25	20	21
Means		18	25	20	21
	1	20	19	20	23
II	2	18	19	21	23
	3	19	19	20	22
Means		19	19	20	23
	1	25	15	23	23
III	2	25	16	20	20
	3	25	16	20	20
Means		25	16	21	21
	1	26	24	17	22
IV	2	25	24	17	22
	3	26	24	19	22
Means		26	24	18	22
	1	15	20	20	20
V	2	16	20	20	21
	3	16	21	20	21
Means		16	20	20	21
	1	23	18	20	19
VI	2	24	17	21	20
	3	23	18	19	20
Means		23	18	20	20
	1	20	22	20	21
VII	2	20	20	21	21
	3	20	20	19	22
Means		20	21	20	21
	1	21	21	20	18
VIII	2	20	20	20	20
	3	20	20	21	20
Means		20	20	20	19
	1	21	18	20	20
IX	2	19	19	20	21
	3	20	20	20	21
Means		20	19	20	21
	1	20	19	20	19
X	2	22	21	21	17
	3	20	20	21	20
Means		21	20	21	19
ean and Range		20.8 +5.2 -4.8	20.2 +4.8 -4.2	20.0 +1 -2	20.8 +2.2 -1.8

Table 5 G.L.C. peak height (mm) produced by CO_2 samples of gases taken from six roux bottles contacted main container were aerated singly, by adding 1, 2 and 3 litres H20 respectively to displace same volume of CO_2/air from each replicate bottle.

x tle	G.L.C. sampling no.	Experiment Number I Litres of H20 added 1	2	3	II Litres of H20 added 1	2	3	III Litres of H20 added 1	2	3
	1	18	22	29	19	25	30	18	23	30
I	2	18	22	29	19	25	30	18	23	30
	3	18	22	29	19	25	30	18	23	30
eans		18	22	29	19	25	30	18	23	30
	1	17	22	29	19	26	30	18	23	30
II	2	18	22	29	19	26	30	18	23	30
	3	18	22	29	19	26	30	18	23	30
leans		18	22	29	19	26	30	18	23	30
	1	18	25	28	19	25	30	18	25	30
II	2	18	25	28	19	25	30	18	25	30
	3	18	25	28	19	25	30	18	25	30
leans		18	25	28	19	25	30	18	25	30
	1	18	25	29	19	25	30	18	23	29
IV	2	17	25	29	19	25	30	18	23	29
	3	18	25	29	19	25	30	18	23	29
leans		18	25	29	19	25	30	18	23	29
	1	18	25	29	18	25	29	18	24	30
V	2	18	25	29	19	24	29	18	24	30
	3	18	25	29	19	24	29	18	24	30
leans		18	25	29	19	24	29	18	24	30
	1	19	24	29	19	24	30	18	24	30
VI	2	18	24	29	19	24	30	18	24	30
	3	18	24	29	18	24	30	19	24	30
leans		18	24	29	19	24	30	18	24	30
an and range		18.00	23.83	28.83	18.88	24.88	29.83	18.05	23.66	29.83
		+1	+1.17	+0.17	+0.12	+1.12	+0.17	+0.95	+1.34	+0.17
		-1	-1.83	-0.83	-0.88	-0.88	-0.83	-0.05	-0.66	-0.83
σ^2		0.0	1.91	0.147	0.104	0.104	0.147	0.055	0.582	0.147

Table 6 Effects of reflooding process on G.L.C. peak heights which produced by CO_2 samples of gases taken from six roux bottles aerated singly directly from the fermenter. Three lit. mixture passed to displace an old CO_2/air mixture into each replicate bottle.

x tle	G.L.C. sampling no.	Reflooding times 1st	2nd	3 rd	4th
	1	30	30	32	30
I	2	30	30	32	30
	3	30	30	32	30
leans		30	30	32	30
	1	30	30	31	30
II	2	30	30	31	30
	3	30	30	32	30
leans		30	30	31	30
	1	30	31	30	30
III	2	30	31	30	30
	3	30	31	30	30
Means		30	31	30	30
	1	29	30	29	29
IV	2	29	30	29	29
	3	29	30	29	29
Means		29	30	29	29
	1	30	30	30	30
V	2	30	30	30	30
	3	30	30	30	30
Means		30	30	30	30
	1	30	30	30	30
VI	2	30	30	30	30
	3	30	30	30	30
Means		30	30	30	30
eans and range		29.83 +0.17 -0.83	30.16 +0.84 -0.16	30.38 +1.68 -1.38	29.83 +0.17 -0.83
σ^2		0.147	0.147	1.075	0.147

APPENDIX II

TABLES OF RESULTS RELEVANT TO

PARTS I AND II

Table 1A Effects of 2-day -old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	20 x 20 = 20	35 x 36 = 35	60 x 60 = 60	82 x 82 = 82
	II	5 x 5 = 5	19 x 21 = 20	35 x 35 = 35	58 x 58 = 58	85 x 85 = 85
	III	5 x 6 = 5	18 x 18 = 18	41 x 42 = 41	60 x 64 = 62	78 x 82 = 80
Means		5	19	37	60	82
paired with *T.viride* 1	I	5 x 5 = 5	13 x 13 = 13	33 x 33 = 33	55 x 55 = 55	77 x 77 = 77
	II	5 x 5 = 5	10 x 10 = 10	29 x 29 = 29	54 x 54 = 54	74 x 74 = 74
	III	5 x 5 = 5	10 x 11 = 10	29 x 30 = 29	54 x 54 = 54	74 x 74 = 74
Means		5	11	30	54	75
Inhibition as % of control		Nil	-42	-19	-10	-9
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	21 x 21 = 21	36 x 40 = 38	50 x 60 = 55	79 x 82 = 80
	II	5 x 5 = 5	20 x 20 = 20	38 x 38 = 38	55 x 55 = 55	81 x 80 = 80
	III	5 x 5 = 5	20 x 20 = 20	38 x 38 = 38	55 x 55 = 55	80 x 80 = 80
Means		5	20	38	55	80
Inhibition or Stimulation as % of control		Nil	+5	+3	-8	-2

Table 2A Effects of 2-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	18 x 22 = 20	32 x 46 = 39	58 x 62 = 60	62 x 68 = 65
	II	5 x 5 = 5	18 x 20 = 19	40 x 46 = 43	49 x 52 = 50	73 x 78 = 75
	III	6 x 6 = 6	20 x 24 = 22	36 x 39 = 37	48 x 53 = 50	71 x 79 = 75
Means		5	20	40	53	72
paired with *T.viride* 1	I	5 x 5 = 5	18 x 18 = 18	28 x 28 = 28	45 x 45 = 45	65 x 65 = 65
	II	5 x 5 = 5	18 x 18 = 18	30 x 31 = 30	46 x 46 = 46	65 x 65 = 65
	III	5 x 5 = 5	17 x 17 = 17	28 x 28 = 28	45 x 45 = 45	65 x 65 = 65
Means		5	18	29	45	65
Inhibition as % of control		Nil	-10	-28	-15	-10
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	21 x 21 = 21	35 x 35 = 35	50 x 50 = 50	73 x 73 = 73
	II	5 x 5 = 5	20 x 20 = 20	34 x 34 = 34	50 x 50 = 50	74 x 74 = 74
	III	5 x 5 = 5	20 x 20 = 20	35 x 35 = 35	50 x 50 = 50	73 x 73 = 73
Means		5	20	35	50	73
Inhibition or Stimulation as % of control		Nil	Nil	-13	-6	+1

Table 1B Effects of 4-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies.

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	22 x 24 = 23	38 x 41 = 39	65 x 65 = 65	86 x 85 = 85
	II	5 x 5 = 5	17 x 18 = 17	28 x 30 = 29	55 x 57 = 56	80 x 80 = 80
	III	5 x 5 = 5	19 x 19 = 19	30 x 30 = 30	58 x 58 = 58	80 x 80 = 80
Means		5	20	33	60	82
paired with *T.viride* 1	I	5 x 5 = 5	17 x 19 = 18	33 x 34 = 33	51 x 51 = 51	60 x 60 = 60
	II	5 x 5 = 5	19 x 20 = 19	30 x 34 = 32	50 x 54 = 52	60 x 63 = 61
	III	5 x 5 = 5	20 x 19 = 19	31 x 32 = 31	52 x 52 = 52	60 x 60 = 60
Means		5	19	32	52	60
Inhibition as % of control		Nil	-5	-3	-13	-27
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	20 x 20 = 20	36 x 37 = 36	54 x 54 = 54	65 x 65 = 65
	II	5 x 5 = 5	20 x 21 = 20	32 x 36 = 34	54 x 55 = 54	65 x 65 = 65
	III	5 x 6 = 5	20 x 20 = 20	35 x 35 = 35	54 x 54 = 54	65 x 65 = 65
Means		5	20	35	54	65
Inhibition or Stimulation as % of control		Nil	Nil	+6	-10	-21

Table 2B Effects of 4-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	18 x 22 = 20	32 x 46 = 39	60 x 64 = 62	80 x 80 = 80
	II	5 x 5 = 5	18 x 20 = 19	40 x 46 = 43	60 x 60 = 60	80 x 81 = 80
	III	5 x 5 = 5	20 x 24 = 22	36 x 38 = 37	61 x 62 = 61	81 x 83 = 82
Means		5	20	40	61	81
paired with *T.viride* 1	I	5 x 5 = 5	17 x 17 = 17	35 x 35 = 35	55 x 55 = 55	77 x 77 = 77
	II	5 x 5 = 5	17 x 17 = 17	33 x 37 = 35	55 x 57 = 56	77 x 77 = 77
	III	5 x 5 = 5	18 x 18 = 18	35 x 35 = 35	55 x 55 = 55	77 x 77 = 77
Means		5	17	35	55	77
Inhibition as % of control		Nil	-15	-13	-10	-5
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	20 x 20 = 20	35 x 35 = 35	58 x 58 = 58	79 x 79 = 79
	II	5 x 5 = 5	20 x 21 = 20	35 x 36 = 35	59 x 60 = 59	80 x 80 = 80
	III	5 x 5 = 5	20 x 20 = 20	35 x 35 = 35	58 x 58 = 58	79 x 79 = 79
Means		5	20	35	58	79
Inhibition as % of control		Nil	Nil	-13	-5	-2

Table 1C Effects of 6-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	6 x 7 = 6	20 x 22 = 21	35 x 35 = 35	63 x 63 = 63	85 x 85 = 85
	II	6 x 6 = 6	20 x 22 = 21	35 x 35 = 35	63 x 63 = 63	90 x 90 = 90
	III	5 x 6 = 5	20 x 20 = 20	35 x 36 = 35	60 x 61 = 60	80 x 80 = 80
Means		6	21	35	62	85
paired with *T.viride* 1	I	5 x 5 = 5	6 x 7 = 6	23 x 23 = 23	39 x 39 = 39	55 x 55 = 55
	II	5 x 5 = 5	8 x 8 = 8	22 x 22 = 22	40 x 41 = 40	55 x 55 = 55
	III	5 x 5 = 5	6 x 6 = 6	22 x 23 = 22	42 x 42 = 42	55 x 56 = 55
Means		5	7	22	40	55
Inhibition as % of control		-17	-67	-37	-35	-35
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	22 x 22 = 22	32 x 32 = 32	58 x 58 = 58	77 x 77 = 77
	II	5 x 5 = 5	19 x 20 = 19	29 x 29 = 29	53 x 53 = 53	75 x 75 = 75
	III	5 x 6 = 5	20 x 20 = 20	29 x 31 = 30	54 x 54 = 54	76 x 75 = 75
Means		5	20	30	55	76
Inhibition as % of control		-17	-5	-14	-11	-11

Table 2C Effects of 6-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	20 x 20 = 20	33 x 36 = 34	60 x 64 = 62	82 x 82 = 82
	II	5 x 5 = 5	20 x 20 = 20	40 x 43 = 41	60 x 60 = 60	80 x 80 = 80
	III	5 x 5 = 5	19 x 20 = 19	36 x 38 = 37	60 x 60 = 60	80 x 81 = 80
Means		5	20	37	61	81
paired with *T.viride* 1	I	5 x 5 = 5	10 x 10 = 10	20 x 20 = 20	34 x 34 = 34	60 x 60 = 60
	II	5 x 5 = 5	10 x 10 = 10	20 x 20 = 20	35 x 35 = 35	60 x 60 = 60
	III	5 x 5 = 5	10 x 10 = 10	20 x 20 = 20	34 x 34 = 34	60 x 60 = 60
Means		5	10	20	34	60
Inhibition as % of control		Nil	-50	-46	-44	-26
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	19 x 19 = 19	35 x 35 = 35	55 x 55 = 55	79 x 79 = 79
	II	5 x 5 = 5	20 x 21 = 20	35 x 36 = 35	55 x 55 = 55	81 x 82 = 81
	III	5 x 5 = 5	18 x 18 = 18	35 x 35 = 35	55 x 55 = 55	76 x 77 = 76
Means		5	19	35	55	79
Inhibition as % of control		Nil	-5	-5	-10	-2

Table 1D Effects of 8-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing 1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	25 x 26 = 25	36 x 36 = 36	66 x 66 = 66	83 x 83 = 83
	II	5 x 5 = 5	20 x 20 = 20	28 x 28 = 28	58 x 58 = 58	78 x 79 = 78
	III	5 x 5 = 5	20 x 20 = 20	28 x 28 = 28	59 x 59 = 59	79 x 79 = 79
Means		5	22	31	61	80
paired with *T.viride* 1	I	5 x 5 = 5	10 x 10 = 10	20 x 20 = 20	48 x 48 = 48	77 x 77 = 77
	II	5 x 5 = 5	10 x 10 = 10	20 x 21 = 20	47 x 48 = 47	73 x 76 = 74
	III	5 x 5 = 5	10 x 10 = 10	19 x 21 = 20	46 x 50 = 48	73 x 77 = 75
Means		5	10	20	48	75
Inhibition as % of control		Nil	-55	-35	-21	-6
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	11 x 11 = 11	20 x 20 = 20	47 x 47 = 47	80 x 80 = 80
	II	5 x 5 = 5	9 x 9 = 9	19 x 19 = 19	45 x 45 = 45	78 x 78 = 78
	III	5 x 5 = 5	13 x 13 = 13	20 x 23 = 21	46 x 52 = 49	80 x 90 = 85
Means		5	11	20	47	81
Inhibition or stimulation as % of control		Nil	-50	-35	-23	+1

Table 2D Effects of 8-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	6 x 7 = 6	20 x 20 = 20	36 x 38 = 37	60 x 60 = 60	80 x 84 = 82
	II	5 x 6 = 5	18 x 19 = 18	32 x 46 = 39	62 x 65 = 63	80 x 80 = 80
	III	5 x 6 = 5	20 x 21 = 20	36 x 40 = 38	60 x 60 = 60	80 x 81 = 80
Means		5	19	38	61	81
paired with *T.viride* 1	I	5 x 5 = 5	12 x 12 = 12	35 x 35 = 35	43 x 43 = 43	75 x 75 = 75
	II	5 x 5 = 5	12 x 12 = 12	35 x 35 = 35	44 x 44 = 44	75 x 75 = 75
	III	5 x 6 = 5	12 x 12 = 12	35 x 35 = 35	42 x 42 = 42	75 x 75 = 75
Means		5	12	35	43	75
Inhibition as % of control		Nil	-37	-8	-30	-7
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	19 x 20 = 19	35 x 35 = 35	52 x 52 = 52	75 x 75 = 75
	II	5 x 6 = 5	16 x 16 = 16	36 x 36 = 36	52 x 52 = 52	75 x 75 = 75
	III	5 x 6 = 5	16 x 18 = 17	35 x 35 = 35	52 x 52 = 52	75 x 75 = 75
Means		5	17	35	52	75
Inhibition as % of control		Nil	-11	-8	-15	-7

Table 1E Effects of 10-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	18 x 19 = 18	30 x 35 = 32	60 x 60 = 60	80 x 84 = 82
	II	5 x 5 = 5	20 x 20 = 20	36 x 38 = 37	64 x 64 = 64	87 x 87 = 87
	III	5 x 5 = 5	16 x 17 = 16	27 x 30 = 28	56 x 60 = 58	80 x 81 = 80
Means		5	18	32	61	83
paired with *T.viride* 1	I	5 x 5 = 5	16 x 17 = 16	30 x 30 = 30	55 x 55 = 55	85 x 86 = 85
	II	5 x 5 = 5	18 x 18 = 18	30 x 30 = 30	52 x 52 = 52	86 x 86 = 86
	III	5 x 5 = 5	13 x 16 = 14	30 x 30 = 30	50 x 50 = 50	85 x 85 = 85
Means		5	16	30	52	85
Inhibition or stimulation as % of control		Nil	-11	-6	-15	+2
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	10 x 10 = 10	30 x 30 = 30	55 x 55 = 55	73 x 73 = 73
	II	5 x 5 = 5	12 x 13 = 12	20 x 21 = 20	40 x 40 = 40	68 x 69 = 68
	III	5 x 5 = 5	16 x 16 = 16	22 x 26 = 24	48 x 48 = 48	70 x 71 = 70
Means		5	13	25	48	70
Inhibition as % of control		Nil	-28	-22	-21	-16

Table 2E Effects of 10-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 6 = 5	20 x 22 = 21	38 x 38 = 38	60 x 64 = 62	80 x 80 = 80
	II	5 x 5 = 5	19 x 19 = 19	36 x 36 = 36	60 x 60 = 60	80 x 80 = 80
	III	5 x 5 = 5	19 x 19 = 19	36 x 37 = 36	62 x 65 = 63	83 x 88 = 85
Means		5	20	37	62	82
paired with *T.viride* 1	I	5 x 5 = 5	12 x 12 = 12	34 x 34 = 34	56 x 56 = 56	80 x 80 = 80
	II	5 x 5 = 5	14 x 14 = 14	34 x 34 = 34	56 x 56 = 56	79 x 80 = 79
	III	5 x 5 = 5	12 x 13 = 12	35 x 35 = 35	54 x 58 = 56	80 x 80 = 80
Means		5	13	34	56	80
Inhibition as % of control		Nil	-35	-8	-10	-2
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	19 x 19 = 19	37 x 37 = 37	59 x 59 = 59	84 x 84 = 84
	II	5 x 5 = 5	17 x 17 = 17	34 x 36 = 35	56 x 60 = 58	86 x 86 = 86
	III	5 x 5 = 5	14 x 14 = 14	34 x 34 = 34	58 x 58 = 58	82 x 84 = 83
Means		5	17	35	58	84
Inhibition or stimulation as % of control		Nil	-15	-5	-6	+2

Table 1F Effects of 12-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	23 x 23 = 23	33 x 33 = 33	64 x 64 = 64	90 x 90 = 90
	II	5 x 5 = 5	20 x 26 = 23	30 x 34 = 32	60 x 64 = 62	80 x 84 = 82
	III	5 x 5 = 5	23 x 23 = 23	31 x 31 = 31	59 x 61 = 60	81 x 81 = 81
Means		5	23	32	62	84
paired with *T.viride* 1	I	5 x 5 = 5	25 x 25 = 25	35 x 35 = 35	65 x 65 = 65	85 x 86 = 85
	II	5 x 5 = 5	15 x 16 = 15	25 x 25 = 25	55 x 55 = 55	85 x 85 = 85
	III	5 x 5 = 5	20 x 20 = 20	30 x 30 = 30	58 x 58 = 58	85 x 85 = 85
Means		5	20	30	59	85
Inhibition or stimulation as % of control		Nil	-13	-6	-5	+1
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	20 x 21 = 20	26 x 27 = 26	58 x 58 = 58	85 x 85 = 85
	II	5 x 5 = 5	20 x 20 = 20	24 x 24 = 24	54 x 54 = 54	85 x 85 = 85
	III	5 x 5 = 5	20 x 20 = 20	24 x 26 = 25	56 x 56 = 56	85 x 85 = 85
Means		5	20	25	56	85
Inhibition or stimulation as % of control		Nil	-13	-22	-10	+1

Table 2F Effects of 12-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	20 x 20 = 20	36 x 36 = 36	60 x 60 = 60	82 x 82 = 82
	II	5 x 5 = 5	21 x 22 = 21	39 x 40 = 39	59 x 61 = 60	85 x 85 = 85
	III	5 x 5 = 5	22 x 22 = 22	38 x 38 = 38	61 x 61 = 61	81 x 82 = 81
Means		5	21	38	60	83
paired with *T.viride* 1	I	5 x 5 = 5	20 x 20 = 20	30 x 30 = 30	47 x 47 = 47	80 x 80 = 80
	II	5 x 5 = 5	20 x 20 = 20	37 x 37 = 37	50 x 50 = 50	80 x 84 = 82
	III	5 x 5 = 5	20 x 20 = 20	34 x 34 = 34	46 x 46 = 46	80 x 80 = 80
Means		5	20	34	48	81
Inhibition as % of control		Nil	-5	-11	-20	-2
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	25 x 25 = 25	42 x 42 = 42	70 x 70 = 70	88 x 88 = 88
	II	5 x 5 = 5	25 x 25 = 25	42 x 42 = 42	71 x 71 = 71	88 x 89 = 88
	III	5 x 5 = 5	25 x 25 = 25	40 x 40 = 40	70 x 70 = 70	86 x 87 = 86
Means		5	25	41	70	87
Stimulation as % of control		Nil	+19	+8	+17	+5

Table 1G Effects of 14-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing 1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	26 x 30 = 28	35 x 39 = 37	64 x 65 = 64	87 x 92 = 89
	II	5 x 5 = 5	30 x 30 = 30	38 x 38 = 38	65 x 65 = 65	89 x 89 = 89
	III	5 x 5 = 5	20 x 21 = 20	31 x 31 = 31	60 x 60 = 60	80 x 82 = 81
Means		5	26	35	63	86
paired with *T.viride* 1	I	5 x 5 = 5	19 x 20 = 19	35 x 35 = 35	50 x 50 = 50	80 x 81 = 80
	II	5 x 5 = 5	19 x 20 = 19	35 x 35 = 35	50 x 50 = 50	80 x 80 = 80
	III	5 x 5 = 5	19 x 19 = 19	33 x 38 = 35	47 x 54 = 50	78 x 83 = 80
Means		5	19	35	50	80
Inhibition as % of control		Nil	-27	Nil	-21	-7
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	22 x 22 = 22	36 x 37 = 36	56 x 55 = 55	85 x 85 = 85
	II	5 x 5 = 5	24 x 24 = 24	34 x 34 = 34	53 x 53 = 53	85 x 85 = 85
	III	5 x 5 = 5	20 x 20 = 20	34 x 36 = 35	53 x 53 = 53	86 x 85 = 85
Means		5	22	35	54	85
Inhibition as % of control		Nil	-15	Nil	-14	-1

Table 2G Effects of 14-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	19 x 21 = 20	37 x 37 = 37	60 x 60 = 60	80 x 80 = 80
	II	6 x 6 = 6	21 x 21 = 21	37 x 37 = 37	57 x 61 = 59	80 x 81 = 80
	III	5 x 6 = 5	20 x 20 = 20	35 x 37 = 36	60 x 62 = 61	82 x 83 = 82
Means		5	20	37	60	81
paired with *T.viride* 1	I	5 x 5 = 5	12 x 12 = 12	37 x 37 = 37	44 x 46 = 45	80 x 84 = 82
	II	5 x 5 = 5	9 x 10 = 9	28 x 28 = 28	40 x 41 = 40	74 x 76 = 75
	III	5 x 5 = 5	14 x 14 = 14	30 x 30 = 30	42 x 42 = 42	76 x 76 = 76
Means		5	12	32	42	78
Inhibition as % of control		Nil	-40	-14	-30	-4
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	18 x 18 = 18	40 x 42 = 41	62 x 62 = 62	90 x 90 = 90
	II	5 x 5 = 5	20 x 20 = 20	39 x 39 = 39	60 x 60 = 60	82 x 84 = 83
	III	5 x 5 = 5	19 x 19 = 19	38 x 38 = 38	60 x 60 = 60	82 x 84 = 83
Means		5	19	39	61	85
Inhibition or stimulation as % of control		Nil	-5	+5	+2	+5

Table 1H Effects of 16-day-old cultures of *Trichoderma viride* 1 and *T.longibranbhiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	20 x 21 = 20	32 x 35 = 33	60 x 65 = 62	94 x 94 = 94
	II	5 x 5 = 5	20 x 20 = 20	35 x 35 = 35	60 x 64 = 62	87 x 92 = 89
	III	5 x 5 = 5	20 x 20 = 20	30 x 30 = 30	60 x 60 = 60	86 x 86 = 86
Means		5	20	33	61	90
paired with *T.viride* 1	I	5 x 5 = 5	15 x 15 = 15	26 x 26 = 26	50 x 50 = 50	65 x 65 = 65
	II	5 x 5 = 5	15 x 15 = 15	23 x 27 = 25	47 x 52 = 49	64 x 67 = 65
	III	5 x 5 = 5	15 x 15 = 15	25 x 25 = 25	49 x 49 = 49	65 x 65 = 65
Means		5	15	25	49	65
Inhibition as % of control		Nil	-25	-24	-20	-28
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	20 x 20 = 20	30 x 31 = 30	56 x 56 = 56	86 x 86 = 86
	II	5 x 6 = 5	20 x 20 = 20	30 x 30 = 30	54 x 57 = 55	84 x 86 = 85
	III	5 x 5 = 5	20 x 20 = 20	30 x 30 = 30	54 x 54 = 54	85 x 85 = 85
Means		5	20	30	55	85
Inhibition as % of control		Nil	Nil	-9	-10	-6

Table 2H Effects of 16-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	20 x 20 = 20	38 x 38 = 38	60 x 60 = 60	80 x 80 = 80
	II	5 x 6 = 5	20 x 24 = 22	39 x 40 = 39	60 x 62 = 61	85 x 85 = 85
	III	5 x 5 = 5	20 x 21 = 20	38 x 38 = 38	62 x 63 = 62	80 x 80 = 80
Means		5	21	38	61	82
paired with *T.viride* 1	I	5 x 5 = 5	18 x 22 = 20	38 x 40 = 39	50 x 50 = 50	80 x 81 = 80
	II	5 x 5 = 5	16 x 16 = 16	32 x 32 = 32	50 x 50 = 50	80 x 80 = 80
	III	5 x 5 = 5	18 x 18 = 18	35 x 35 = 35	51 x 51 = 51	80 x 80 = 80
Means		5	18	35	50	80
Inhibition as % of control		Nil	-14	-8	-18	-2
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	22 x 25 = 23	34 x 36 = 35	60 x 64 = 62	83 x 83 = 83
	II	5 x 5 = 5	22 x 22 = 22	34 x 34 = 34	60 x 60 = 60	85 x 85 = 85
	III	5 x 5 = 5	20 x 20 = 20	35 x 35 = 35	59 x 59 = 59	80 x 80 = 80
Means		5	22	35	60	83
Inhibition or Stimulation as % of control		Nil	+5	-8	-2	+1

Table 1 I Effects of 18-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	12 x 12 = 12	32 x 32 = 32	59 x 60 = 59	80 x 83 = 81
	II	4 x 6 = 5	13 x 17 = 15	27 x 34 = 30	60 x 69 = 64	80 x 80 = 80
	III	5 x 5 = 5	10 x 10 = 10	20 x 20 = 20	56 x 60 = 58	75 x 75 = 75
Means		5	12	27	60	79
paired with *T.viride* 1	I	5 x 5 = 5	10 x 10 = 10	26 x 26 = 26	46 x 46 = 46	65 x 65 = 65
	II	5 x 5 = 5	15 x 15 = 15	28 x 28 = 28	48 x 48 = 48	66 x 66 = 66
	III	5 x 5 = 5	9 x 12 = 10	18 x 22 = 20	40 x 45 = 42	63 x 67 = 65
Means		5	12	25	45	65
Inhibition as % of control		Nil	Nil	-7	-25	-18
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	12 x 12 = 12	33 x 33 = 33	57 x 57 = 57	75 x 75 = 75
	II	5 x 5 = 5	10 x 10 = 10	30 x 30 = 30	56 x 56 = 56	76 x 76 = 76
	III	5 x 5 = 5	10 x 11 = 10	28 x 28 = 28	53 x 53 = 53	75 x 75 = 75
Means		5	11	30	55	75
Inhibition or stimulation as % of control		Nil	-8	+11	-8	-5

Table 2 I Effects of 18-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 5 = 5	18 x 18 = 18	35 x 35 = 35	60 x 61 = 60	80 x 82 = 81
	II	6 x 6 = 6	17 x 18 = 17	40 x 40 = 40	65 x 65 = 65	78 x 80 = 79
	III	5 x 5 = 5	19 x 19 = 19	38 x 39 = 38	60 x 61 = 60	81 x 81 = 81
Means		5	18	38	62	80
paired with *T.viride* 1	I	5 x 5 = 5	15 x 15 = 15	33 x 33 = 33	52 x 55 = 53	78 x 82 = 80
	II	5 x 5 = 5	16 x 16 = 16	28 x 32 = 30	50 x 50 = 50	75 x 75 = 75
	III	5 x 5 = 5	15 x 17 = 16	30 x 34 = 32	50 x 54 = 52	79 x 79 = 79
Means		5	16	32	52	78
Inhibition as % of control		Nil	-11	-16	-16	-3
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	17 x 17 = 17	35 x 37 = 36	59 x 65 = 62	82 x 86 = 84
	II	5 x 6 = 5	16 x 16 = 16	35 x 36 = 35	58 x 58 = 58	82 x 82 = 82
	III	5 x 5 = 5	16 x 16 = 16	35 x 35 = 35	59 x 59 = 59	84 x 84 = 84
Means		5	16	35	60	83
Inhibition or stimulation as % of control		Nil	-11	-8	-3	+4

Table 1J Effects of 20-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 6 = 5	12 x 12 = 12	32 x 32 = 32	62 x 62 = 62	85 x 85 = 85
	II	5 x 5 = 5	9 x 10 = 9	30 x 30 = 30	62 x 62 = 62	86 x 87 = 86
	III	5 x 6 = 5	10 x 10 = 10	20 x 25 = 22	58 x 62 = 60	82 x 89 = 85
Means		5	10	28	61	85
paired with *T.viride* 1	I	5 x 5 = 5	10 x 10 = 10	26 x 27 = 26	48 x 48 = 48	81 x 81 = 81
	II	5 x 5 = 5	10 x 10 = 10	25 x 25 = 25	44 x 44 = 44	76 x 76 = 76
	III	5 x 5 = 5	10 x 11 = 10	25 x 25 = 25	44 x 44 = 44	78 x 78 = 78
Means		5	10	25	45	78
Inhibition as % of control		Nil	Nil	-11	-26	-8
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	13 x 10 = 11	26 x 26 = 26	56 x 56 = 56	83 x 83 = 83
	II	5 x 5 = 5	12 x 10 = 11	26 x 26 = 26	55 x 55 = 55	83 x 83 = 83
	III	5 x 6 = 5	10 x 10 = 10	23 x 24 = 23	54 x 54 = 54	83 x 83 = 83
Means		5	11	25	55	83
Inhibition or stimulation as % of control		Nil	+10	-11	-10	-2

Table 2J Effects of 20-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576, on linear growth of *Rhizoctonia solani* in paired Petri dish assemblies

Treatment	Assembly No.	Individual and mean colony diameter (mm) measured at stated days after pairing				
		1	2	4	6	8
paired with uninoculated 2% malt agar (Control)	I	5 x 6 = 5	20 x 20 = 20	30 x 30 = 30	60 x 60 = 60	80 x 80 = 80
	II	5 x 5 = 5	19 x 19 = 19	34 x 34 = 34	60 x 60 = 60	86 x 86 = 86
	III	5 x 6 = 5	20 x 20 = 20	32 x 36 = 34	62 x 63 = 62	80 x 82 = 81
Means		5	20	33	61	82
paired with *T.viride* 1	I	5 x 5 = 5	18 x 18 = 18	30 x 30 = 30	51 x 51 = 51	72 x 72 = 72
	II	5 x 5 = 5	19 x 19 = 19	32 x 32 = 32	50 x 53 = 51	70 x 76 = 73
	III	5 x 5 = 5	18 x 18 = 18	30 x 30 = 30	49 x 49 = 49	72 x 72 = 72
Means		5	18	31	50	72
Inhibition as % of control		Nil	-10	-6	-18	-12
paired with *T.longibranchiatum* WBC 4576	I	5 x 5 = 5	20 x 20 = 20	30 x 30 = 30	54 x 54 = 54	85 x 87 = 86
	II	5 x 5 = 5	20 x 22 = 21	30 x 34 = 32	55 x 60 = 57	88 x 93 = 90
	III	5 x 5 = 5	20 x 22 = 21	29 x 29 = 29	54 x 54 = 54	80 x 80 = 80
Means		5	21	30	55	85
Inhibition or Stimulation as % of control		Nil	+5	-9	-10	+4

Table 3 Peak heights of volatile organic compounds identified in gases above 7-day-old pure cultures of two species of Trichoderma

Constituents	Peak heights (mm) at 7th day (a)																	
	T.viride 1									T.longibranchiatum WBC 4576								
	Expt. I bottle no.			Expt. II bottle no.			Expt. III bottle no.			Expt. I bottle no.			Expt. II bottle no.			Expt. III (b) bottle no.		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Ethylene	2	2	2	5	6	4	3	4	5	1	1	1	2	2	3	1	1	0
Ammonia	26	32	38	12	20	27	34	27	20	6	8	7	3	5	7	2	6	9
Acetaldehyde	36	49	23	20	41	62	22	31	39	20	23	27	12	20	27	18	26	34
Acetone	50	32	15	32	24	22	26	27	27	38	29	21	11	10	17	28	19	20
Ethyl acetate	1	2	4	7	11	12	0	1	2	1	1	2	2	1	0	2	3	4
Ethanol	78	126	156	206	153	99	110	130	149	68	57	46	54	60	72	60	54	48
n-propanol	2	2	2	2	3	4	3	2	2	1	1	1	1	3	3	2	1	1
Iso-butanol	5	5	4	2	3	4	3	4	6	1	1	1	2	1	1	1	2	3
Methanol	10	13	16	2	9	15	14	12	10	9	12	15	3	10	17	6	9	12
Carbon dioxide	40	38	36	40	44	47	48	39	30	21	26	31	30	25	20	26	28	30
Oxygen	70	63	65	60	64	68	70	65	61	70	70	75	72	74	76	70	79	73

(a) All peak heights were measured at attentuation 5 x 10^2 for first 8 compounds, at attentuation 50 x 1 for ethylene, and at $^1/_{32}$ for CO_2 and O_2 .

(b) Each experiment consisted of the examination of 3 replicate sealed Roux bottles for each fungus, as shown.

Table 4A Record of growth of *Rhizoctonia solani* colonies in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ethylene.

Ethylene (ml /l.air) used	Assembly No.	1st day * GLC peak height (mm)	Daily observations of colony diam.(mm) 1	2	3	4	5	6	7
Nil Control	I	6	30	50	77	104	120	135	160
	II	5	28	50	75	104	120	135	158
	III	2	29	50	74	103	118	131	155
Means		4	29	50	75	104	119	134	158
0.001	I	9	32	52	78	110	125	143	170
	II	8	29	52	75	106	125	140	167
	III	10	31	52	73	100	118	136	163
Means		9	31	52	75	105	123	140	167
Stimulation as % of control			+7	+4	Nil	+1	+3	+5	+6
0.01	I	360	30	52	77	104	120	137	162
	II	290	32	52	76	104	120	135	162
	III	322	29	50	74	106	124	139	164
Means		324	30	51	76	105	121	137	163
Stimulation as % of control			+3	+2	+1	+1	+2	+2	+3
0.1	I	3200	29	51	75	100	115	130	155
	II	3440	31	53	76	105	122	143	165
	III	2580	30	54	77	105	125	140	160
Means		3073	30	53	76	103	121	138	160
Inhibition or Stimulation as % of control			+3	+6	+1	-1	+2	+3	+1
1.0	I	37400	27	50	76	104	120	136	158
	II	37000	28	52	76	106	124	139	162
	III	36800	28	52	77	105	125	140	165
Means		37067	28	51	76	105	123	138	162
Inhibition or Stimulation as % of control			-3	+2	+1	+1	+3	+3	+3

*Attenuation was 50 x 1.

Table 4B Record of growth of Rhizoctonia solani colonies in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ammonia.

Ammonia (ml liq./l.air) used	Assembly No.	1st day GLC peak * height (mm)	Daily observations of colony diam.(mm) 1	2	3	4	5	6	7
Nil Control	I	0.0	25	46	68	97	135	165	190
	II	0.0	26	51	74	102	140	170	196
	III	0.0	27	53	74	103	138	170	197
Means		0.0	26	50	72	101	138	168	194
0.001	I	0.0	25	50	72	100	136	170	196
	II	0.0	26	51	74	103	139	171	198
	III	0.0	25	52	75	105	140	172	200
Means		0.0	25	51	74	103	138	171	198
Inhibition or Stimulation as % of control			-4	+2	+2	+2	Nil	+2	+2
0.01	I	1	27	54	77	105	139	172	190
	II	1	25	52	73	100	138	171	200
	III	1	27	55	80	110	142	176	205
Means		1	26	54	77	105	140	173	198
Stimulation as % of control			Nil	+7	+7	+4	+2	+3	+2
0.1	I	300	20	50	78	108	138	171	200
	II	295	5	48	66	100	135	168	189
	III	308	10	43	65	102	135	168	190
Means		301	12	47	70	103	136	169	193
Inhibition or Stimulation as % of control			-55	-6	-3	+2	-1	+1	-1
1.0	I	1050	5	5	5	5	5	5	5
	II	1170	5	5	5	5	5	5	5
	III	1130	5	5	5	5	5	5	5
Means		1120	5	5	5	5	5	5	5
Inhibition as % of control			-81	-90	-93	-95	-96	-97	-97

*Measurements were taken at attentuation 2 x 10^2

Table 4C Record of growth of Rhizoctonia solani colonies in test assemblies with known concentrations of authentic acetaldehyde.

Acetaldehyde (ml liq./l.air) used	Assembly No.	1st day col. diam. (mm)	1st day peak height (mm)	7th day col. diam. (mm)	7th day peak height (mm)	14th day col. diam. (mm)	14th day peak height (mm)
Nil Control	I	10	2	140	4	200	6
	II	8	1	131	8	180	3
	III	9	1	130	6	185	7
Means		9	1	134	6	188	5
0.001	I	11	60	133	38	180	15
	II	9	70	138	35	196	16
	III	9	78	135	35	182	20
Means		10	69	135	36	186	17
Stimulation or Inhibition as % of control		+11		+1		-1	
0.005	I	9	200	132	140	180	60
	II	8	168	133	130	175	50
	III	9	166	138	130	182	80
Means		9	178	134	133	179	53
Inhibition as % of control		Nil		Nil		-5	
0.01	I	6	300	130	260	176	100
	II	9	260	128	220	180	125
	III	7	290	131	250	175	110
Means		7	283	130	243	177	112
Inhibition as % of control		-22		-3		-6	

Table 4D Record of growth of *Rhizoctonia solani* colonies in test assemblies with known concentrations of authentic acetone.

Acetone (ml liq./l.air) used	Assembly No.	1st day col. diam. (mm)	1st day peak height (mm)	7th day col. diam. (mm)	7th day peak height (mm)	14th day col. diam. (mm)	14th day peak height (mm)
Nil Control	I	8	2	138	2	180	3
	II	8	1	130	2	177	2
	III	10	1	140	1	184	2
Means		9	1	136	2	180	2
0.001	I	8	60	126	30	184	10
	II	9	42	136	21	179	11
	III	12	68	142	35	190	18
Means		10	57	135	29	184	13
Stimulation or Inhibition as % of control		+11		-1		+2	
0.005	I	11	160	144	60	190	50
	II	9	182	135	108	188	93
	III	10	150	135	110	188	42
Means		10	164	138	93	189	62
Stimulation as % of control		+11		+1		+5	
0.01	I	8	300	140	160	190	80
	II	9	250	138	150	193	60
	III	9	280	138	142	184	92
Means		9	277	139	151	189	77
Stimulation as % of control		Nil		+2		+5	

Table 4E Record of growth of Rhizoctonia solani colonies in test assemblies with known concentrations of authentic ethanol.

Ethanol (ml liq./l.air) used.	Assembly No.	1st day col. diam. (mm)	1st day peak height (mm)	7th day col. diam. (mm)	7th day peak height (mm)	14th day col. diam. (mm)	14th day peak height (mm)
Nil (Control)	I	5	1	110	8	200	6
	II	7	2	109	8	190	8
	III	5	1	111	10	206	5
Means		6	1	110	9	199	6
0.03	I	6	70	109	50	189	30
	II	5	80	108	60	185	30
	III	6	72	108	50	186	26
Means		6	74	108	53	187	29
Inhibition as % of control		Nil		-2		-6	
0.1	I	5	220	99	180	167	140
	II	5	250	99	180	176	128
	III	5	244	95	186	188	145
Means		5	238	98	182	177	138
Inhibition as % of control		-17		-11		-11	
0.3	I	5	580	98	230	180	230
	II	5	520	95	220	170	200
	III	5	550	96	250	165	250
Means		5	550	96	233	172	227
Inhibition as % of control		-17		-13		-14	

Table 4F Record of growth of Rhizoctonia solani colonies in test assemblies with known concentrations of authentic carbon dioxide.

CO_2 vol./vol. of air space used	Assembly No.	1st day col. diam. (mm)	1st day peak height (mm)	7th day col. diam. (mm)	7th day peak height (mm)	14th day col. diam. (mm)	14th day peak height (mm)
Normal air (Control)	I	6	7	110	27	198	35
	II	7	8	115	29	202	36
	III	6	7	111	26	200	35
Means		6	7	112	27	200	35
10%	I	5	18	100	35	190	36
	II	5	20	112	38	200	36
	III	6	18	105	35	198	36
Means		5	19	106	36	196	36
Inhibition as % of control		-17		-5		-2	
20%	I	5	30	85	40	162	41
	II	5	34	82	40	160	42
	III	5	31	86	43	176	42
Means		5	32	84	41	166	42
Inhibition as % of control		-17		-25		-17	
30%	I	5	48	70	49	120	46
	II	5	51	60	48	110	47
	III	5	49	68	48	118	46
Means		5	49	66	48	116	46
Inhibition as % of control		-17		-41		-42	

Table 5A Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentration of 0.0005 ml liquid acetaldehyde / litre space of air and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
Treatment									
I	20	18	38	60	82	110	130	142	5
II	26	25	41	68	90	115	128	144	15
III	18	23	40	70	95	116	128	148	6
IV	14	23	40	68	88	115	127	150	6
V	22	20	40	67	88	120	132	155	10
Means	20	22	40	67	89	115	129	148	8
Control									
I	2	20	32	60	85	110	130	152	4
II	1	22	42	70	90	115	128	151	1
III	2	20	40	68	92	115	129	155	8
IV	2	15	40	68	94	115	130	152	5
V	2	22	41	67	98	120	132	153	4
Means	2	20	39	67	92	115	130	153	5
Inhibition or Stimulation as % of control		+10	+3	Nil	-3	Nil	-1	-3	

Table 5B Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentration of 0.001 ml liquid acetaldehyde / litre space of air and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day GLC peak height (mm)	Daily observation of colony diameter (mm)							7th day GLC peak height (mm)
	Acetaldehyde	1	2	3	4	5	6	7	Acetaldehyde
Treatment									
I	60	20	38	65	90	110	128	146	5
II	65	23	40	72	96	115	131	155	30
III	80	23	40	70	93	115	130	154	34
IV	48	23	40	72	95	116	130	154	35
V	46	22	40	73	95	116	131	155	5
Means	60	22	40	70	94	114	130	153	22
Control									
I	2	20	32	60	85	110	130	152	4
II	1	22	42	70	90	115	128	151	1
III	2	20	40	68	92	115	129	155	8
IV	2	15	40	68	94	115	130	152	5
V	2	22	41	67	98	120	132	153	4
Means	2	20	39	67	92	115	130	153	4
Inhibition or Stimulation as % of control		+10	+3	+6	+2	-1	Nil	Nil	

Table 5C Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentration of 0.005 ml liquid acetaldehyde / litre space of air and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
Treatment									
I	200	22	40	70	95	115	130	145	140
II	195	21	40	70	96	118	131	146	130
III	180	25	46	72	91	112	129	145	120
IV	168	26	46	70	90	118	138	150	120
V	166	26	47	74	97	122	140	156	115
Means	182	24	44	71	94	117	134	148	125
Control									
I	2	22	42	70	91	112	130	150	4
II	2	24	46	74	94	113	120	145	2
III	1	16	34	68	88	100	115	149	3
IV	1	28	49	68	90	110	125	151	5
V	6	25	46	68	90	110	125	150	4
Means	2	23	43	70	91	109	123	149	4
Inhibition or Stimulation as % of control		+4	+2	+2	+4	+7	+9	-1	

Table 5D Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentration 0.01 ml liquid acetaldehyde / litre space of air and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
Treatment									
I	300	8	20	49	60	85	100	115	260
II	250	9	22	50	62	90	110	119	250
III	290	10	30	55	66	91	110	118	240
IV	350	7	28	50	61	87	108	118	280
V	330	8	28	50	62	89	108	120	293
Means	304	8	26	51	62	88	107	118	264
Control									
I	2	8	22	49	65	86	108	122	10
II	1	6	25	52	70	90	108	120	2
III	1	10	31	55	72	92	110	120	2
IV	1	10	34	55	70	93	115	130	2
V	1	10	33	58	72	92	115	130	2
Means	1	9	29	54	70	91	111	124	4
Inhibition as % of control		-11	-12	-6	-11	-3	-4	-5	

Table 5E Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentration 0.05 ml liquid acetaldehyde / litre space of air. and with uninoculated 2% malt agar used as controls

Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
Treatment									
I	1108	5	5	5	5	5	6	6	1000
II	1080	5	5	5	5	6	6	7	980
III	1100	5	5	5	5	6	6	7	980
IV	1111	5	5	5	5	5	5	6	970
V	1120	5	5	5	5	5	5	5	1000
Means	1104	5	5	5	5	5	5	6	986
Control									
I	1	6	24	48	65	85	107	120	10
II	1	8	20	50	69	88	108	119	12
III	2	10	28	54	70	90	110	120	3
IV	1	9	25	53	73	91	120	122	2
V	1	10	25	55	74	95	112	125	1
Means	1	9	24	52	70	90	111	121	6
Inhibition as % of control		-44	-79	-90	-93	-94	-95	-95	

Table 5F Record growth of Rhizoctonia solani in test assemblies with known concentrations of authentic acetone

etone liq./l.air) sed	Assembly No.	1st day GLC peak height (mm) Acetone	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetone
Nil (Control)	I	1	8	29	48	65	85	107	120	1
	II	2	8	29	50	69	88	108	119	1
	III	1	10	30	54	70	90	110	120	1
	IV	1	9	25	53	71	90	112	125	2
	V	1	10	28	54	74	95	114	126	2
Means		1	9	28	52	70	90	110	123	1
0.0005	I	10	9	28	48	70	80	111	120	3
	II	22	7	24	44	65	80	115	120	10
	III	20	9	25	48	68	80	114	122	6
	IV	18	9	28	48	72	89	120	130	6
	V	16	14	36	52	78	95	120	132	7
Means		17	10	28	48	71	85	116	125	6
ibition or mulation as f control			+7	Nil	-8	+1	-6	+5	+2	
0.001	I	60	14	36	50	78	90	112	122	30
	II	48	8	28	48	74	88	110	123	30
	III	45	8	28	48	70	83	111	120	20
	IV	60	6	28	47	70	85	109	121	30
	V	52	10	30	52	80	89	112	125	22
Means		53	9	30	53	74	87	111	122	26
ibition or mulation as f control			Nil	+7	+2	+6	+3	+1	-1	
0.005	I	170	11	34	50	62	80	100	118	72
	II	169	9	33	46	62	80	105	120	70
	III	190	9	28	51	75	97	114	128	59
	IV	165	9	30	54	82	100	119	135	60
	V	172	10	31	58	87	105	118	135	77
Means		173	10	31	52	74	92	111	127	68
mulation as f control			+7	+11	Nil	+6	+2	+1	+3	

Table 5G Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentrations of 0.03 ml and of 0.1 ml liquid ethanol/litre space of air.

Ethanol (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Ethanol
	I	1	9	30	44	64	90	110	125	1
	II	1	10	34	53	74	100	120	135	1
Nil	III	2	10	32	51	70	98	120	133	1
(Control)	IV	2	10	33	50	70	97	117	130	1
	V	1	11	35	53	73	100	118	130	1
Means		1	10	33	50	70	97	117	131	1
	I	80	9	28	44	60	90	111	120	60
	II	70	10	32	49	68	96	112	130	22
0.03	III	70	13	36	55	75	100	120	130	48
	IV	92	9	28	48	72	100	116	130	34
	V	81	12	35	52	72	96	116	132	28
Means		79	11	32	50	69	96	115	128	38
Inhibition or stimulation as % of control			+6	-3	Nil	-1	-1	-2	-2	
	I	290	8	24	45	60	78	98	120	148
	II	270	10	34	50	70	92	109	130	145
0.1	III	268	12	32	50	69	94	114	130	149
	IV	170	10	32	48	70	98	118	134	138
	V	270	12	32	48	69	95	115	132	135
Means		254	10	31	48	68	91	111	129	143
Inhibition as % of control			Nil	-6	-4	-3	-6	-5	-2	

Table 5H Record of growth of Rhizoctonia solani colonies in test assemblies with initial concentrations of 0.3 and of 1.0 ml liquid ethanol/litre space of air.

ıanol liq./l.air) ed	Assembly No.	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Ethanol
	I	4	8	20	49	65	85	107	120	9
	II	1	6	20	51	69	90	107	119	8
Nil	III	1	10	25	53	70	90	110	120	9
Control)	IV	2	10	25	52	72	95	112	125	7
	V	1	10	25	55	75	92	111	126	9
Means		2	9	23	52	70	90	109	122	8
	I	557	9	23	56	70	89	109	121	340
	II	558	9	22	52	69	89	110	120	307
0.3	III	565	8	20	50	69	90	111	124	343
	IV	490	7	20	48	66	85	108	118	340
	V	561	7	19	48	66	85	107	118	340
Means		546	8	21	51	68	88	109	120	334
ıibition as of control			-11	-9	-2	-3	-2	Nil	-2	
	I	982	8	20	40	60	82	93	102	952
	II	977	8	20	40	60	79	90	105	948
1.0	III	974	8	20	42	62	80	95	106	953
	IV	985	9	20	50	65	82	94	106	853
	V	980	6	22	44	64	81	94	105	950
Means		980	8	20	43	62	81	93	105	931
ıibition as of control			-11	-13	-17	-11	-10	-15	-14	

Table 5I Record of growth of Rhizoctonia solani colonies in test assemblies with known concentrations of authentic carbon dioxide.

CO_2% vol./vol. of air space used	Assembly No.	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	GLC peak height (mm) CO_2 0	2	4	6
	I	7	28	45	72	101	125	147	0	0	0	0
Normal air	II	5	25	55	78	109	132	155	0	0	0	0
(Control)	III	7	28	51	74	105	126	146	0	0	0	0
	IV	7	26	46	73	101	121	143	0	0	0	0
	V	7	27	50	75	100	125	150	0	0	1	0
Means		7	27	49	74	103	126	148	0	0	0	0
	I	5	20	40	58	78	95	110	18	17	20	16
	II	5	18	40	57	78	96	112	18	19	20	20
10	III	6	22	42	60	79	96	111	18	19	20	20
	IV	6	18	36	56	72	94	108	18	19	20	20
	V	6	18	35	56	73	93	100	18	20	20	19
Means		6	19	39	57	76	95	108	18	19	20	19
Inhibition as % of control		-14	-30	-20	-23	-26	-25	-27				
	I	5	15	24	35	50	61	70	28	27	25	28
	II	5	15	24	34	50	62	74	27	25	22	27
20	III	5	16	26	35	50	64	74	23	25	22	27
	IV	5	14	20	32	50	62	72	28	28	26	28
	V	5	17	27	37	56	64	74	27	26	27	28
Means		5	15	24	35	51	63	73	27	26	24	28
Inhibition as % of control		-29	-44	-51	-53	-50	-50	-51				
	I	5	12	20	30	42	50	60	39	42	40	39
	II	5	12	21	30	43	54	62	41	44	40	38
25	III	5	11	20	29	39	50	58	42	44	40	38
	IV	6	14	25	35	44	58	64	38	42	40	37
	V	5	13	19	30	41	49	56	40	42	42	37
Means		5	12	21	31	42	52	60	40	43	40	38
Inhibition as % of control		-29	-56	-57	-58	-59	-59	-59				
	I	5	5	6	11	16	20	26	47	49	48	50
	II	5	5	6	11	16	22	29	46	50	41	48
30	III	5	5	7	12	16	22	28	46	45	42	46
	IV	5	5	6	13	16	22	26	45	45	45	40
	V	5	5	6	11	15	19	25	45	45	45	48
Means		5	5	6	12	16	21	27	46	47	44	46
Inhibition as % of control		-29	-81	-88	-84	-84	-83	-82				

Table 6 Record of growth of Rhizoctonia solani colonies in test assemblies with mixture of maximum concentrations of authentic metabolites identified in pure cultures of T.viride 1 and T.longibranchiatum WBC 4576 respectively.

Volatile comps. used	Ass. No.	1st day col. diam. (mm)	1st day GLC peak height mm (a) ETH.	(b) ACET.	(c) ETOH	CO_2	7th day col. diam. (mm)	7th day GLC peak height mm ETH.	ACET.	ETHO	CO_2	14th day col. diam. (mm)	14th day GLC peak height mm ETH.	ACET.	ETØH.	CO_2
Nil (Control)	I	8	0.0	2, 1	1	1	110	1	2, 1	8	25	200	1	5, 4	9	34
	II	10	0.0	1, 2	2	2	115	1	3, 1	8	24	190	1	6, 2	10	32
	III	7	0.0	1, 1	1	1	111	1	5, 2	6	22	209	1	6, 1	8	34
Means		8	0.0	1, 1	1	1	112	1	3, 1	7	24	200	1	6, 2	9	33
0.001 ml/l.ethylene +0.005 ml/l.ammonia +0.001 ml/l.acetaldehyde +0.001 ml/l.acetone +0.1 ml/l.ethanol + 30% v/v.CO2	I	5	6	70,20	220	48	38	7	40, 6	200	48	70	2	20, 2	150	46
	II	5	20	60,36	200	47	40	16	40, 17	160	45	75	17	12,16	160	45
	III	5	11	64,29	200	50	41	10	29, 12	168	45	77	8	18, 3	130	44
Means		5	12	65,28	207	48	40	11	36, 12	176	46	74	9	17, 7	147	45
Inhibition as % of control		-38					-64					-63				
0.001 ml/l.ethylene +0.001 ml/l.ammonia +0.001 ml/l.acetaldehyde +0.001 ml/l.acetone +0.03 ml/l.ethanol +20% v/v.CO2	I	6	5	20,16	70	34	70	2	30, 6	60	33	130	3	20, 2	38	30
	II	7	8	28,20	58	30	73	10	30, 2	30	32	125	3	6, 8	32	35
	III	5	6	19,12	74	34	70	3	21, 8	38	32	134	6	8, 5	32	35
Means		6	6	22,16	67	33	71	5	27, 5	43	32	130	4	11, 5	34	33
Inhibition as % of control		-25					-37					-35				

(a) ETH. =Ethylene

(b) ACET. = Acetaldehyde peak height followed by acetone

(c) ETØH. = Ethanol

Table 7A Record of growth of colonies of Rhizoctonia solani in test assemblies measured 1, 7 & 14 days after inoculation and pairing with 6-day -old cultures of T.viride 1 and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day			7th day			14th day		
	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2
Treatment									
I	5	20	6	70	50	40	110	260	50
II	15	35	7	70	50	45	100	250	55
III	10	25	6	60	90	38	140	200	55
IV	10	20	5	60	70	40	90	250	55
V	10	18	9	65	70	40	140	190	55
VI	10	29	4	60	60	40	100	230	55
Means	10	25	6	64	65	41	113	230	54
Control									
I	10	1	1	115	16	16	220	12	40
II	10	1	1	110	15	20	210	15	40
III	12	1	1	116	13	16	220	12	40
Means	11	1	1	114	15	17	217	13	40
Inhibition as % of control	-9			-44			-48		

Table 7B Record of growth of colonies of Rhizoctonia solani in test assemblies measured 1, 7 & 14 days after inoculation and pairing with 7-day -old cultures of T.viride 1 and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day			7th day			14th day		
	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2
Treatment									
I	9	30	10	67	230	35	105	220	45
II	8	40	12	70	230	40	105	225	45
III	10	30	10	62	240	40	65	270	50
IV	10	20	8	54	255	40	70	260	50
V	8	35	10	67	250	45	100	220	40
VI	10	30	10	67	235	40	70	280	40
Means	9	31	10	65	240	40	85	246	45
Control									
I	10	1	1	109	18	15	190	18	30
II	10	1	2	115	15	15	190	15	30
III	10	1	1	120	13	15	190	12	30
Means	10	1	1	115	15	15	190	15	30
Inhibition as % of control	-10			-43			-55		

Table 7C Record of growth of colonies of Rhizoctonia solani in test assemblies measured 1, 7 & 14 days after inoculation and pairing with 8-day -old cultures of T.viride 1 and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day			7th day			14th day		
	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2
Treatment									
I	10	25	5	72	50	40	115	130	45
II	5	35	5	70	40	40	105	135	45
III	10	30	8	75	50	45	115	125	50
IV	12	25	5	70	50	45	105	110	45
V	10	35	3	75	40	45	115	130	45
VI	11	31	5	76	40	40	120	120	50
Means	10	30	5	73	45	43	113	125	47
Control									
I	10	1	1	115	10	15	200	15	35
II	10	1	2	122	10	15	200	10	35
III	10	1	2	120	5	15	200	5	35
Means	10	1	1	119	8	15	200	10	35
Inhibition as % of control	Nil			-39			-44		

Table 7D Record of growth of colonies of Rhizoctonia solani in test assemblies measured 1, 7 & 14 days after inoculation and pairing with 6-day -old cultures of T.longibranchiatum WBC 4576 and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day			7th day			14th day		
	R.solani colony diam. (mm)	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm)	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm)	GLC peak height (mm) Ethanol	CO_2
Treatment									
I	10	25	5	160	16	24	190	28	25
II	10	25	5	150	16	26	190	28	25
III	9	30	10	110	24	40	160	35	43
IV	12	22	8	110	18	43	150	30	45
V	10	25	6	140	24	35	165	35	43
VI	10	24	6	100	22	44	120	40	40
Means	10	25	7	128	20	35	163	33	37
Control									
I	10	1	1	140	2	23	190	2	35
II	9	1	1	160	3	25	200	2	33
III	10	1	1	170	2	22	200	2	25
Means	10	1	1	157	2	23	197	2	31
Inhibition as % of control	Nil			-18			-17		

Table 7E Record of growth of colonies of Rhizoctonia solani in test assemblies measured 1, 7 & 14 days after inoculation and pairing with 7-day -old cultures of T.longibranchiatum WBC 4576, and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day			7th day			14th day		
	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC Peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2
Treatment									
I	10	20	7	110	40	42	150	100	42
II	12	20	7	125	45	35	160	100	42
III	10	15	10	120	45	34	150	100	44
IV	9	25	9	120	40	35	120	120	42
V	10	22	8	115	45	40	160	60	43
VI	10	20	8	130	44	32	160	65	40
Means	10	20	8	120	45	36	150	91	42
Control									
I	10	1	1	140	20	18	210	5	32
II	12	2	1	150	20	20	210	5	33
III	10	1	1	145	22	20	210	3	33
Means	11	1	1	145	21	19	210	4	33
Inhibition as % of control	-9			-17			-29		

Table 7F Record of growth of colonies of Rhizoctonia solani in test assemblies measured 1, 7 & 14 days after inoculation and pairing with 8-day -old cultures of T.longibranchiatum WBC 4576 and with uninoculated 2% malt agar used as controls.

Assembly No.	1st day			7th day			14th day		
	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2	R.solani colony diam. (mm) Mean	GLC peak height (mm) Ethanol	CO_2
Treatment									
I	9	35	8	115	40	35	130	70	40
II	12	30	6	105	40	35	130	70	40
III	10	30	8	120	33	33	190	55	35
IV	10	35	9	105	38	30	130	45	40
V	8	42	8	105	35	35	130	60	45
VI	8	40	8	100	38	30	130	68	40
Means	10	35	8	108	37	33	140	61	40
Control									
I	12	1	1	150	22	18	210	5	30
II	11	1	1	130	24	20	210	5	30
III	10	1	1	135	20	15	209	5	35
Means	11	1	1	138	22	18	210	5	32
Inhibition as % of control	-9			-22			-33		

Table 8A Record of growth of *Rhizoctonia solani* colonies paired with 7-day-old cultures of *Trichoderma viride* 1 and with uninoculated 2% malt agar used as controls

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily obs. of colony diam. (mm) 14	14th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2
paired	I	1	2	2	3	10	30	60	88	112	132	150	2	3	8	16	230	4	3	4	28
with un-	II	1	2	3	6	6	28	58	88	114	133	150	2	2	2	14	230	3	2	2	30
inoculated	III	2	2	3	8	8	30	62	90	112	135	151	2	2	2	18	230	2	2	3	30
2% malt	IV	1	3	4	2	6	29	56	87	112	130	145	3	2	2	17	230	3	4	3	30
agar	V	1	1	2	4	10	31	62	85	112	135	152	2	2	2	18	230	2	3	3	30
(Control)																					
Means		1	2	3	3	8	30	60	88	112	133	150	2	2	3	16	230	2	3	3	29
paired	I	15	49	135	29	8	20	34	39	44	50	53	15	5	160	40	67	5	5	175	36
with	II	16	29	35	29	6	27	32	36	42	48	50	15	5	65	39	66	4	6	130	36
T. viride 1	III	14	23	34	29	7	18	31	37	42	47	50	15	5	90	39	69	4	3	85	36
	IV	58	4	265	38	5	10	24	34	37	45	50	8	3	185	44	60	10	5	130	38
	V	66	2	35	34	5	16	29	35	38	45	49	8	7	110	43	57	9	7	188	36
Means		34	21	101	32	6	18	30	36	40	47	50	12	5	122	41	64	6	5	142	36
Inhibition as % of control						-25	-40	-50	-59	-64	-64	-67					-72				

Table 8B Record of growth of *Rhizoctonia solani* colonies paired with 7-day-old cultures of *Trichoderma longibranchiatum* WBC 4576 and with uninoculated 2% malt agar used as controls

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily obs. of colony diam.(mm) 14	14th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2
paired	I	1	1	3	1	10	28	54	70	90	110	130	2	3	3	15	230	2	2	2	25
with un-	II	1	2	2	1	10	28	54	72	100	115	135	3	2	2	15	230	2	3	3	25
inoculated	III	2	1	1	1	9	30	50	70	100	115	136	2	2	2	17	230	2	2	4	28
2% malt agar	IV	1	1	1	1	9	31	54	73	105	120	140	2	2	2	16	230	2	2	2	28
(Control)	V	1	2	1	1	10	31	55	72	106	120	138	1	2	3	15	230	2	2	3	26
Means		1	1	2	1	10	30	53	71	100	116	136	2	2	2	15	230	2	2	3	26
paired with	I	9	2	55	23	10	22	40	53	62	76	92	3	3	53	33	116	2	2	75	32
T.longibran-	II	9	23	85	23	10	24	42	54	62	77	93	2	3	82	31	116	2	2	78	30
chiatum	III	12	44	25	24	6	19	40	52	61	75	91	2	3	62	32	114	4	2	107	31
WBC 4576	IV	12	3	27	24	7	22	42	53	62	76	94	3	2	56	30	111	4	2	96	31
	V	2	13	25	29	7	12	36	53	63	77	94	5	2	52	30	114	2	2	81	31
Means		9	17	43	25	8	20	40	53	62	76	93	3	3	61	31	114	3	2	87	31
Inhibition as % of control						-20	-33	-25	-25	- 38	-34	-32					-50				

Table 9A Record of growth of Pyronema domesticum colonies in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ethylene.

C_2H_4 (ml /l.air) used	Assembly No.	1st day * GLC peak height (mm) Ethylene	Daily observations of colony diameter (mm) 1	2	3	4
Nil (Control)	I	1	20	105	220	230
	II	2	26	140	230	230
	III	1	30	145	230	230
Means		2	25	130	227	230
0.001	I	38	32	140	230	230
	II	82	30	135	230	230
	III	58	23	125	230	230
Means		59	28	133	230	230
Stimulation as % of control			+12	+2	+1	Nil
0.01	I	190	34	150	230	230
	II	182	30	135	225	230
	III	204	28	145	230	230
Means		192	31	143	228	230
Stimulation as % of control			+21	+10	+1	Nil
0.1	I	2000	23	105	220	230
	II	3480	32	150	230	230
	III	3080	30	155	230	230
Means		2853	28	137	227	230
Stimulation as % of control			+12	+5	Nil	Nil
1.0	I	20000	33	165	230	230
	II	24000	24	129	230	230
	III	21200	25	130	230	230
Means		21733	27	141	230	230
Stimulation as % of control			+8	+8	+1	Nil

* Attenuation used was 50 x 1.

Table 9B Record of growth of Pyronema domesticum in test assemblies with known concentrations of authentic acetaldehyde.

Acetaldehyde (ml liq/l.air) used	Assembly No	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4*	5	6	7	7th day GLC peak height (mm) Acetaldehyde
	I	1	38	120	190	230	230	230	230	2
Nil	II	2	38	125	200	230	230	230	230	3
(Control)	III	3	38	124	200	230	230	230	230	2
	IV	2	33	120	200	230	230	230	230	2
	V	2	35	130	210	230	230	230	230	1
Means		2	36	124	200	230	230	230	230	2
	I	20	26	90	230	230	230	230	230	6
	II	30	18	82	220	230	230	230	230	12
0.0005	III	22	30	91	230	230	230	230	230	7
	IV	25	33	98	230	230	230	230	230	3
	V	24	31	100	230	230	230	230	230	6
Means		24	28	92	228	230	230	230	230	7
Inhibition or Stimulation as % of control			-22	-26	+14	Nil	Nil	Nil	Nil	
	I	20	20	75	180	200	230	230	230	2
	II	35	25	80	180	200	230	230	230	12
0.001	III	25	18	75	170	200	230	230	230	12
	IV	30	20	70	170	200	230	230	230	12
	V	48	25	80	180	200	230	230	230	5
Means		31	22	76	176	200	230	230	230	9
Inhibition as % of control			-39	-39	-12	-13	Nil	Nil	Nil	
	I	95	15	30	70	140	220	230	230	22
	II	150	16	25	60	125	200	230	230	49
0.005	III	95	15	30	75	140	220	230	230	32
	IV	90	15	30	75	140	220	230	230	23
	V	95	15	30	75	140	210	230	230	22
Means		105	15	29	71	137	214	230	230	30
Inhibition as % of control			-58	-77	-65	-40	-7	Nil	Nil	
	I	236	12	15	48	100	140	200	230	200
	II	189	15	18	52	110	150	210	230	170
0.01	III	189	15	19	52	110	150	200	230	180
	IV	228	14	20	65	115	160	220	230	200
	V	232	15	20	55	110	160	220	230	200
Means		215	14	18	54	109	152	210	230	190
Inhibition as % of control			-61	-85	-73	-53	-34	-9	Nil	
	I	550	5	5	5	5	5	5	5	340
	II	550	5	5	5	5	5	5	5	440
0.05	III	601	5	5	5	5	5	5	5	580
	IV	600	5	5	5	5	5	5	5	550
	V	544	5	5	5	5	5	5	5	500
Means		569	5	5	5	5	5	5	5	482
Inhibition as % of control			-86	-96	-98	-98	-98	-98	-98	

*Agar surface completely covered with the mycelium

Table 9C Record of growth of Pyronema domesticum in test assemblies with known concentrations of authentic acetone.

Acetone (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetone	Daily observation of colony diameter (mm) 1	2	3	4	5	7th day GLC peak height (mm) Acetone
Nil (Control)	I	1	26	150	200	220	230	1
	II	2	30	160	200	230	230	1
	III	1	30	160	200	230	230	2
	IV	2	30	160	205	230	230	1
	V	1	32	160	200	220	230	2
Means		1	30	158	201	226	230	1
0.0005	I	12	37	170	200	230	230	4
	II	20	26	162	195	230	230	10
	III	22	30	173	202	230	230	10
	IV	26	28	160	193	230	230	6
	V	24	32	166	198	230	230	7
Means		21	31	166	198	230	230	7
Inhibition or Stimulation as % of control			+3	+5	-1	+2	Nil	
0.001	I	40	40	172	200	230	230	15
	II	30	29	170	200	230	230	20
	III	48	28	160	190	230	230	28
	IV	44	37	166	200	230	230	30
	V	46	24	160	200	230	230	26
Means		42	32	166	198	230	230	24
Inhibition or Stimulation as % of control			+7	+5	-1	+2	Nil	
0.005	I	136	30	160	190	230	230	90
	II	86	30	165	200	230	230	62
	III	85	35	170	200	230	230	72
	IV	86	38	170	200	230	230	80
	V	80	25	150	190	230	230	30
Means		95	32	163	196	230	230	67
Inhibition or Stimulation as % of control			+7	+3	-2	+2	Nil	

Table 9D Record of growth of *Pyronema domesticum* in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq/l.air) used	Assembly No	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	7th day GLC peak height (mm) Ethanol
	I	2	26	150	200	220	230	2
	II	1	30	160	200	230	230	1
Nil	III	2	30	160	200	230	230	1
(Control)	IV	2	30	160	205	230	230	4
	V	1	32	160	200	220	230	2
Means		2	30	158	201	226	230	2
	I	62	30	120	190	230	230	33
	II	77	36	140	200	230	230	32
0.03	III	70	37	138	200	230	230	40
	IV	68	29	130	180	230	230	28
	V	66	28	135	200	230	230	22
Means		69	32	133	194	230	230	31
Inhibition or Stimulation as % of control			+7	-16	-3	+2	Nil	
	I	85	32	120	195	230	230	70
	II	85	33	120	200	230	230	65
0.1	III	100	32	120	200	230	230	90
	IV	80	32	118	195	230	230	60
	V	100	32	120	200	230	230	85
Means		90	32	120	198	230	230	74
Inhibition or Stimulation as % of control			+7	-24	-1	+2	Nil	
	I	500	29	100	180	220	230	380
	II	600	29	100	180	230	230	265
0.3	III	600	29	100	180	220	230	265
	IV	560	34	105	195	230	230	260
	V	565	34	105	195	230	230	260
Means		565	31	102	187	226	230	274
Inhibition or Stimulation as % of control			+3	-35	-7	Nil	Nil	
	I	1345	28	80	170	225	230	1100
	II	1290	28	80	170	225	230	960
1.0	III	1480	30	86	172	230	230	961
	IV	1440	26	75	168	220	230	962
	V	1490	29	80	171	220	230	958
Means		1409	28	80	170	224	230	988
Inhibition as % of control			-7	-49	-15	-1	Nil	

Table 9E Record of growth of Pyronema domesticum colonies in test assemblies with known concentrations of authentic carbon dioxide.

CO_2 % (vol./vol.) used	Assembly No.	Daily observation of colony diameter. (mm) 1	2	3	4	5	6	7	GLC peak height (mm) CO_2 0	2	4	6
	I	88	220	230	230	230	230	230	0	0	0	1
Normal air	II	80	220	230	230	230	230	230	0	1	1	0
(control)	III	90	230	230	230	230	230	230	0	1	1	1
	IV	92	230	230	230	230	230	230	0	1	0	0
	V	90	230	230	230	230	230	230	0	1	0	0
Means		88	226	230	230	230	230	230	0	1	0	0
	I	65	165	230	230	230	230	230	18	20	22	20
	II	65	165	230	230	230	230	230	18	20	20	20
10	III	62	162	230	230	230	230	230	19	20	23	19
	IV	62	162	230	230	230	230	230	18	20	23	20
	V	62	162	230	230	230	230	230	18	20	23	20
Means		63	163	230	230	230	230	230	18	20	22	20
Inhibition as % of control		-28	-28	Nil	Nil	Nil	Nil	Nil				
	I	43	112	150	180	200	230	230	28	26	32	31
	II	43	115	152	182	209	230	230	28	26	31	31
20	III	40	116	153	185	208	230	230	28	20	30	31
	IV	42	111	152	182	200	230	230	28	26	32	32
	V	45	111	150	180	200	230	230	28	26	32	31
Means		43	113	151	182	203	230	230	28	25	31	31
Inhibition as % of control		-51	-50	-34	-21	-12	Nil	Nil				
	I	15	35	55	70	80	88	93	48	45	50	50
	II	16	35	56	70	80	88	93	48	43	50	52
30	III	16	37	56	72	82	90	93	47	45	49	50
	IV	15	37	56	72	82	91	95	47	45	49	49
	V	16	37	56	72	82	89	93	47	45	49	49
Means		16	36	56	71	81	89	93	47	45	49	50
Inhibition as % of control		-82	-84	-76	-69	-65	-61	-60				

Table 9F Record of growth of colonies of Pythium ultimum in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ethylene

C_2H_4 (ml/l.air) used	Assembly No.	1st day * GLC peak height (mm)	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7
Nil (Control)	I	5	35	80	124	150	155	160	165
	II	2	40	83	123	150	157	164	167
	III	2	50	90	127	154	159	165	169
Means		3	42	84	124	151	157	163	167
0.001	I	90	44	89	125	154	160	163	167
	II	86	43	89	123	151	158	162	167
	III	86	40	82	117	152	159	168	169
Means		87	42	87	122	152	159	164	168
Inhibition or Stimulation as % of control			Nil	+4	-2	+1	+1	+1	+1
0.01	I	124	45	85	125	155	159	163	167
	II	128	47	88	127	157	159	168	172
	III	123	43	86	126	154	159	166	170
Means		125	45	86	126	155	159	166	170
Inhibition as % of control			+7	+2	+2	+3	+1	+2	+2
0.1	I	2120	40	80	118	150	155	159	166
	II	2660	43	86	123	152	156	160	166
	III	2380	43	81	124	155	160	160	165
Means		2387	42	82	122	152	157	160	166
Inhibition or Stimulation as % of control			Nil	-2	-2	+1	Nil	-2	-1
1.0	I	27000	46	88	128	156	161	165	169
	II	28000	41	82	120	150	160	164	168
	III	29200	40	82	121	149	158	165	169
Means		28067	42	84	123	152	160	165	169
Inhibition or Stimulation as % of control			Nil	Nil	-1	+1	+2	+1	+1

* Attenuation used was 50 x 1.

Table 9G Record of growth of *Pythium ultimum* in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
Nil (Control)	I	2	41	86	120	156	175	188	220	2
	II	1	40	85	119	155	175	190	220	2
	III	1	45	92	124	160	180	195	230	2
	IV	2	45	92	125	160	180	195	230	2
	V	1	39	85	120	155	176	190	220	2
Means		1	42	88	122	157	177	192	224	2
0.0005	I	20	44	100	120	149	165	175	220	10
	II	25	39	101	120	150	168	180	225	12
	III	17	48	108	130	166	179	198	230	12
	IV	10	45	99	118	148	165	180	222	6
	V	28	45	98	120	157	177	200	220	6
Means		20	44	101	122	154	171	187	223	9
Inhibition or Stimulation as % of control			+5	+15	Nil	-2	-3	-3	Nil	
0.001	I	70	45	110	130	155	165	175	182	41
	II	60	42	110	140	165	175	186	192	39
	III	65	44	100	130	160	172	185	195	22
	IV	65	40	93	124	155	166	175	183	33
	V	80	49	110	140	162	172	186	194	36
Means		68	44	105	133	159	170	181	189	34
Inhibition or Stimulation as % of control			+5	+19	+9	+1	-4	-5	-16	
0.005	I	145	50	100	129	160	172	183	190	112
	II	150	45	98	125	160	173	185	192	102
	III	165	50	102	130	160	171	182	190	104
	IV	149	49	100	128	159	170	180	189	112
	V	190	35	88	110	145	165	175	186	122
Means		160	46	98	124	157	170	181	189	110
Inhibition or Stimulation as % of control			+10	+11	+2	Nil	-4	-6	-16	
0.01	I	335	28	63	105	155	179	190	206	228
	II	332	29	68	114	160	180	198	210	220
	III	332	29	68	115	160	180	199	210	220
	IV	329	30	68	114	160	181	200	210	140
	V	360	28	62	106	154	179	190	205	240
Means		338	29	66	111	158	180	195	208	210
Inhibition or Stimulation as % of control			-31	-25	-9	Nil	-2	+2	-7	
0.05	I	1000	22	45	60	92	141	160	180	860
	II	990	24	46	62	94	141	162	180	800
	III	900	24	46	62	94	141	162	182	800
	IV	850	25	47	65	96	142	165	184	750
	V	900	25	46	65	96	142	166	185	740
Means		928	24	46	63	94	141	163	182	790
Inhibition as % of control			-43	-48	-48	-40	-20	-15	-19	

Table 9H Record of growth of Pythium ultimum in test assemblies with known concentrations of authentic acetone

Acetone (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetone	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetone
	I	2	44	90	120	138	161	183	196	1
	II	2	43	88	120	138	162	185	198	2
Nil	III	1	42	88	119	138	162	185	198	2
(Control)	IV	2	38	86	116	134	158	180	195	2
	V	2	39	87	116	135	160	184	196	2
Means		2	41	88	118	137	161	183	197	2
	I	20	44	92	121	143	164	186	196	10
	II	25	42	91	122	144	164	182	195	10
0.0005	III	10	40	88	120	143	164	182	194	7
	IV	30	42	90	120	138	160	179	190	6
	V	31	45	95	125	150	171	188	200	16
Means		23	43	91	122	144	165	183	195	10
Inhibition or Stimulation as % of control			+5	+3	+3	+5	+2	Nil	-1	
	I	40	46	98	120	138	166	186	196	20
	II	55	39	90	111	122	160	180	192	20
0.001	III	38	44	92	112	122	160	179	192	20
	IV	59	47	98	118	135	166	188	196	33
	V	50	48	98	121	135	168	188	200	12
Means		48	45	95	116	130	164	184	195	21
Inhibition or Stimulation as % of control			+10	+8	-2	-5	+2	Nil	-1	
	I	120	42	91	122	140	160	180	195	40
	II	100	40	88	120	140	160	180	194	50
0.005	III	140	44	90	120	140	160	181	195	40
	IV	100	42	90	120	141	162	184	198	40
	V	90	45	95	125	145	164	185	200	22
Means		110	43	91	121	141	161	182	196	38
Inhibition or Stimulation as % of control			+5	+3	+3	+3	Nil	-1	Nil	

Table 9I Record of growth of Pythium ultimum in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Ethanol
	I	1	44	90	120	138	161	183	196	1
	II	4	43	88	120	138	162	185	198	1
Nil	III	2	42	88	119	138	162	185	198	2
(Control)	IV	1	38	86	116	134	158	182	195	1
	V	1	39	87	116	135	160	184	196	1
Means		2	41	88	118	137	161	184	197	1
	I	60	40	85	118	136	160	180	198	30
	II	70	39	85	118	138	161	180	193	30
0.03	III	80	41	86	118	138	165	188	200	30
	IV	58	41	83	116	137	160	179	193	12
	V	50	37	82	116	139	159	182	197	33
Means		64	40	84	117	138	161	182	196	27
Inhibition or Stimulation as % of control			-2	-5	-1	+1	Nil	-1	-1	
	I	200	40	84	118	136	160	180	191	80
	II	220	38	85	119	138	161	180	190	95
0.1	III	200	40	85	120	138	162	180	191	70
	IV	230	37	82	117	136	160	179	188	80
	V	290	41	86	120	138	161	180	190	75
Means		208	39	84	119	137	161	180	190	80
Inhibition or Stimulation as % of control			-5	-5	+1	Nil	Nil	-2	-4	
	I	552	38	80	110	130	156	177	187	300
	II	554	40	81	110	131	158	178	188	280
0.3	III	520	43	82	110	131	158	178	188	300
	IV	540	42	81	110	130	157	172	185	300
	V	510	43	85	115	135	160	180	190	290
Means		537	41	82	111	131	158	177	188	294
Inhibition as % of control			Nil	-7	-6	-4	-2	-4	-5	
	I	900	38	73	95	118	140	164	175	800
	II	1100	35	70	93	116	138	160	172	900
1.0	III	1050	35	70	93	116	138	161	174	820
	IV	1000	38	73	95	119	140	164	175	900
	V	999	38	71	92	116	128	161	173	800
Means		1010	37	71	94	117	139	162	174	844
Inhibition as % of control			-10	-19	-20	-14	-14	-12	-12	

Table 9J Record of growth of *Pythium ultimum* in test assemblies with known concentrations of authentic carbon dioxide

CO_2 % (vol./vol.) used	Assembly No.	Daily observation of colony diam. (mm) 1	2	3	4	5	6	7	GLC peak height (mm) CO_2 0	2	4	6
	I	46	130	143	180	205	220	230	0	0	0	0
	II	46	128	142	180	205	220	230	0	0	0	0
Normal air	III	47	128	142	180	205	220	230	0	0	1	0
(Control)	IV	45	128	142	181	206	230	230	0	0	1	0
	V	45	128	142	181	208	230	230	0	0	1	0
Means		46	128	142	180	206	224	230	0	0	1	0
	I	38	110	121	150	180	200	230	18	24	20	22
	II	38	106	121	150	180	200	230	18	24	20	22
10	III	38	106	121	150	180	200	230	18	29	20	22
	IV	40	110	123	151	180	203	230	18	30	20	22
	V	39	105	122	150	180	200	230	18	30	20	22
Means		39	107	122	150	180	201	230	18	27	20	22
Inhibition as % of control		-15	-16	-14	-17	-13	-13	Nil				
	I	27	87	102	127	150	162	172	26	30	30	30
	II	29	89	102	127	150	162	172	26	32	30	30
20	III	28	90	102	128	150	163	172	26	32	30	31
	IV	29	92	102	128	150	163	172	24	32	30	28
	V	24	90	107	126	148	162	172	28	32	30	28
Means		27	90	103	127	150	162	172	26	32	30	29
Inhibition as % of control		-41	-30	-28	-30	-27	-28	-25				
	I	22	66	75	88	110	121	134	47	47	51	50
	II	24	70	80	90	112	122	130	47	47	50	50
30	III	22	67	75	88	110	122	130	49	47	51	50
	IV	22	67	75	88	111	121	135	48	46	50	50
	V	22	67	75	88	111	123	130	50	47	50	50
Means		22	67	76	88	111	122	132	48	47	50	50
Inhibition as % of control		-52	-48	-47	-51	-46	-46	-43				

Table 9K Record of growth of colonies of Fomes annosus in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ethylene

C_2H_4 (ml /l.air) used	Assembly No.	1st day* GLC peak height (mm)	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7
Nil (Control)	I	2	5	10	16	23	28	32	38
	II	3	5	9	14	23	29	32	42
	III	4	5	11	16	23	28	32	38
Means		3	5	10	15	23	28	32	39
0.001	I	110	5	10	16	21	29	32	38
	II	122	5	9	16	21	28	30	40
	III	143	6	10	16	22	30	31	38
Means		125	5	10	16	21	29	31	39
Inhibition or Stimulation as % of control			Nil	Nil	+7	-9	+4	-3	Nil
0.01	I	480	5	11	16	25	31	38	42
	II	500	5	9	16	22	28	34	40
	III	444	5	11	18	24	28	33	38
Means		475	5	10	17	24	29	35	40
Stimulation as % of control			Nil	Nil	+13	+4	+4	+9	+2
0.1	I	3700	5	10	15	22	28	34	42
	II	3400	5	12	18	24	29	34	42
	III	3440	6	10	17	23	29	35	42
Means		3513	5	11	17	23	29	34	42
Stimulation as % of control			Nil	+10	+13	Nil	+4	+7	+8
1.0	I	39000	5	9	16	21	27	32	40
	II	41000	5	10	16	22	29	31	40
	III	41800	5	9	15	22	28	32	41
Means		40600	5	9	16	22	28	32	40
Inhibition or Stimulation as % of control			Nil	-10	+7	-4	Nil	Nil	+3

*Attenuation used was 50 x 1

Table 9L Record of growth of *Fomes annosus* in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
	I	2	5	6	10	15	20	23	33	2
Nil	II	2	5	5	6	14	16	20	25	2
(Control)	III	2	5	5	8	15	16	21	26	2
	IV	3	5	6	8	15	17	23	30	2
	V	2	5	6	9	14	20	24	29	2
Means		2	5	6	8	15	18	22	29	2
	I	25	5	6	8	16	20	25	30	20
	II	20	5	6	8	16	20	23	28	20
0.0005	III	16	5	6	9	13	16	22	28	10
	IV	5	5	8	10	16	20	26	33	1
	V	24	5	6	8	15	20	24	30	9
Means		18	5	6	9	15	19	24	30	12
Stimulation as % of control			Nil	Nil	+13	Nil	+6	+8	+3	
	I	25	5	6	8	15	19	24	29	25
	II	55	5	6	8	15	20	24	29	25
0.001	III	60	5	6	7	12	17	21	26	42
	IV	60	5	6	7	12	18	20	26	48
	V	80	5	6	8	16	21	23	29	22
Means		56	5	6	8	14	19	22	28	32
Inhibition or Stimulation as % of control			Nil	Nil	Nil	-7	+6	Nil	-3	
	I	185	5	5	6	12	16	24	30	65
	II	145	5	5	8	14	18	25	30	24
0.005	III	175	5	5	7	12	17	22	27	40
	IV	120	5	6	8	12	16	23	30	36
	V	175	5	5	7	12	16	23	27	30
Means		160	5	5	7	12	17	23	29	39
Inhibition or Stimulation as % of control			Nil	-17	-13	-20	-6	+5	Nil	
	I	283	5	5	6	14	20	22	26	130
	II	285	5	5	6	14	20	21	25	130
0.01	III	295	5	5	5	10	14	19	25	130
	IV	280	5	5	8	16	20	23	25	125
	V	288	5	5	8	15	18	21	25	125
Means		286	5	5	7	14	18	21	25	128
Inhibition as % of control			Nil	-17	-13	-7	Nil	-5	-14	
	I	705	5	5	5	5	5	9	15	620
	II	900	5	5	5	5	5	8	15	700
0.05	III	810	5	5	5	5	5	6	12	709
	IV	1022	5	5	5	5	5	5	10	715
	V	910	5	5	5	5	5	6	14	709
Means		869	5	5	5	5	5	7	13	691
Inhibition as % of control			Nil	-17	-38	-67	-72	-68	-55	

Table 9M Record of growth of Fomes annosus in test assemblies with known concentrations of authentic acetone

Acetone (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetone	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetone
Nil (Control)	I	1	5	8	16	20	24	28	32	3
	II	2	5	9	15	21	25	28	32	2
	III	1	5	8	12	20	23	27	32	2
	IV	1	5	9	16	20	24	29	33	2
	V	1	5	7	13	19	23	27	30	3
Means		1	5	8	15	20	24	28	32	2
0.0005	I	14	5	8	13	18	20	27	30	10
	II	30	6	10	16	21	23	30	33	19
	III	12	6	11	16	22	25	31	36	9
	IV	13	5	8	14	20	23	29	31	8
	V	18	5	7	14	21	23	29	32	8
Means		17	5	9	15	20	23	29	32	11
Inhibition or Stimulation as % of control			Nil	+13	Nil	Nil	-4	+4	Nil	
0.001	I	60	7	10	15	20	25	28	31	20
	II	55	10	13	17	24	28	32	35	30
	III	30	5	8	14	20	25	29	31	20
	IV	50	5	8	14	18	22	26	30	22
	V	48	6	8	15	20	24	30	32	24
Means		49	7	9	15	20	25	29	32	23
Stimulation as % of control			+40	+13	Nil	Nil	+4	+4	Nil	
0.005	I	180	5	8	12	18	24	26	30	140
	II	180	5	8	12	20	24	28	33	80
	III	195	5	8	10	16	23	25	30	145
	IV	170	5	9	12	20	24	28	32	150
	V	190	5	9	12	20	25	28	33	160
Means		183	5	8	12	19	24	27	32	137
Inhibition as % of control			Nil	Nil	-21	-5	Nil	-3	Nil	

Table 9N Record of growth of Fomes annosus in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Ethanol
Nil (Control)	I	1	5	8	16	20	24	28	32	2
	II	2	5	9	16	21	25	28	32	2
	III	2	5	8	12	20	23	27	32	3
	IV	2	5	9	16	20	24	29	33	3
	V	2	5	7	13	19	23	27	30	2
Means		2	5	8	15	20	24	28	32	2
0.03	I	60	5	7	14	20	25	26	30	35
	II	66	5	7	13	20	25	28	33	35
	III	80	7	11	18	25	30	33	36	33
	IV	50	5	8	15	20	24	25	30	30
	V	55	5	6	11	18	22	25	30	31
Means		62	5	8	14	21	25	27	32	27
Inhibition or Stimulation as % of control			Nil	Nil	-7	+5	+4	-3	Nil	
0.1	I	220	5	7	14	20	23	26	31	100
	II	224	5	5	9	18	21	25	30	100
	III	227	5	9	18	22	25	28	33	99
	IV	220	5	10	18	23	26	27	30	80
	V	230	5	8	11	19	21	26	30	90
Means		224	5	8	14	20	23	26	31	94
Inhibition as % of control			Nil	Nil	-7	Nil	-4	-7	-3	
0.3	I	560	5	5	11	18	22	24	29	340
	II	555	5	5	10	18	22	25	29	310
	III	456	5	6	12	20	23	25	30	310
	IV	553	5	6	10	18	21	23	29	300
	V	561	5	5	9	16	20	22	28	282
Means		537	5	5	10	18	22	24	29	308
Inhibition as % of control			Nil	-38	-33	-10	-8	-14	-9	
1.0	I	1160	5	5	5	15	20	24	30	1000
	II	1150	5	5	5	15	20	24	30	1000
	III	1049	5	5	6	15	19	23	30	900
	IV	1050	5	5	7	18	21	24	30	820
	V	1145	5	5	5	12	20	23	30	1000
Means		1111	5	5	6	15	20	24	30	944
Inhibition as % of control			Nil	-38	-62	-25	-16	-15	-6	

Table 9 0 Record of growth of Fomes annosus in test assemblies with known concentrations of authentic carbon dioxide

CO_2% (vol./vol.) used	Assembly No.	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	GLC peak height (mm) CO_2 0	2	4	6
	I	5	10	14	18	22	26	34	0	0	0	0
Normal air	II	5	11	16	25	28	31	38	0	0	0	0
(Control)	III	5	11	18	22	30	35	38	0	0	0	0
	IV	5	10	16	25	30	35	40	0	0	0	0
	V	5	11	19	26	29	35	41	0	0	0	0
Means		5	11	17	23	28	32	38	0	0	0	0
	I	5	10	18	22	28	36	40	17	18	18	20
	II	5	10	19	23	30	37	40	16	18	18	20
10	III	5	8	12	20	29	35	39	16	16	18	20
	IV	5	8	13	20	25	34	38	16	14	20	20
	V	5	9	15	20	26	34	38	16	18	20	20
Means		5	9	15	21	28	35	39	16	17	19	20
Inhibition or Stimulation as % of control		Nil	-18	-12	-9	Nil	+9	+3				
	I	5	8	10	18	20	25	30	28	30	29	30
	II	5	9	15	19	24	26	31	27	28	28	29
20	III	5	8	9	18	21	25	30	28	28	29	30
	IV	5	8	10	18	20	25	30	28	28	28	30
	V	5	8	12	19	22	25	30	27	28	30	30
Means		5	8	11	18	21	25	30	28	28	29	30
Inhibition as % of control		Nil	-27	-35	-22	-25	-22	-21				
	I	5	5	6	12	15	18	20	48	44	50	48
	II	5	5	8	12	16	24	30	48	46	48	49
30	III	5	6	10	18	21	30	32	48	48	49	49
	IV	5	5	10	18	22	30	32	48	44	49	49
	V	5	6	11	19	22	30	31	48	48	49	49
Means		5	5	9	16	19	26	29	48	46	49	49
Inhibition as % of control		Nil	-55	-47	-30	-32	-19	-24				

Table 9 P Record of growth of colonies of Mucor hiemalis in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ethylene

C_2H_4 (ml/l.air) used	Assembly No.	1st day* GLC peak height (mm) Ethylene	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7
Nil (Control)	I	5	10	43	61	80	100	119	127
	II	6	12	44	63	82	99	120	132
	III	5	12	42	63	82	102	119	129
Means		5	11	43	62	81	100	119	129
0.001	I	145	12	45	65	83	103	122	132
	II	121	12	44	64	84	103	120	128
	III	111	13	45	64	82	103	120	129
Means		126	12	45	64	83	103	121	130
Stimulation as % of control			+9	+5	+3	+2	+3	+2	+1
0.01	I	446	12	43	64	84	104	122	128
	II	490	12	45	64	85	105	124	129
	III	508	12	44	63	82	103	121	126
Means		481	12	44	64	84	104	122	128
Inhibition or Stimulation as % of control			+9	+2	+3	+3	+4	+3	-1
0.1	I	3660	14	46	65	86	105	122	129
	II	3480	14	47	66	86	104	122	131
	III	3700	12	46	64	86	105	122	131
Means		3613	13	46	65	86	105	122	130
Stimulation as % of control			+18	+7	+5	+6	+5	+3	+1
1.0	I	42200	11	43	61	82	103	122	128
	II	38400	14	46	64	83	102	123	132
	III	41400	14	45	64	83	103	123	131
Means		40667	13	45	63	83	103	123	130
Stimulation as. % of control			+18	+5	+1	+2	+3	+3	+1

* Attenuation used was 50 x 1.

Table 9Q Record of growth of Mucor hiemalis in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
	I	5	20	41	65	78	97	102	112	7
	II	7	20	42	67	80	98	104	116	8
Nil	III	12	19	40	64	77	96	102	110	10
(Control)	IV	15	20	43	69	80	98	104	115	20
	V	15	20	43	68	79	97	102	112	9
Means		11	20	42	67	79	97	103	113	11
	I	20	20	48	72	80	100	103	109	10
	II	31	22	48	70	80	99	103	116	10
0.0005	III	22	22	46	70	79	96	99	115	9
	IV	6	21	46	69	77	95	98	115	8
	V	35	24	50	72	82	99	100	108	12
Means		23	22	48	71	80	98	101	113	10
Inhibition or Stimulation as % of control			+10	+14	+6	+1	+1	-2	Nil	
	I	31	23	49	72	91	106	120	135	30
	II	46	22	49	69	90	103	118	130	40
0.001	III	38	22	50	71	90	102	118	130	30
	IV	40	20	48	70	88	100	116	125	50
	V	40	24	48	71	90	105	120	138	45
Means		39	22	49	71	90	103	118	132	39
Stimulation as % of control			+10	+17	+6	+14	+6	+15	+17	
	I	175	24	50	72	92	110	120	130	70
	II	170	24	52	72	92	110	120	130	75
0.005	III	175	22	55	76	94	113	122	134	50
	IV	200	22	52	72	92	110	120	130	70
	V	180	24	52	74	93	112	122	133	70
Means		180	23	52	73	93	111	121	131	67
Stimulation as % of control			+15	+24	+9	+18	+14	+18	+16	
	I	270	20	40	62	73	90	102	110	220
	II	280	18	38	61	72	88	100	110	250
0.01	III	275	17	38	61	72	88	100	110	210
	IV	280	17	37	60	72	86	99	108	220
	V	265	20	39	60	72	88	101	113	220
Means		274	18	38	61	72	88	100	110	224
Inhibition as % of control			-10	-10	-9	-9	-9	-3	-3	
	I	1120	5	28	38	44	53	66	76	800
	II	900	10	30	39	44	53	66	77	800
0.05	III	950	5	28	36	41	52	66	77	770
	IV	900	6	29	36	41	52	66	77	760
	V	900	6	30	40	45	53	68	79	800
Means		954	6	29	38	43	53	66	77	786
Inhibition as % of control			-70	-31	-43	-46	-45	-36	-32	

Table 9R Record of growth of *Mucor hiemalis* in test assemblies with known concentrations of authentic acetone

Acetone (ml liq/l.air) used	Assembly No.	1st day GLC peak height (mm) Acetone	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetone
	I	1	18	43	60	82	98	110	125	5
	II	1	19	45	62	84	100	110	125	5
Nil	III	2	20	44	61	84	99	109	124	4
(Control)	IV	1	19	46	62	84	100	110	125	9
	V	1	20	43	60	81	98	109	123	6
Means		1	19	44	61	83	99	110	124	6
	I	20	20	45	60	84	89	108	123	20
	II	4	20	46	60	84	91	108	124	20
0.0005	III	24	20	45	59	82	88	102	122	18
	IV	32	21	48	64	89	100	114	126	15
	V	22	21	47	64	88	100	114	127	16
Means		20	20	46	61	85	94	109	124	18
Inhibition or Stimulation as % of control			+5	+5	Nil	+3	-5	-1	Nil	
	I	58	24	49	68	83	98	108	124	50
	II	78	18	39	60	79	92	106	124	40
0.001	III	50	22	48	68	80	92	106	123	48
	IV	50	21	48	67	80	93	106	123	44
	V	50	22	49	70	85	100	111	128	39
Means		57	21	47	67	81	95	107	124	44
Inhibition or Stimulation as % of control			+11	+7	+10	-2	-4	-3	Nil	
	I	99	20	45	58	83	98	114	126	62
	II	89	20	44	58	82	97	112	125	80
0.005	III	108	21	45	62	85	100	116	129	90
	IV	95	21	47	64	86	103	116	130	65
	V	85	20	43	58	85	98	114	126	30
Means		95	20	45	60	84	99	114	127	65
Inhibition or Stimulation as % of control			+5	+2	-1	+1	Nil	+4	+2	

Table 98 Record of growth of *Mucor hiemalis* in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq/l.air) used	Assembly No	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Ethanol
Nil (Control)	I	1	18	43	60	82	98	110	125	420
	II	1	19	45	62	84	100	110	125	425
	III	1	20	44	61	84	99	109	124	422
	IV	1	19	46	62	84	100	110	125	325
	V	2	20	43	60	81	98	109	123	426
Means		1	19	44	61	83	99	110	124	404
0.03	I	80	21	48	61	82	100	109	123	550
	II	76	21	49	64	85	105	110	129	560
	III	52	17	44	58	82	104	110	130	500
	IV	65	19	48	59	80	100	108	120	508
	V	70	20	48	62	84	104	109	120	500
Means		69	20	47	61	83	103	109	124	524
Inhibition or Stimulation as % of control			+5	+7	Nil	Nil	+4	-1	Nil	
0.1	I	285	20	48	63	83	100	112	123	399
	II	280	20	50	65	85	102	115	126	500
	III	200	17	43	62	83	99	112	123	440
	IV	298	19	44	62	83	100	114	125	550
	V	290	19	44	62	83	99	114	125	410
Means		291	19	46	63	83	100	113	124	459
Stimulation as % of control			Nil	+5	+3	Nil	+1	+3	Nil	
0.3	I	572	18	40	58	80	95	102	120	480
	II	580	18	41	59	81	95	104	120	485
	III	500	15	38	46	75	89	99	115	480
	IV	420	20	43	59	82	96	105	121	480
	V	574	19	40	58	80	94	102	118	400
Means		529	18	40	56	80	94	102	119	465
Inhibition as % of control			-5	-9	-8	-4	-5	-7	-4	
1.0	I	1110	17	38	54	76	87	97	113	1200
	II	890	18	40	55	78	88	99	115	1000
	III	1000	16	36	50	75	85	95	110	1100
	IV	900	18	39	54	77	86	97	114	980
	V	1009	16	37	50	74	83	95	109	1100
Means		982	17	38	53	76	86	97	112	1076
Inhibition as % of control			-11	-14	-14	-8	-13	-12	-10	

Table 9T Record of growth of Mucor hiemalis in test assemblies with known concentrations of authentic carbon dioxide

CO_2 % (vol./vol.) used	Assembly No.	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	GLC peak height (mm) CO_2 0	2	4	6
	I	22	45	68	85	105	121	134	0	0	1	2
	II	20	45	70	86	106	122	133	0	0	11	2
Normal air	III	22	46	68	85	105	121	133	0	0	1	2
(Control)	IV	22	45	68	85	105	121	133	0	0	1	2
	V	21	46	70	87	106	122	135	0	0	2	0
Means		21	45	69	86	105	121	134	0	0	1	2
	I	18	38	56	75	95	108	114	18	18	22	20
	II	18	37	55	75	95	108	114	18	18	20	20
10	III	18	38	58	76	95	109	114	18	18	20	20
	IV	20	38	58	76	95	109	116	17	16	19	19
	V	20	38	59	78	96	109	116	18	16	19	18
Means		19	38	57	76	95	109	115	18	17	20	19
Inhibition as % of control		-10	-16	-17	-12	-10	-10	-14				
	I	15	30	44	55	70	83	94	28	29	30	31
	II	15	28	45	57	70	83	94	28	29	30	31
20	III	14	26	42	54	66	80	92	28	29	30	31
	IV	15	30	48	59	71	85	97	28	29	29	29
	V	14	30	48	59	71	84	95	28	29	29	30
Means		15	29	45	57	70	83	94	28	29	30	30
Inhibition as % of control		-29	-36	-34	-34	-33	-32	-30				
	I	9	22	32	42	50	60	68	48	48	49	51
	II	10	22	32	42	50	60	68	48	48	48	50
30	III	10	22	32	44	53	63	70	48	50	49	51
	IV	10	22	31	41	48	59	68	48	48	49	50
	V	10	22	33	43	50	60	69	48	48	49	50
Means		10	22	32	42	50	60	69	48	49	49	50
Inhibition as % of control		-52	-51	-54	-51	-52	-50	-49				

Table 9U Record of growth of colonies of Fusarium oxysporum in test assemblies measured every day for 7 days after inoculation and enclosing with known concentrations of authentic ethylene

C_2H_4 (ml /l.air) used	Assembly No	1st day*GLC peak height (mm)	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7
Nil (Control)	I	15	12	25	30	50	62	72	83
	II	6	10	23	30	50	60	70	80
	III	5	11	25	30	48	58	70	82
Means		9	11	24	30	49	60	71	82
0.001	I	115	12	24	30	50	60	71	84
	II	88	11	25	31	53	63	71	84
	III	80	12	25	32	49	62	72	83
Means		94	12	25	31	51	62	71	84
Stimulation as % of control			+9	+4	+3	+4	+3	Nil	+2
0.01	I	390	12	25	31	52	62	72	83
	II	376	10	22	30	50	59	70	81
	III	404	13	24	30	50	61	70	82
Means		390	12	24	30	51	61	71	82
Stimulation as % of control			+9	Nil	Nil	+4	+2	Nil	Nil
0.1	I	3480	13	24	30	50	61	71	82
	II	3400	13	24	30	51	60	70	81
	III	3500	13	23	32	52	62	71	84
Means		3460	13	24	31	51	61	71	82
Stimulation as % of control			+18	Nil	+3	+4	+2	Nil	Nil
1.0	I	37400	12	23	29	49	61	71	83
	II	39200	12	24	31	50	60	72	82
	III	36600	10	23	30	49	62	72	83
Means		37733	11	23	30	49	61	72	83
Inhibition or Stimulation as % of control			Nil	-4	Nil	Nil	+2	+1	+1

*Attenuation used was 50 x 1.

Table 9V Record of growth of Fusarium oxysporum in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq./l.air) used	Assembly No	1st day GLC peak height (mm) Acetaldehyde	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetaldehyde
	I	5	12	24	32	42	59	70	81	30
	II	2	12	25	33	45	60	72	82	35
Nil	III	3	11	23	32	43	60	72	82	36
(Control)	IV	4	12	24	33	45	62	73	84	32
	V	5	11	25	33	45	63	73	84	32
Means		4	12	24	33	44	61	72	83	33
	I	25	14	26	32	44	61	70	83	35
	II	24	14	28	33	46	64	74	84	36
0.0005	III	22	15	28	33	45	64	75	84	30
	IV	20	10	25	31	40	62	70	82	40
	V	36	12	25	30	40	63	72	82	42
Means		25	13	26	32	43	63	72	83	37
Inhibition or Stimulation as % of control			+8	+8	-3	-2	+3	Nil	Nil	
	I	65	12	23	34	45	62	73	83	35
	II	40	12	24	35	45	62	73	83	40
0.001	III	45	12	25	34	44	60	72	83	35
	IV	40	13	26	36	46	62	73	84	30
	V	55	13	26	36	45	62	73	84	35
Means		51	12	25	35	45	62	73	83	35
Stimulation as % of control			Nil	+4	+6	+2	+2	+1	Nil	
	I	184	13	24	34	45	60	70	80	128
	II	180	12	23	33	45	60	71	82	126
0.005	III	170	12	24	34	47	62	73	84	132
	IV	165	12	22	35	48	62	73	84	136
	V	175	12	24	35	48	62	73	84	132
Means		175	12	23	34	47	61	72	83	131
Inhibition or Stimulation as % of control			Nil	-4	+3	+7	Nil	Nil	Nil	
	I	250	10	23	30	39	60	70	79	110
	II	280	6	20	27	36	57	66	78	130
0.01	III	290	6	20	27	36	57	66	78	120
	IV	260	9	22	29	38	60	69	79	120
	V	250	10	23	30	38	60	69	79	100
Means		266	8	22	29	37	59	68	79	116
Inhibition as % of control			-33	-8	-12	-16	-3	-6	-5	
	I	1000	5	5	6	7	22	38	50	800
	II	990	5	5	6	7	23	40	50	800
0.05	III	900	5	6	7	8	25	41	50	800
	IV	850	5	7	9	15	29	44	51	660
	V	900	5	5	5	8	22	38	48	600
Means		928	5	6	7	9	24	40	50	732
Inhibition as % of control			-58	-75	-79	-80	-61	-44	-40	

Table 9W Record of growth of *Fusarium oxysporum* in test assemblies with known concentrations of authentic acetone

Acetone (ml liq./l.air) used	Assembly No.	1st day GLC peak height (mm) Acetone	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acetone
	I	1	8	27	38	48	58	69	80	5
Nil	II	1	9	31	41	49	60	70	82	3
(Control)	III	1	11	32	42	50	61	72	82	2
	IV	1	10	32	42	50	60	71	82	2
	V	1	10	29	40	49	59	70	80	2
Means		1	10	30	41	49	60	70	81	3
	I	20	6	26	40	48	60	69	80	22
	II	20	11	36	44	49	60	70	80	28
0.0005	III	48	10	36	42	40	60	68	80	28
	IV	36	9	32	41	46	58	67	78	30
	V	22	9	32	42	50	59	67	80	30
Means		25	9	32	42	48	59	68	80	28
Inhibition or Stimulation as % of control			-10	+7	+2	-2	-2	-3	-1	
	I	52	14	33	44	50	60	70	80	40
	II	58	8	30	43	49	60	70	79	41
0.001	III	53	8	31	43	50	60	69	80	48
	IV	42	13	34	44	52	63	73	82	40
	V	46	12	35	45	52	63	72	80	39
Means		50	11	33	44	51	61	71	80	42
Inhibition or Stimulation as % of control			+10	+10	+7	+4	+2	+1	-1	
	I	182	10	32	44	52	62	72	80	130
	II	200	10	30	44	52	61	72	80	130
0.005	III	190	11	32	44	52	62	72	81	120
	IV	200	9	30	40	50	59	70	80	140
	V	190	10	31	45	54	63	72	80	150
Means		192	10	31	43	52	61	72	80	134
Inhibition or Stimulation as % of control			Nil	+3	+5	+6	+2	+3	-1	

Table 9X Record of growth of Fusarium oxysporum in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq/l.air) used	Assembly No	1st day GLC peak height (mm) Ethanol	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Ethanol
	I	2	8	27	38	48	58	69	80	256
Nil	II	3	9	31	41	49	60	70	82	260
(Control)	III	3	11	32	42	50	61	72	82	258
	IV	2	10	32	42	50	60	71	82	261
	V	1	10	29	40	49	59	70	80	250
Means		2	10	30	41	49	60	70	81	257
	I	80	6	30	40	51	60	70	80	320
	II	80	12	38	48	56	62	74	82	450
0.03	III	78	8	29	41	53	60	70	81	420
	IV	60	9	34	44	53	62	70	81	440
	V	62	9	34	44	53	61	74	81	430
Means		72	9	33	43	53	61	72	81	412
Inhibition or Stimulation as % of control			-10	+10	+5	+8	+2	+3	Nil	
	I	280	10	30	39	52	63	72	79	270
	II	290	10	32	40	52	63	72	80	240
0.1	III	300	9	32	42	53	64	74	80	245
	IV	300	8	29	39	51	62	73	80	260
	V	280	9	30	40	52	62	72	79	225
Means		290	9	31	40	52	63	73	80	248
Inhibition or Stimulation as % of control			-10	+3	-2	+6	+5	+4	-1	
	I	661	9	26	36	45	56	66	75	340
	II	550	8	26	36	45	56	66	75	350
0.3	III	590	9	30	40	48	58	67	77	342
	IV	545	8	28	38	46	56	65	74	350
	V	560	9	30	40	49	58	68	77	350
Means		581	9	28	38	47	57	66	76	346
Inhibition as % of control			-10	-7	-7	-4	-5	-6	-6	
	I	900	8	22	30	42	52	62	70	995
	II	1000	7	22	30	40	50	60	69	900
1.00	III	950	8	24	32	42	52	62	70	900
	IV	1100	5	22	30	40	50	60	69	998
	V	880	9	27	34	44	53	63	70	999
Means		966	7	23	31	42	51	61	70	958
Inhibition as % of control			-30	-23	-24	-14	-15	-13	-14	

Table 9Y Record of growth of Fusarium oxysporum in test assemblies with known concentrations of authentic carbon dioxide

CO_2% (vol./vol.) used	Assembly No.	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	GLC peak height (mm) CO_2 0	2	4	6
	I	12	25	35	47	60	72	82	0	0	2	2
Normal air	II	10	23	35	47	60	72	81	0	0	1	1
(Control)	III	10	23	35	46	60	72	81	0	0	1	2
	IV	9	21	32	44	59	70	80	0	0	1	2
	V	10	22	34	44	59	70	80	0	0	2	2
Means		10	23	34	46	60	71	81	0	0	1	2
	I	11	22	33	43	56	67	77	15	15	22	19
	II	10	20	32	42	56	67	75	15	16	24	20
10	III	10	20	32	43	56	67	77	16	18	25	21
	IV	9	19	31	42	56	67	75	16	15	20	21
	V	9	18	30	41	55	66	75	16	15	20	21
Means		10	20	32	42	56	67	76	16	16	22	20
Inhibition as % of control		Nil	-13	-6	-9	-7	-6	-6				
	I	8	19	28	36	48	58	66	25	28	30	30
	II	10	18	27	37	48	57	67	24	30	29	30
20	III	11	20	28	37	48	58	66	25	26	26	30
	IV	8	19	28	39	49	59	67	20	30	30	30
	V	8	20	28	39	47	58	66	22	30	30	30
Means		9	19	28	38	48	58	66	23	29	29	30
Inhibition as % of control		-10	-17	-18	-17	-20	-19	-19				
	I	6	15	25	34	40	51	59	47	50	49	51
	II	9	18	26	34	41	52	59	48	50	50	51
30	III	7	15	25	34	40	50	57	48	50	50	51
	IV	7	15	25	33	41	50	57	48	50	50	50
	V	7	17	26	33	41	51	58	48	50	50	51
Means		7	16	25	34	41	51	58	48	50	50	51
Inhibition as % of control		-30	-30	-26	-26	-32	-28	-28				

Table 10A Record of growth of *Pyronema domesticum* colonies paired with 7-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily observation of colony diameter (mm) 1	2	3	4*	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (control)	I	2	3	8	2	32	160	220	230	230	230	230	5	2	3	36
	II	2	2	4	2	31	170	220	230	230	230	230	2	2	3	35
	III	3	2	2	3	32	180	230	230	230	230	230	3	2	4	35
	IV	3	2	2	3	33	190	230	230	230	230	230	2	2	2	35
	V	2	2	2	2	32	158	210	230	230	230	230	3	3	3	35
Means		2	2	4	2	32	172	222	230	230	230	230	3	3	3	35
paired with *T.viride* 1	I	25	2	145	36	5	5	20	70	80	85	90	15	5	89	39
	II	5	12	38	30	5	12	49	70	78	85	90	14	23	100	31
	III	12	5	169	30	5	15	42	72	81	88	92	18	5	140	32
	IV	15	2	190	36	5	6	38	66	70	78	89	10	4	95	34
	V	10	5	69	32	5	8	49	65	70	78	89	20	7	89	32
Means		13	5	122	33	5	9	39	69	76	83	90	15	8	103	34
Inhibition as % of control						-84	-95	-83	-70	-67	-64	-61				
paired with *T.longibranchiatum* WBC4576	I	5	3	128	27	18	92	128	160	180	200	210	4	2	54	38
	II	7	13	88	21	19	92	120	156	180	198	210	2	2	60	37
	III	20	3	46	20	20	100	123	165	190	210	230	7	4	65	38
	IV	11	23	29	22	18	98	120	165	180	210	230	2	2	50	38
	V	6	4	39	29	15	90	120	155	180	198	210	2	2	65	38
Means		10	9	65	24	18	94	123	160	184	203	218	3	2	59	38
Inhibition as % of control						-44	-45	-45	-30	-20	-12	-5				

*Agar surface completely covered with mycelium in controlled assemblies

Table 10B Record of growth of *Pythium ultimum* colonies paired with 7-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	2	1	2	2	35	88	138	180	195	205	210	2	2	2	35
	II	2	2	2	2	32	90	140	180	196	209	215	2	2	2	35
	III	1	2	1	2	38	89	140	186	197	220	230	2	2	2	35
	IV	2	1	1	1	32	92	135	182	199	220	220	2	2	2	34
	V	2	3	1	2	34	85	130	180	190	210	220	2	2	2	34
Means		2	2	1	2	34	89	137	182	195	213	219	2	2	2	34
paired with *T.viride* 1	I	2	3	134	38	22	52	82	99	105	110	116	6	3	90	40
	II	2	5	42	28	22	64	92	98	105	111	113	7	3	130	38
	III	3	12	25	29	22	62	90	95	102	109	113	6	3	118	40
	IV	23	13	250	29	15	55	90	87	94	98	104	15	3	138	39
	V	42	42	35	29	24	65	91	99	106	109	114	15	4	134	40
Means		14	15	98	31	21	60	89	96	102	107	112	10	3	122	39
Inhibition as % of control						-39	-33	-35	-48	-48	-49	-49				
paired with *T.longibranchiatum* WBC 4576	I	22	3	14	24	26	66	105	130	155	170	195	2	2	12	36
	II	12	2	83	24	25	65	105	130	154	171	195	2	2	35	36
	III	12	42	20	25	24	64	103	138	150	178	194	2	2	40	35
	IV	12	33	80	29	25	65	100	135	150	178	194	2	2	30	36
	V	18	4	27	24	25	65	105	132	156	173	196	6	5	15	38
Means		15	17	45	25	25	65	104	133	153	174	195	3	2	26	36
Inhibition as % of control						-27	-27	-24	-27	-22	-18	-11				

Table 10C Record of growth of *Fomes annosus* colonies paired with 7-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2
paired with	I	1	1	1	1	5	5	6	12	24	28	34	1	1	1	9
uninoculated	II	2	1	1	2	5	6	6	14	24	28	34	1	1	1	9
2% malt agar	III	1	2	1	1	5	6	7	16	25	30	35	1	1	1	11
(Control)	IV	1	1	1	2	5	8	9	18	26	30	36	1	1	2	10
	V	1	1	1	2	5	8	9	18	26	30	36	1	1	1	12
Means		1	1	1	2	5	7	7	16	25	29	35	1	1	1	10
paired with	I	52	3	80	38	5	5	6	10	15	20	25	6	3	130	39
T.viride 1	II	25	15	148	29	5	5	5	11	15	20	25	20	15	130	26
	III	16	3	38	29	5	5	7	12	16	20	26	25	4	110	37
	IV	8	23	36	38	5	5	5	12	16	22	26	6	5	133	39
	V	6	4	138	29	5	5	5	11	15	20	25	6	5	113	36
Means		21	6	88	33	5	5	6	11	15	20	25	13	6	123	37
Inhibition as % of control						Nil	-29	-14	-31	-40	-31	-29				
paired with	I	20	3	15	24	5	6	7	12	20	28	34	2	2	28	30
T.longibran-	II	21	2	13	25	5	6	6	10	20	26	34	4	2	33	30
chiatum	III	2	2	40	23	5	5	7	12	20	27	33	2	3	55	32
WBC 4576	IV	5	3	65	23	5	6	8	13	21	28	35	3	2	45	32
	V	5	3	15	28	5	6	6	12	20	27	33	2	2	66	32
Means		10	3	30	25	5	6	7	12	20	27	34	3	2	45	31
Inhibition as % of control						Nil	-14	Nil	-25	-20	-7	-3				

Table 10D Record of growth of *Mucor hiemalis* colonies paired with 7-day-old cultures of *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.Eth.		CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.Eth.		CO_2
paired with uninoculated 2% malt agar (Control)	I	1	2	3	3	22	42	69	86	106	116	126	28	5	480	40
	II	2	3	3	4	20	41	68	85	103	116	128	30	6	460	40
	III	3	1	2	3	20	40	68	85	102	115	130	25	10	480	41
	IV	4	2	3	3	22	42	69	86	106	116	128	24	6	425	40
	V	2	2	4	3	23	43	71	88	108	120	130	18	5	440	40
Means		2	2	3	3	21	42	69	86	105	117	128	25	6	457	40
paired with *T.viride* 1	I	40	42	50	31	18	34	58	69	72	77	84	20	10	320	41
	II	13	22	65	39	18	33	59	69	72	78	85	10	10	332	39
	III	90	14	55	34	18	35	60	70	74	79	85	10	10	336	40
	IV	20	14	265	36	18	33	58	69	72	77	83	10	10	320	39
	V	13	14	150	30	19	38	60	69	76	80	87	24	5	340	44
Means		35	21	117	34	18	35	59	69	73	78	85	15	9	330	41
Inhibition as % of control						-15	-17	-15	-20	-30	-33	-34				
paired with *T.longibranchiatum* WBC 4576	I	18	12	20	23	19	39	64	75	88	110	118	2	2	320	40
	II	2	22	30	24	20	36	62	75	90	113	119	2	2	303	36
	III	21	11	20	28	19	33	60	74	89	110	118	9	2	263	39
	IV	2	12	41	24	18	39	62	74	89	111	120	4	2	340	36
	V	12	11	120	25	20	39	63	75	90	113	120	2	2	300	36
Means		8	14	46	25	19	37	62	75	89	111	119	4	2	301	37
Inhibition as % of control						-10	-12	-10	-13	-15	-4	-7				

Table 10E Record of growth of *Fusarium oxysporum* colonies paired with 7-day-old cultures of *Trichoderma viride* 1 and *T. longibranchiatum* WBC 4576

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	2	2	1	3	10	22	40	50	60	70	82	28	3	170	30
	II	1	2	2	3	9	20	39	48	59	68	80	30	3	180	30
	III	3	2	1	4	11	23	41	51	60	70	83	28	3	160	31
	IV	2	2	1	3	10	22	40	50	60	70	82	25	4	175	30
	V	1	1	2	2	10	22	40	50	60	70	82	30	3	175	31
Means		2	2	1	3	10	22	40	50	60	70	82	28	3	172	30
paired with *T.viride* 1	I	10	12	45	30	10	20	35	45	53	59	70	14	5	440	32
	II	19	23	45	29	11	23	35	46	53	60	72	20	6	460	34
	III	10	64	75	30	8	19	32	42	52	60	71	20	5	420	39
	IV	12	13	160	34	8	19	32	42	51	59	70	12	6	320	33
	V	112	13	270	36	8	18	30	42	51	59	70	12	6	340	33
Means		33	25	119	32	9	20	33	43	52	59	71	16	6	396	34
Inhibition as % of control						-10	-9	-17	-13	-13	-15	-13				
paired with *T.longibranchiatum* WBC 4576	I	11	14	23	23	10	20	38	49	59	66	73	10	2	177	36
	II	12	45	27	22	11	22	40	50	60	66	73	2	2	250	38
	III	9	23	46	22	10	20	40	49	58	66	73	3	2	250	35
	IV	10	13	28	29	9	20	38	50	58	65	75	3	2	160	36
	V	26	14	84	29	9	20	40	50	58	65	75	2	2	175	35
Means		14	22	41	25	10	20	39	49	59	66	74	4	2	202	36
Inhibition as % of control						Nil	-9	-2	-2	-2	-6	-10				

Table 11. Record of growth of *Rhizoctonia solani* colonies paired with 7-day-old cultures of *Trichoderma viride* 14, *T.polysporum* 2 (active), *T. pseudokoningii* A/196-1, *T.polysporum* 74 (least active).

Treatment	Assembly No.	2nd day GLC peak height (mm) Acet-ald.	Acet.Eth.		CO_2	Daily observation of colony diameter (mm) 1	2	3	4	5	6	7	7th day GLC peak height (mm) Acet-ald.	Acet.Eth.		CO_2
paired with	I	1	1	1	1	15	38	61	93	112	128	140	3	10	10	30
uninoculated	II	1	1	2	1	16	40	62	96	115	129	142	2	2	5	26
2% malt agar	III	1	1	1	1	16	39	60	93	114	130	144	2	2	5	25
(Control)	IV	1	2	1	1	18	40	61	94	115	130	143	1	2	6	25
	V	1	1	2	1	20	42	64	96	114	129	141	2	5	6	24
Means		1	1	1	1	17	40	62	94	114	129	142	2	4	6	26
paired with *T.viride* 14	I	22	12	100	39	9	20	35	42	50	60	62	13	7	53	35
	II	31	59	105	36	10	20	38	49	60	70	75	10	20	50	33
	III	12	12	250	35	11	24	39	51	60	71	75	15	15	169	39
	IV	32	69	98	35	11	22	38	47	57	69	72	33	13	85	36
	V	52	14	120	36	10	19	34	45	56	69	72	63	12	48	36
Means		30	33	135	36	10	21	37	47	57	68	71	27	13	81	37
Inhibition as % of control						-41	-48	-40	-50	-50	-47	-50				
paired with *T.polysporum* 2	I	12	15	120	28	12	26	34	50	59	64	68	3	10	51	34
	II	15	35	245	33	9	24	31	46	55	61	67	2	11	145	34
	III	66	16	92	36	8	20	29	48	55	60	66	3	15	120	38
	IV	27	17	120	39	10	20	30	50	60	66	72	2	6	40	33
	V	22	12	100	30	10	24	31	54	65	69	75	5	6	74	35
Means		28	19	135	33	10	23	31	50	59	64	70	3	10	86	35
Inhibition as % of control						-41	-43	-50	-47	-48	-50	-51				
paired with *T.pseudo-koningii* A/196-1	I	12	13	20	30	17	32	42	58	65	68	70	1	7	18	32
	II	12	16	20	30	18	34	44	64	80	90	98	1	3	15	31
	III	40	55	25	30	19	34	42	63	75	77	80	2	5	20	31
	IV	9	9	98	32	15	29	42	59	67	74	80	3	3	40	31
	V	5	9	22	31	15	29	45	65	68	75	82	2	3	12	30
Means		16	20	41	31	17	32	43	62	71	77	82	2	4	21	31
Inhibition as % of control						Nil	-20	-31	-34	-38	-40	-42				
paired with *T.polysporum* 74	I	5	5	5	25	18	32	40	54	68	80	90	1	1	2	30
	II	3	61	25	24	18	30	39	54	70	89	98	1	3	3	29
	III	1	39	3	24	18	40	49	68	78	92	100	1	5	5	32
	IV	1	3	33	27	16	30	38	54	75	90	100	1	1	1	33
	V	1	5	4	27	15	30	38	54	75	90	100	1	1	2	32
Means		2	23	14	25	17	32	41	57	73	88	98	1	2	3	31
Inhibition as % of control						Nil	-20	-34	-39	-36	-32	-31				

Table 12A Record of means of peak heights from samples of identified volatile metabolites present over pure cultures of *Trichoderma* strains incubated in sealed bottles. Strains are arranged due to amounts of CO_2 produced by each on seventh day after inoculation. inoculation.

No.	Trichoderma species tested	Mean of peak heights (mm) for samples taken on days																											
		1st day												7th day												14th day			
		Acetaldehyde Bottle No.			Acetone Bottle No.			Ethanol Bottle No.			CO_2 Bottle No.			Acetaldehyde Bottle No.			Acetone Bottle No.			Ethanol Bottle No.			CO_2 Bottle No.			Acetaldehyde Bottle No			Acetone Bottle No			Ethanol Bottle No			CO_2 Bottle No.		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	T.viride 14	0	2	2	3	10	5	2	4	1	2	2	2	3	2	4	8	20	7	240	259	250	40	36	43	6	16	7	16	12	19	360	320	370	42	45	48
2	T.koningii 6	1	1	0	5	4	5	1	0	3	2	1	2	6	11	8	18	20	15	230	198	247	41	40	40	8	6	11	16	12	8	230	200	261	40	42	41
3	T.viride 1	1	1	0	16	12	16	11	8	9	2	2	3	22	22	23	12	22	11	198	167	204	40	42	39	36	25	27	15	31	28	240	200	280	44	44	43
4	T.koningii C/57693	1	1	2	10	12	8	5	2	7	4	6	2	20	22	17	2	16	11	189	150	200	40	36	45	12	15	2	15	20	9	182	130	228	42	40	39
5	T.viride C/16198	1	1	2	3	3	3	0	1	1	3	3	3	2	8	3	20	22	23	200	256	250	38	40	39	10	9	10	22	24	20	280	230	210	39	40	39
6	T.koningii 7	1	0	2	2	2	2	10	8	11	2	5	2	2	8	2	33	62	84	220	168	212	39	41	38	20	12	28	33	51	35	300	266	335	39	38	38
7	T.koningii B/6242	2	0	4	10	22	4	6	8	6	2	4	3	1	1	1	9	27	15	200	182	219	38	39	38	6	8	6	15	9	20	272	226	252	38	40	37
8	T.polysporum 2	0	1	1	3	20	9	3	3	2	3	3	3	3	3	2	16	10	27	200	259	217	36	38	34	3	2	3	16	20	10	300	262	338	39	40	37
9	T.viride A	4	3	4	4	14	5	2	7	5	2	4	3	15	30	29	9	12	9	170	200	155	36	34	37	20	32	22	10	22	13	180	220	156	38	37	39
10	T.viride B/4555	1	0	1	12	4	7	2	4	3	2	1	2	2	1	2	0	9	8	180	122	162	36	37	36	2	0	6	6	8	7	188	140	153	36	36	37
11	T.viride D/109551	1	2	1	2	12	9	2	3	4	2	2	1	5	6	3	3	13	6	100	162	158	35	38	36	2	4	3	10	10	10	222	302	316	40	43	43
12	T.polysporum 5	0	1	3	11	2	9	6	10	8	2	3	3	2	2	2	18	8	27	125	130	121	34	36	35	2	8	3	11	4	17	142	148	130	34	36	36
13	T.harzianum 1	1	0	2	3	13	13	2	1	2	2	3	4	2	1	3	20	50	64	108	140	98	34	37	35	5	4	5	50	22	77	116	146	128	34	39	37
14	T.longibranchiatum WBC 4576	1	2	1	10	23	4	2	12	4	1	2	1	12	3	12	27	20	32	90	110	94	32	37	35	5	6	5	20	31	16	98	112	90	34	35	36
15	T.koningii A/73022	1	0	3	18	22	15	2	4	3	2	3	3	2	0	2	18	30	31	81	102	88	35	35	35	3	4	3	26	25	26	90	112	75	36	37	36
16	T.polysporum C/306	1	2	1	7	7	7	2	3	3	2	2	1	1	2	0	20	31	25	82	100	88	35	35	36	5	6	5	20	22	9	88	110	102	35	35	35
17	T.ranzian 129	1	1	1	2	12	11	2	3	3	3	3	2	2	2	3	20	25	16	65	75	56	34	37	35	4	3	5	18	26	17	80	101	83	36	39	39
18	T.viride 3	1	1	1	14	3	26	2	10	4	2	3	2	2	1	1	16	12	18	50	68	63	35	30	39	2	1	1	12	4	45	62	120	118	36	32	40
19	T.koningii SHD/M/2629	2	0	2	3	0	10	2	0	3	6	2	3	2	1	4	20	8	39	62	32	26	37	34	35	3	3	3	25	11	40	39	35	31	36	35	35
20	T.piluliferum SHD/M/2636	1	1	2	8	8	9	2	6	3	3	5	3	6	7	6	10	24	9	70	90	74	33	35	34	2	9	5	22	19	80	110	130	121	34	36	36
21	T.hamatum JMD/12	1	2	1	6	7	6	2	4	3	3	3	3	3	3	4	8	7	8	40	46	50	32	33	34	5	2	9	10	11	10	121	162	92	35	35	35
22	T.pseudokoningii A/196/1	2	3	2	2	2	2	0	0	2	2	1	5	1	2	1	15	17	12	35	30	54	34	31	34	4	3	5	12	20	14	102	120	98	32	30	36
23	T.hamatum 127	1	0	3	5	6	4	2	7	5	3	3	3	1	1	1	30	42	45	60	86	64	28	32	31	2	3	2	26	38	56	71	88	67	34	36	35
24	T.polysporum 74	1	2	1	1	1	1	0	8	3	2	4	3	1	2	1	2	3	2	44	62	33	30	30	30	2	7	6	10	9	12	78	96	66	34	36	35

Table 12B Record of means of peak heights from samples of primary volatile metabolites (ethanol and carbon dioxide), present over pure cultures of Trichoderma strains incubated in cotton wool plugged bottles. Strains are arranged due to amount of CO_2 produced by each on seventh day after inoculation.

No.	Trichoderma species tested.	Mean of peak heights (mm) for samples taken on days											
		7th day						14th day					
		Ethanol Bottle No.			CO_2 Bottle No.			Ethanol Bottle No.			CO_2 Bottle No.		
		1	2	3	1	2	3	1	2	3	1	2	3
1	T.viride 1	142	120	158	32	28	30	148	132	140	35	36	35
2	T.koningii 7	121	165	134	28	32	31	80	96	94	29	32	30
3	T.harzianum 1	102	120	108	26	30	33	92	62	116	35	36	35
4	T.viride A	102	179	139	26	31	28	60	100	140	26	34	31
5	T.viride 14	122	82	156	28	26	29	90	62	118	20	25	22
6	T.koningii 6	62	100	79	26	25	27	60	81	40	26	25	25
7	T.koningii B/6242	60	92	88	24	25	24	40	68	13	22	26	27
8	T.viride C/16198	100	88	112	22	25	23	90	98	113	30	32	34
9	T.koningii C/54693	76	72	62	20	26	21	40	39	42	20	22	19
10	T.viride B/45553	92	120	118	20	20	20	50	30	70	20	24	22
11	T.viride D/109551	90	88	92	22	20	19	70	65	74	20	16	25
12	T.polysporum 2	50	62	38	19	20	19	66	51	72	20	20	20
13	T.polysporum 5	40	80	61	18	16	19	70	81	60	10	8	13
14	T.longibranchiatum WBC 4576	70	60	81	18	17	17	60	62	59	20	16	23
15	T.koningii A/73022	90	108	101	16	15	17	80	96	65	23	22	29
16	T.harzianum 129	90	95	86	15	17	16	60	92	88	20	18	23
17	T.viride 3	82	79	110	16	15	16	60	58	63	20	16	29
18	T.koningii SHD/M/2629	66	73	71	12	13	12	80	75	86	5	14	10
19	T.piluliferum SHD/M/2636	93	104	104	11	12	11	88	78	76	15	19	12
20	T.polysporum C/306	80	88	73	10	9	12	62	50	69	11	10	10
21	T.pseudokoningii A/196-1	46	40	53	10	12	9	32	49	40	10	10	11
22	T.hamatum JMD/12	69	90	80	9	9	10	70	72	69	6	5	6
23	T.hamatum 127	60	68	53	9	8	9	68	73	70	6	6	7
24	T.polysporum 74	50	37	64	9	9	10	60	70	79	15	16	15

APPENDIX TABLES 13

Test of effects of gases from cultures of Trichoderma viride 1 and Trichoderma longibranchiatum WBC 4576 on the macroscopic growth of Erwinia species on agar.

(2) experiments were carried out. Each comprised 3 paired assemblies of the Trichoderma species under test with one of each of E.amylovora, E.aroideae, E.atroseptica, E.carotovora, E.tracheiphila and appropriate controls.

There were no visible differences between the growth in treated and control assemblies in any case.

Table 14A Record of growth of Erwinia amylovora in test assemblies with known concentrations of authentic acetaldehyde

cetaldehyde l liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	1	1	1	1	3
		2	1	1	1	1	3
		3	1	1	1	1	3
	II	1	1	1	2	2	3
		2	1	1	2	2	3
		3	1	1	2	2	3
	III	1	1	1	2	2	3
		2	1	1	2	2	3
		3	1	1	1	2	3
0.01	I	1	1	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
0.05	I	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0

For other details see text.

Table 14B Record of growth of Erwinia amylovora in test assemblies with known concentrations of authentic acetone.

Acetone (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.05	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.25	I	1	1	2	2	2	3
		2	1	2	2	2	3
		3	1	2	2	2	3
	II	1	1	2	2	2	3
		2	1	2	2	2	3
		3	1	2	2	2	3
	III	1	1	2	2	2	3
		2	1	2	2	2	3
		3	1	2	2	2	3
0.5	I	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	II	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3

For other details see text.

Table 14C Record of growth of Erwinia amylovora in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq/l.air) used	Bottle No	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.03	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.1	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.3	I	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	II	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
0.6	I	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2

For other details see text.

Table 14D Record of growth of Erwinia aroideae in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	1	1	1	2	3
		2	0	0	2	3	3
		3	0	0	1	3	3
	II	1	0	0	1	3	3
		2	1	1	1	3	3
		3	0	0	1	3	3
	III	1	1	1	1	3	3
		2	1	1	1	2	3
		3	0	0	1	2	3
0.01	I	1	1	0	0	0	2
		2	1	0	0	0	2
		3	1	0	0	0	2
	II	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	III	1	0	0	0	1	2
		2	0	0	0	0	2
		3	0	0	0	0	2
0.05	I	1	1	0	0	0	2
		2	1	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	III	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1

For other details see text.

Table 14E Record of growth of Erwinia aroideae in test assemblies with known concentrations of authentic acetone.

Acetone (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.05	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.25	I	1	2	2	2	3	3
		2	2	2	2	3	3
		3	2	2	2	2	3
	II	1	2	2	2	3	3
		2	2	2	2	2	3
		3	1	2	2	3	3
	III	1	2	2	2	3	3
		2	2	2	2	3	3
		3	2	2	2	2	3
0.5	I	1	1	0	0	0	2
		2	1	0	0	0	3
		3	1	0	0	0	3
	II	1	1	0	0	0	3
		2	1	0	0	0	3
		3	1	0	0	0	3
	III	1	1	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3

For other details see text.

Table 14F Record of growth of *Erwinia aroideae* in test assemblies with known concentrations of authentic ethanol.

ethanol (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.03	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.1	I	1	2	3	3	3	3
		2	2	3	3	3	3
		3	2	3	3	3	3
	II	1	2	3	3	3	3
		2	2	2	3	3	3
		3	2	2	3	3	3
	III	1	2	3	3	3	3
		2	2	3	3	3	3
		3	1	3	3	3	3
0.3	I	1	1	1	1	2	3
		2	1	1	1	2	3
		3	1	1	1	1	3
	II	1	1	1	1	2	3
		2	1	1	1	2	3
		3	1	1	1	1	3
	III	1	1	1	1	2	3
		2	1	1	1	2	3
		3	1	1	1	1	3
0.6	I	1	1	1	1	1	3
		2	1	1	1	1	3
		3	1	0	0	0	3
	II	1	1	1	0	0	3
		2	1	0	0	0	3
		3	1	0	0	0	3
	III	1	1	0	0	0	3
		2	1	0	0	0	3
		3	1	0	0	0	3
0.9	I	1	1	1	1	1	3
		2	1	1	1	1	3
		3	1	1	1	1	3
	II	1	1	0	0	0	2
		2	1	0	0	0	2
		3	1	0	0	0	2
	III	1	1	0	0	0	3
		2	0	0	0	0	2
		3	0	0	0	0	2

For other details see text.

Table 14 Record of growth of *Erwinia atroseptica* in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	1	0	0	0	3
		2	1	0	0	0	3
		3	1	0	0	0	3
	II	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	1	0	0	0	3
		2	1	0	0	0	3
		3	0	0	0	0	3
0.01	I	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
0.05	I	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0

For other details see text.

Table 14H Record of growth of Erwinia atroseptica in test assemblies with known concentrations of authentic acetone

Acetone (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.05	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.25	I	1	1	0	0	0	3
		2	1	0	0	0	3
		3	1	0	0	0	3
	II	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	1	0	0	0	3
		2	1	0	0	0	3
		3	0	0	0	0	3
0.5	I	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	III	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2

For other details see text.

Table 14I Record of growth of Erwinia atroseptica in test assemblies with known concentrations of authentic ethanol

Ethanol (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.03	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.1	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	2	2	2	3	3
		2	2	2	2	2	3
		3	2	2	2	2	3
	III	1	2	2	2	3	3
		2	2	2	2	2	3
		3	2	2	2	2	3
0.3	I	1	1	0	0	0	2
		2	1	0	0	0	2
		3	1	0	0	0	2
	II	1	1	0	0	0	2
		2	1	0	0	0	2
		3	1	0	0	0	1
	III	1	1	0	0	0	2
		2	0	0	0	0	1
		3	1	0	0	0	1
0.6	I	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0

For other details see text.

Table 14J Record of growth of *Erwinia carotovora* in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	1	0	0	0	3
		2	1	1	1	0	3
		3	0	0	0	0	3
	II	1	1	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	1	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
0.01	I	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	II	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
0.05	I	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	II	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	III	1	0	0	0	0	1
		2	0	0	0	0	0
		3	0	0	0	0	0

For other details see text

Table 14K Record of growth of Erwinia carotovora in test assemblies with known concentrations of authentic acetone.

Acetone (ml liq /l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.05	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.25	I	1	0	0	1	1	3
		2	0	0	1	1	3
		3	0	0	1	1	3
	II	1	0	1	1	1	3
		2	0	1	1	1	3
		3	0	1	1	1	3
	III	1	0	0	1	1	3
		2	0	0	1	1	3
		3	0	0	1	1	3
0.5	I	1	0	0	0	1	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	II	1	0	0	0	1	3
		2	0	0	0	1	3
		3	0	0	0	0	2
	III	1	0	0	0	1	2
		2	0	0	0	0	2
		3	0	0	0	0	2

For other details see text.

Table14L Record of growth of Erwinia carotovora in test assemblies with known concentrations of authentic ethanol 208

Ethanol (ml liq /l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.03	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.1	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	2	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.3	I	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
	II	1	0	0	0	0	3
		2	0	0	1	1	3
		3	0	0	0	0	3
	III	1	0	0	0	1	3
		2	0	0	0	0	3
		3	0	0	0	0	3
0.6	I	1	0	0	0	0	2
		2	0	0	0	0	1
		3	0	0	0	0	1
	II	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	III	1	0	0	0	0	2
		2	0	0	0	0	1
		3	0	0	0	0	1
0.9	I	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	1
		2	0	0	0	0	0
		3	0	0	0	0	0
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0

For other details see text.

Table 14M Record of growth of *Erwinia tracheiphila* in test assemblies with known concentrations of authentic acetaldehyde

Acetaldehyde (ml liq/l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	3
		2	0	0	0	0	2
		3	0	0	0	0	2
	III	1	0	0	0	0	3
		2	0	0	0	0	2
		3	0	0	0	0	2
0.01	I	1	0	0	0	0	1
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	2
		2	0	0	0	0	1
		3	0	0	0	0	1
	III	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
0.05	I	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
	II	1	0	0	0	0	1
		2	0	0	0	0	0
		3	0	0	0	0	0
	III	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0

For other details see text.

Table 14 N Record of growth of Erwinia tracheiphila in test assemblies with known concentrations of authentic acetone

Acetone (ml liq /l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media.
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.001	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.005	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.05	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.25	I	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	2
	II	1	0	0	0	0	2
		2	0	0	0	0	3
		3	0	0	0	0	3
	III	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
0.5	I	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	1
	III	1	0	0	0	0	1
		2	0	0	0	0	2
		3	0	0	0	0	1

For other details see text.

Tables 14 0 Record of growth of Erwinia tracheiphila in test assemblies with known concentrations of authentic ethanol.

Ethanol (ml liq /l.air) used	Bottle No.	Agar Block No.	Daily observation of bacterial growth 1	2	3	4	Bacterial viability after re-cultured on fresh media
Nil (Control)	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.03	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.1	I	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	II	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
	III	1	3	3	3	3	3
		2	3	3	3	3	3
		3	3	3	3	3	3
0.3	I	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	III	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	3
0.6	I	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	II	1	0	0	0	0	2
		2	0	0	0	0	2
		3	0	0	0	0	2
	III	1	0	0	0	0	3
		2	0	0	0	0	3
		3	0	0	0	0	2
0.9	I	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	II	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1
	III	1	0	0	0	0	1
		2	0	0	0	0	1
		3	0	0	0	0	1

For other details see text.

Table 15A Record of effects of 7-day-old T.viride 1 on growth of Erwinia tracheiphila on 7th day after inoculation in agar and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g agar ($no. \times 10^8$)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	388	10	2	2	2	30	3	1	23
	II	420	12	4	4	2	26	3	1	24
	III	366	6	3	8	2	12	9	2	24
Means		391	9	3	5	2	23	5	1	24
paired with T.viride 1	I	424	40	16	178	28	108	6	185	45
	II	312	20	5	202	29	83	8	175	46
	III	320	58	22	162	29	100	19	212	50
Means		352	39	14	181	29	97	11	191	47
Inhibition as % of control		-10								

Table 15B Record of effects of 7-day-old T.viride 1 on growth of Erwinia tracheiphila on 7th day after inoculation in loam and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g loam (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	220	1	1	2	3	1	2	2	8
	II	188	2	1	2	3	1	1	1	10
	III	236	1	1	2	3	.2	2	2	10
Means		215	1	1	2	3	1	2	2	9
paired with T.viride 1	I	130	30	10	118	27	12	8	193	46
	II	121	48	10	176	28	10	8	248	47
	III	103	21	9	155	28	26	16	200	47
Means		118	33	10	150	28	16	11	214	47
Inhibition as % of control		-45								

Table 15C Record of effects of 7-day-old T.viride 1 on growth of Erwinia tracheiphila on 7th day after inoculation in clay and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g clay (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	ETh.	CO_2
paired with uninoculated 2% malt agar	I	60	2	1	1	1	2	5	2	5
	II	82	2	1	2	1	2	5	2	4
	III	48	1	1	1	1	1	2	1	4
Means		63	2	1	1	1	2	4	2	4
paired with T.viride 1	I	60	14	17	102	28	4	7	159	46
	II	41	8	12	198	28	5	7	121	50
	III	57	19	6	124	28	18	3	120	50
Means		53	14	12	141	28	9	6	133	49
Inhibition as % of control		-16								

Table 15D Record of effects of 7-day-old T.viride 1 on growth of Erwinia tracheiphila on 7th day after inoculation in sand and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g sand (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	36	1	1	1	2	2	8	3	4
	II	58	1	2	1	2	1	10	3	6
	III	49	1	1	1	1	2	6	2	6
Means		48	1	1	1	2	2	8	3	5
paired with T.viride 1	I	41	26	12	228	24	40	13	235	50
	II	33	6	8	181	27	16	10	255	49
	III	40	7	9	152	27	8	14	250	50
Means		38	13	10	187	26	21	12	247	50
Inhibition as % of control		-21								

Table 15E Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia tracheiphila on 7th day after inoculation in agar and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g agar ($no. \times 10^8$)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	300	22	2	3	3	32	2	3	25
	II	252	12	8	4	2	26	9	4	20
	III	268	14	2	6	.2	9	6	1	22
Means		273	16	4	4	2	22	6	3	22
paired with T.longibranchiatum WBC 4576	I	222	12	6	70	22	29	16	100	43
	II	278	22	8	82	21	32	22	112	39
	III	271	25	9	53	21	30	28	92	40
Means		257	20	8	68	21	30	22	101	41
Inhibition as % of control		-6								

Table 15F Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia tracheiphila on 7th day after inoculation in loam and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g loam (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar	I	208	1	1	1	3	1	2	2	9
	II	166	2	2	1	2	2	1	1	10
	III	186	1	1	1	2	2	1	1	7
Means		187	1	1	1	2	2	1	1	9
paired with T.longibranchiatum WBC 4576	I	128	8	20	60	22	16	6	120	36
	II	109	19	18	98	24	29	8	130	38
	III	126	16	22	59	20	11	7	99	35
Means		121	14	20	72	22	19	7	116	36
Inhibition as % of control		-35								

Table 15G Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia tracheiphila on 7th day after inoculation in clay and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g clay (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	66	2	1	1	1	2	2	3	3
	II	52	2	2	1	2	1	3	2	4
	III	33	1	1	1	1	2	4	2	2
Means		50	2	1	1	1	2	3	2	3
paired with T.longibranchiatum WBC 4576	I	46	16	6	68	19	6	12	82	32
	II	34	9	7	72	24	7	7	98	36
	III	58	18	17	92	22	16	19	92	32
Means		46	14	10	77	22	10	13	91	33
Inhibition as % of control		-8								

Table 15H Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia tracheiphila on 7th day after inoculation in sand and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g sand (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	30	1	1	2	1	2	5	4	4
	II	48	1	1	2	1	1	6	3	6
	III	26	1	1	2	3	2	3	6	3
Means		35	1	1	2	2	2	5	4	4
paired with T.longibranchiatum WBC 4576	I	30	26	18	82	21	32	16	120	36
	II	33	42	32	62	19	48	28	148	36
	III	24	22	16	77	24	36	23	96	40
Means		29	30	22	74	21	39	22	121	37
Inhibition as % of control		-17								

Table 15I Record of effects of 7-day-old *Trichoderma viride* 1 on growth of *Erwinia aroideae* on 7th day after inoculation in agar and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g agar ($no. \times 10^8$)	1st day peak height (mm)				7th day peak height (mm)			
			Acet - ald.	Acet.	Eth.	CO_2	Acet- ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	426	9	3	36	3	19	18	22	16
	II	388	9	2	16	3	8	12	36	11
	III	416	8	2	33	3	18	25	48	10
Means		410	9	2	28	3	15	18	35	12
paired with *T.viride* 1	I	398	60	6	115	26	38	16	220	46
	II	353	65	5	177	29	32	11	200	48
	III	380	79	5	165	29	22	3	164	45
Means		377	68	5	152	28	31	10	195	46
Inhibition as % of control		-8								

Table 15J. Record of effects of 7-day-old Trichoderma viride 1 on growth of Erwinia aroideae on 7th day after inoculation in loam and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g loam ($no. \times 10^6$)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	120	9	4	10	3	4	8	12	12
	II	166	12	3	8	3	2	7	10	12
	III	152	6	3	9	3	6	6	15	12
Means		146	9	3	9	3	4	7	12	12
paired with T.viride 1	I	132	28	10	120	26	8	10	298	46
	II	120	28	10	114	29	12	14	230	45
	III	78	24	8	118	28	18	12	258	49
Means		110	27	9	117	28	13	12	262	47
Inhibition as % of control		-25								

Table 15K Record of effects of 7-day-old Trichoderma viride 1 on growth of Erwinia aroideae on 7th day after inoculation in clay and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g clay ($no.x10^6$)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	58	2	2	6	2	12	2	16	4
	II	31	1	2	7	2	3	3	17	1
	III	39	1	1	3	1	4	2	8	2
Means		43	1	2	5	2	6	2	14	2
paired with T.viride 1	I	39	12	16	100	26	4	6	159	43
	II	32	33	26	122	27	16	13	188	46
	III	49	19	6	98	25	8	2	110	44
Means		40	21	16	107	26	9	7	152	44
Inhibition as % of control		-7								

Table 15L Record of effects of 7-day-old *Trichoderma viride* 1 on growth of *Erwinia aroideae* on 7th day after inoculation in sand and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating.

Treatment	Assembly No.	Mean bacterial cell numbers/g sand (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	20	2	1	2	2	2	2	9	3
	II	6	1	1	6	3	2	2	8	4
	III	28	1	1	3	1	2	2	12	3
Means		18	1	1	4	2	2	2	10	3
paired with *T.viride* 1	I	33	6	12	220	25	28	6	252	47
	II	4	24	18	151	27	49	34	182	53
	III	9	16	9	162	27	26	20	235	51
Means		15	15	13	178	26	34	20	223	50
Inhibition as % of control		-17								

Table 15M Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia aroideae on 7th day after inoculation in agar and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g agar ($no. \times 10^8$)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	360	9	2	22	2	22	6	26	18
	II	240	3	2	12	4	13	8	38	12
	III	298	8	2	30	2	18	9	32	16
Means		299	7	2	21	3	18	8	32	15
paired with T.longibranchiatum WBC 4576	I	260	2	3	60	20	21	13	92	40
	II	312	29	14	92	22	38	22	118	38
	III	262	20	16	68	22	34	18	100	40
Means		278	17	11	73	21	31	18	103	39
Inhibition as % of control		-7								

Table 15N Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia aroideae on 7th day after inoculation in loam and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g loam (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	266	8	4	12	3	2	8	12	14
	II	320	8	3	18	3	3	6	3	11
	III	286	18	3	9	4	6	7	4	11
Means		291	11	3	13	3	4	7	6	12
paired with T.longibranchiatum WBC 4576	I	268	23	12	66	23	16	6	121	38
	II	252	25	11	78	25	29	18	132	40
	III	230	25	24	68	25	18	11	91	39
Means		250	24	16	71	24	21	12	115	39
Inhibition as % of control		-14								

Table 15 0 Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia aroideae on 7th day after inoculation in clay and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g clay (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	46	1	2	3	2	12	4	19	2
	II	52	1	1	3	2	3	5	6	3
	III	38	1	1	4	1	2	6	28	3
Means		45	1	1	3	1	6	5	18	2
paired with T.longibranchiatum WBC 4576	I	42	29	6	48	22	6	12	58	31
	II	38	22	17	72	24	3	8	108	35
	III	53	9	7	98	20	5	9	136	30
Means		44	20	10	73	22	5	10	101	32
Inhibition as % of control		-2								

Table 15P Record of effects of 7-day-old T.longibranchiatum WBC 4576 on growth of Erwinia aroideae on 7th day after inoculation in sand and G.L.C. analysis of volatile metabolites present immediately after pairing and before plating

Treatment	Assembly No.	Mean bacterial cell numbers/g sand (no. x 10^6)	1st day peak height (mm)				7th day peak height (mm)			
			Acet-ald.	Acet.	Eth.	CO_2	Acet-ald.	Acet.	Eth.	CO_2
paired with uninoculated 2% malt agar (Control)	I	22	1	1	1	2	1	2	9	3
	II	18	2	2	11	4	1	3	9	6
	III	28	2	1	9	4	1	3	12	5
Means		23	2	1	7	3	1	3	10	5
paired with T.longibranchiatum WBC 4576	I	20	32	18	89	20	30	19	100	35
	II	26	42	30	60	22	21	48	116	37
	III	18	40	36	68	23	44	48	120	41
Means		21	38	28	72	22	32	38	112	38
Inhibition as % of control		-9								

Table 15Q Record of survival of Erwinia tracheiphila stored with 7-day-old Trichoderma viride 1 and Trichoderma longibranchiatum WBC 4576 respectively for 7 days after inoculation in agar and equivalent layers of soil

Treatment	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered											
		(no. x 10^8) Agar Expt. No.			(no. x) Loam Expt. No.			(no. x 10^6) Clay Expt. No.			(no. x) Sand Expt. No.		
		I	II	III	I x10^6	II x10^8	III x10^8	I	II	III	I x10^6	II x10^4	III x10^6
paired with uninoculated 2% malt agar (Control)	I	388	443	432	220	533	500	60	467	120	36	549	100
	II	420	432	363	188	520	471	82	481	144	58	690	160
	III	366	489	363	236	557	479	48	310	86	49	622	141
Means		391	455	386	215	537	483	63	419	117	48	620	134
paired with T.viride 1	I	424	282	249	130	248	291	60	228	88	41	444	100
	II	312	247	179	121	152	237	41	201	93	33	397	122
	III	320	320	192	103	164	217	57	242	101	40	383	84
Means		352	283	207	118	188	248	53	224	94	38	408	102
Inhibition as % of control		-10	-38	-46	-45	-65	-49	-16	-47	-20	-21	-34	-24
paired with uninoculated 2% malt agar (Control)	I	300	520	-	208	369	-	66	63	-	30	57	-
	II	252	381	-	166	389	-	52	42	-	48	32	-
	III	268	411	-	186	412	-	33	46	-	26	48	-
Means		273	437	-	187	390	-	50	50	-	35	46	-
paired with T.longibranchiatum WBC 4576	I	222	390	-	128	280	-	46	30	-	30	49	-
	II	278	289	-	109	290	-	34	58	-	33	20	-
	III	271	422	-	126	260	-	58	38	-	24	42	-
Means		257	367	-	121	277	-	46	42	-	29	37	-
Inhibition as % of control		-6	-16	-	-35	-29	-	-8	-16	-	-17	-20	-

Table 15R Record of survival of *Erwinia aroideae* stored with 7-day-old *Trichoderma viride* 1 and *T.longibranchiatum* WBC 4576 respectively for 7 days after inoculation in agar and equivalent layers of soils

Treatment	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered									
		(no. x 10^8) Agar Expt. No.			(no. x 10^6) Loam Expt. No.			(no.x 10^6) Clay Expt. No.		(no. x 10^6) Sand Expt. No.	
		I	II	III	I	II	III	I	II	I	II
paired with uninoculated 2% malt agar (Control)	I	426	378	163	120	463	687	58	76	20	37
	II	388	384	233	166	525	627	31	73	6	31
	III	416	410	161	152	438	543	39	64	28	39
Means		410	391	186	146	475	619	43	71	18	36
paired with *T.viride* 1	I	398	281	120	132	340	520	39	69	33	40
	II	353	289	200	120	389	460	32	48	4	22
	III	380	240	110	78	300	300	49	66	9	32
Means		377	270	143	110	343	427	40	61	15	31
Inhibition as % of control		-8	-31	-23	-25	-28	-31	- 7	-14	-17	-14
paired with uninoculated 2% malt agar (Control)	I	360	378	-	266	282	-	46	109	22	33
	II	240	384	-	320	310	-	52	96	18	29
	III	298	410	-	286	278	-	38	98	28	42
Means		299	391	-	291	290	-	45	101	23	35
paired with *T.longibranchiatum* WBC 4576	I	260	260	-	268	250	-	42	99	20	33
	II	312	400	-	252	212	-	38	102	26	40
	III	262	384	-	230	252	-	53	82	18	18
Means		278	348	-	250	238	-	44	94	21	30
Inhibition as % of control		-7	-11	-	-14	-18	-	-2	-7	-9	-14

Table 16A Record of survival of Erwinia tracheiphila stored with known concentrations of authentic acetaldehyde for 7 days after inoculation in agar and equivalent layers of soil.

Acetaldehyde (ml liquid/l.air) used	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered											
		(no. x 10^8) Agar Expt. No.			(no. x 10^6) Loam Expt. No.			(no. x 10^6) Clay Expt. No.			(no. x 10^6) Sand Expt. No.		
		I	II	III	I	II	III	I	II	III	I	II	III
Nil (Control)	I	230	232	163	226	178	163	150	155	120	68	147	180
	II	220	200	181	177	229	180	183	153	170	42	130	113
	III	288	215	125	173	170	126	128	103	127	72	96	133
Means		246	216	156	192	193	156	154	137	139	61	124	142
0.001	I	280	242	128	182	200	208	170	144	110	23	130	89
	II	238	188	162	166	180	126	140	120	158	69	132	142
	III	232	184	144	216	170	100	146	144	130	88	116	182
Means		250	205	145	188	183	145	152	136	133	60	126	138
Inhibition or Stimulation as % of control		+2	-5	-7	-2	-5	-7	-1	-1	-4	-2	+2	-3
0.005	I	224	220	126	163	184	166	120	88	92	68	120	120
	II	276	168	158	208	152	98	166	148	98	44	120	140
	III	233	176	124	148	156	111	160	142	176	58	116	128
Means		244	188	136	173	164	125	149	126	122	57	119	129
Inhibition as % of control		-1	-13	-13	-10	-15	-20	-3	-8	-12	-7	-4	-9
0.01	I	204	166	133	98	120	118	130	100	96	50	100	120
	II	166	182	144	142	82	69	98	138	178	60	125	140
	III	146	150	125	168	114	70	148	62	89	52	120	106
Means		172	166	134	136	105	86	125	100	121	54	115	122
Inhibition as % of control		-30	-23	-14	-29	-45	-45	-19	-27	-13	-12	-7	-14
0.05	I	0.0	0.0	49	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	II	0.0	0.0	58	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	III	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Means		Nil	Nil	36	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Inhibition as % of control		-100	-100	-77	-100	-100	-100	-100	-100	-100	-100	-100	-100

Table 16B Record of survival of Erwinia tracheiphila stored with known concentrations of authentic acetone for 7 days after inoculation in agar and equivalent layers of soil.

Acetone (ml liquid/l.air) used	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered											
		(no. x 10^8) Agar Expt. No.			(no. x 10^6) Loam Expt. No.			(no. x 10^6) Clay Expt. No.			(no. x 10^6) Sand Expt. No.		
		I	II	III	I	II	III	I	II	III	I	II	III
Nil (Control)	I	330	288	380	400	420	340	156	216	142	48	122	93
	II	280	320	406	362	460	320	198	167	166	31	85	77
	III	296	346	362	380	396	286	136	192	124	33	108	112
Means		302	318	383	381	425	315	163	192	144	37	105	94
0.001	I	268	312	400	380	408	312	136	160	120	32	108	88
	II	330	298	377	400	418	280	186	220	204	33	128	96
	III	326	362	417	384	436	335	146	190	120	42	70	84
Means		308	324	398	388	421	309	156	190	148	36	102	89
Inhibition or Stimulation as % of control		+2	+2	+4	+2	-1	-2	-4	-1	+3	-3	-3	-5
0.05	I	300	260	378	288	400	300	160	232	200	38	100	80
	II	260	342	405	236	396	282	182	164	120	31	61	88
	III	328	344	332	562	404	280	126	156	102	40	136	76
Means		296	315	372	362	400	287	156	184	141	36	99	81
Inhibition as % of control		-2	-1	-3	-5	-6	-9	-4	-4	-2	-3	-6	-14
0.1	I	232	268	300	308	300	208	178	208	208	22	60	72
	II	244	222	312	280	280	200	148	174	108	29	82	77
	III	304	284	285	258	314	224	140	143	64	30	68	52
Means		260	258	299	282	298	211	155	175	127	27	70	67
Inhibition as % of control		-14	-19	-22	-26	-30	-33	-5	-9	-12	-27	-33	-29

Table 16C Record of survival of Erwinia tracheiphila stored with known concentrations of authentic ethanol for 7 days after inoculation in agar and equivalent layers of soil.

Ethanol (ml liquid/l.air) used	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered (no. x 10^8) Agar Expt. No. I	II	III	(no. x 10^6) Loam Expt. No. I	II	III	(no. x 10^6) Clay Expt. No. I	II	III	(no. x 10^6) Sand Expt. No. I	II	III
Nil (Control)	I	380	329	181	360	323	225	226	214	244	82	186	99
	II	420	277	277	388	194	246	160	275	188	59	108	61
	III	388	270	361	362	280	258	192	227	208	66	133	89
Means		396	292	273	370	266	243	193	239	213	69	142	83
0.03	I	292	266	360	360	225	240	200	214	200	98	130	88
	II	380	300	280	358	266	214	180	240	232	89	166	82
	III	552	319	170	370	244	224	204	242	201	23	126	74
Means		408	295	270	363	245	226	195	232	211	70	141	81
Inhibition or Stimulation as % of control		+3	+1	-1	-2	-8	-7	+1	-3	-1	+1	-1	-4
0.1	I	420	320	224	328	230	200	200	120	280	66	100	77
	II	360	300	288	368	266	192	166	208	200	80	159	67
	III	384	230	298	370	215	199	202	323	126	58	128	75
Means		388	283	270	355	235	197	189	217	202	68	129	73
Inhibition as % of control		-2	-3	-1	-4	-12	-19	-2	-9	-5	-2	-9	-12
0.3	I	300	102	208	228	118	140	150	200	130	50	90	55
	II	255	206	170	250	116	142	200	162	208	80	108	20
	III	300	188	154	232	116	136	112	184	188	36	94	75
Means		285	165	177	237	117	139	154	182	175	55	97	50
Inhibition as % of control		-28	-43	-35	-36	-56	-43	-20	-24	-18	-20	-32	-40
0.6	I	220	100	88	166	40	88	87	120	92	2	30	21
	II	282	140	68	142	44	81	144	161	122	41	36	1
	III	164	102	9	158	36	87	93	141	120	22	32	22
Means		222	114	55	155	40	85	108	141	111	22	33	15
Inhibition as % of control		-44	-61	-80	-58	-85	-65	-44	-41	-48	-68	-77	-82

Table 16D Record of survival of Erwinia tracheiphila stored with known concentrations of authentic CO_2 for 7 days after inoculation in agar and equivalent layers of soil

CO_2 in air mixture (vol./vol.) put with each treatment	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered											
		(no. x 10^8) Agar Expt. No.			(no. x 10^6) Loam Expt. No.			(no. x 10^6) Clay Expt. No.			(no. x 10^6) Sand Expt. No.		
		I	II	III	I	II	III	I	II	III	I	II	III
Air (0.03%)	I	422	447	304	229	360	486	332	354	420	180	127	88
	II	386	450	285	280	329	446	260	375	380	200	147	128
	III	392	484	233	266	314	471	240	353	388	166	193	116
Means		400	460	274	258	335	468	277	261	396	182	156	111
20%	I	410	320	200	188	220	300	200	221	444	166	120	80
	II	350	410	222	180	182	256	228	232	360	158	142	82
	III	332	374	220	176	180	314	254	186	276	147	104	72
Means		364	368	214	181	194	290	227	213	360	157	122	78
Inhibition as % of control		-9	-20	-22	-30	-42	-38	-18	-18	-9	-14	-22	-30
30%	I	380	226	120	140	120	200	250	208	300	140	108	66
	II	300	312	156	146	102	226	268	222	330	122	120	98
	III	328	286	126	146	130	192	148	162	260	164	74	37
Means		336	275	134	144	117	206	222	197	297	142	101	67
Inhibition as % of control		-16	-40	-51	-44	-65	-56	-20	-24	-25	-22	-35	-40

Table 16E Record of survival of *Erwinia aroideae* stored with known concentrations of authentic acetaldehyde for 7 days after inoculation in agar and equivalent layers of soils

Acetaldehyde (ml liquid/l.air) used	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered (no. x 10^8) Agar Expt. No. I	II	III	(no. x 10^6) Loam Expt. No. I	II	III	(no. x 10^6) Clay Expt. No. I	II	(no. x 10^6) Sand Expt. No. I	II
Nil (Control)	I	380	256	274	260	209	185	166	228	120	98
	II	428	285	297	298	223	203	123	166	144	128
	III	400	236	263	222	220	224	143	178	132	116
Means		403	259	278	260	217	204	144	191	132	114
0.001	I	400	302	270	258	200	212	92	188	124	91
	II	426	242	312	232	248	188	166	111	132	144
	III	407	218	242	260	190	194	182	262	124	100
Means		411	254	275	250	213	198	147	187	127	112
Inhibition or Stimulation as % of control		+2	-2	-1	-4	-2	-3	+2	-2	-4	-2
0.005	I	426	300	260	252	206	160	96	208	122	100
	II	358	228	280	231	188	212	156	168	142	60
	III	316	234	270	228	230	152	170	180	116	172
Means		367	254	270	237	208	175	141	185	127	111
Inhibition as % of control		-9	-2	-3	-9	-4	-14	-2	-3	-4	-3
0.01	I	422	260	280	220	210	127	130	66	94	116
	II	290	212	210	132	198	158	98	168	122	118
	III	330	242	270	212	109	156	156	294	144	84
Means		347	238	253	188	172	147	128	176	120	106
Inhibition as % of control		-14	-8	-9	-28	-21	-28	-11	-8	-9	-7
0.05	I	88	24	30	0.0	0.0	0.0	0.0	22	0.0	2
	II	67	78	4	0.0	48	0.0	32	102	0.0	14
	III	88	70	50	0.0	81	0.0	19	95	0.0	14
Means		81	57	28	0.0	43	0.0	17	73	0.0	10
Inhibition as % of control		-80	-78	-90	-100	-80	-100	-88	-62	-100	-91

Table 16F Record of survival of Erwinia aroideae stored with known concentrations of authentic acetone for 7 days after inoculation and equivalent layers of soils

Acetone (ml liquid/l.air) used	Assembly No.	Mean bacterial cell numbers/ gram of substrate recovered (no. x 10^8) Agar Expt. No. I	II	III	(no. x 10^6) Loam Expt. No. I	II	III	(no.x 10^6) Clay Expt. No. I	II	(no. x 10^6) Sand Expt. No. I	II
Nil (Control)	I	420	301	267	320	215	296	180	200	89	122
	II	388	244	288	390	265	275	162	158	63	144
	III	392	252	238	362	230	273	103	166	106	108
Means		400	266	264	357	237	281	148	175	86	125
0.001	I	400	306	300	340	228	290	98	186	82	120
	II	388	260	288	362	256	262	182	162	85	144
	III	448	250	212	357	242	267	160	192	88	108
Means		412	272	267	353	242	273	147	180	85	124
Inhibition or Stimulation as % of control		+3	+3	+1	-1	+2	-3	-1	+3	-1	-1
0.05	I	412	300	220	320	250	260	68	132	88	120
	II	320	260	258	360	226	251	160	92	63	132
	III	444	200	290	358	230	257	204	292	100	117
Means		392	253	256	346	235	256	144	172	84	123
Inhibition as % of control		-2	-5	-3	-3	-1	-9	-3	-2	-2	-2
0.1	I	400	260	232	340	218	240	34	200	88	150
	II	226	230	240	380	220	260	180	122	63	42
	III	550	260	224	298	216	200	202	182	92	122
Means		392	250	232	339	218	233	139	168	81	105
Inhibition as % of control		-2	-6	-12	-5	-8	-17	-6	-4	-6	-16

Table 16G Record of survival of Erwinia aroideae stored with known concentrations of authentic ethanol for 7 days after inoculation in agar and equivalent layers of soils.

Ethanol (ml liquid/l.air) used	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered									
		(no. x 10^8) Agar Expt. No.			(no. x 10^6) Loam Expt. No.			(no. x 10^6) Clay Expt. No.		(no. x 10^6) Sand Expt. No.	
		I	II	III	I	II	III	I	II	I	II
Nil (Control)	I	260	276	377	172	236	164	180	192	88	92
	II	312	216	377	228	188	176	152	122	98	144
	III	252	226	379	206	238	157	132	177	124	86
Means		275	239	378	202	221	166	155	164	103	107
0.03	I	300	222	412	214	208	162	160	212	88	138
	II	226	282	360	180	249	165	84	142	132	146
	III	275	207	338	200	200	163	236	130	80	25
Means		267	237	370	198	219	163	160	161	100	103
Inhibition or Stimulation as % of control		-3	-1	-2	-2	-1	-2	+3	-2	-3	-4
0.1	I	256	252	188	175	208	132	106	93	31	121
	II	316	220	389	162	190	140	186	183	112	48
	III	205	224	488	184	193	130	158	202	144	116
Means		259	232	355	174	197	134	150	159	96	95
Inhibition as % of control		-6	-3	-6	-14	-11	-19	-3	-3	-7	-11
0.3	I	254	186	320	159	180	106	133	152	92	88
	II	222	226	266	139	156	96	202	98	98	86
	III	260	212	344	158	134	132	73	202	86	83
Means		245	208	310	152	157	111	136	151	92	86
Inhibition as % of control		-11	-13	-18	-25	-29	-33	-12	-8	-11	-20
0.6	I	200	151	204	88	102	40	109	120	66	52
	II	185	166	254	81	82	85	98	100	68	48
	III	201	142	222	80	74	10	108	86	59	53
Means		195	153	227	83	86	45	105	102	64	51
Inhibition as % of control		-29	-36	-40	-59	-61	-73	-32	-38	-38	-52

Table 16H Record of survival of Erwinia aroideae stored with known concentrations of authentic carbon dioxide for 7 days after inoculation in agar and equivalent layers of soils

CO_2 in air mixture (vol./vol.) put with each treatment	Assembly No.	Mean bacterial cell numbers/gram of substrate recovered									
		(no. x 10^8) Agar Expt. No.			(no. x 10^6) Loam Expt. No.			(no.x10^6) Clay Expt. No.		(no. x 10^6) Sand Expt. No.	
		I	II	III	I	II	III	I	II	I	II
Air (0.03%)	I	402	433	516	320	453	411	186	226	99	68
	II	360	433	482	346	438	386	265	180	87	128
	III	366	467	480	381	428	386	166	188	127	106
Means		376	444	493	349	440	394	206	198	104	101
20%	I	350	312	514	300	358	300	208	186	62	92
	II	321	400	440	288	324	332	166	185	104	98
	III	355	420	494	332	362	352	188	180	128	89
Means		342	377	483	307	348	328	187	184	98	93
Inhibition as % of control		-9	-15	-2	-12	-21	-17	-9	-7	-6	-8
30%	I	332	322	386	281	288	280	184	166	88	92
	II	294	277	472	300	282	292	166	182	62	68
	III	367	362	474	278	300	247	194	138	110	83
Means		331	320	444	286	290	273	181	162	87	81
Inhibition as % of control		-12	-28	-10	-18	-34	-31	-12	-18	-16	-20

Table 17A Changes in G.L.C. peak heights produced by samples of volatiles of authentic acetaldehyde (0.01 ml liquid/ litre of air space) in test assemblies in agar, loam, clay and sand compared to dry air under uniform conditions.

Substrate	Bottle No.	G.L.C. mean peak height (mm)			
		Immediately after assemble	1st day	4th day	7th day
Dry air	I	290	282	270	252
	II	320	330	302	268
	III	274	288	269	260
Means		295	300	280	260
Agar	I	260	180	130	100
	II	240	186	120	112
	III	292	198	146	103
Means		264	188	132	105
Loam	I	300	252	240	195
	II	260	245	236	200
	III	286	260	242	188
Means		282	252	239	194
Clay	I	270	230	206	180
	II	298	232	200	160
	III	284	240	220	168
Means		284	234	209	169
Sand	I	330	292	280	264
	II	292	290	280	270
	III	312	300	292	268
Means		311	294	284	267

Table 17B Changes in G.L.C. peak heights produced by samples of gases of authentic CO_2 (30% volume/volume) in test assemblies of agar, loam, clay and sand compared to dry air under uniform conditions.

Substrate	Bottle No.	G.L.C. mean peak height (mm)			
		Immediately after assemble	1st day	4th day	7th day
Dry air	I	45	45	44	44
	II	51	49	48	48
	III	48	47	46	46
Means		48	47	46	46
Agar	I	48	44	40	38
	II	45	40	35	33
	III	46	40	34	32
Means		46	41	36	34
Loam	I	52	48	47	46
	II	50	46	45	44
	III	51	48	47	45
Means		51	47	46	45
Clay	I	48	45	40	40
	II	52	48	44	44
	III	47	46	43	42
Means		49	46	42	42
Sand	I	50	48	48	45
	II	52	50	50	47
	III	50	50	49	45
Means		51	49	49	46