MODELLING THE INTERACTION OF STREPTOMYCETES AND THEIR PHAGE

'Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Lesley Noelle Manchester.'

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SUMMARY

The interaction of streptomycetes and their phage in a natural environment, the soil, was examined by the application of the Nicholson-Bailey model for host-instect parasitoids utilizing data obtained from both broth and soil experiments. The experimental work undertaken included examination of phage and streptomycete spore survival, phage replication cycles, streptomycete growth in soil and phage adsorption under varying streptomycete and phage densities.

Simple soil experiments were also performed to examine the extent and nature of the streptomycete-phage interaction occurring in a soil and to provide additional data for use in modelling the streptomycete-phage systems. Simple increases in phage numbers similar to those obtained in one-step growth experiments was observed, the increase occurring within the first 72h of inoculation. This increase was concurrent with a decrease in the host population.

The experimentally obtained data together with logical alterations of the model parameters were used to provide information on the relative importance and the theoretical role of the parameters on the final outcome of the interaction, and also suggested further areas for investigation. It was possible to obtain a sustained but chaotic interaction with the simple model. Parameter "a", the infection efficiency was observed to play a considerable role in the outcome of the interaction. The introduction of both constant number and proportion refuges in order to provide some account of the heterogeneity of the environment and populations indicated that it is possible to obtain a stable interaction between streptomycetes and their phage within a soil system.

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CHAPTER 1

INTRODUCTION

Microbial communities in soil ecosystems display many of the properties and characteristics of higher organisms. Disturbance, habitat size and competition are all features common to both (Gooding & McCalla, 1945; Finstein & Alexander, 1962; Clark, 1965; Mayfield, 1969; Mayfield et al., 1972). Predation in the sense of one organism utilizing another as a primary resource for growth or reproduction is an additional feature. The interaction between phage and bacteria constitutes one such example.

Within any environment, either natural or in laboratory culture, the precise nature of the interaction of bacterial and phage populations is likely to be of considerable importance in the survival, persistence and evolution of both the bacterial and phage populations. Some bacterial-phage systems have already been investigated experimentally in natural environments, such as *Bacillus* phage in soil (Reanney & Marsh, 1973; Tan & Reanney, 1976), *Arthrobacter globiformis* phage in soil (Casida & Liu, 1974), rumen bacteria and their associated phage (Orpin & Munn, 1974). Various laboratory cultures have been investigated but the *Escherichia coli* + phage systems have received by far the most attention (Roper & Marshall, 1974; Gaspar et al., 1979). However there is little or no information on the interaction of streptomycetes and their phage in natural environments, possibly because of the difficulties involved with working with a filamentous microbe as opposed to unicellular ones. Work which is available concentrates mainly on individual aspects of the interaction, such as the effect of soil pH and colloids on actinophage (Sykes & Williams,

197[illegible]; Sykes, Lanning & Williams, [illegible]; Williams & Lanning, [illegible]), and the growth and germination of streptomycete spores in soil, (Mayfield _et al._, 1972).

The lytic action of phage upon streptomycete was observed in the 1930's and 1940's, when phage were first detected in soil by Wiebols & Wieringe (1936), when studying potato scab and later by Muhlens (1940). However actinophage did not assume any importance until they were observed as a contaminant during the industrial production of antibiotics by _Streptomyces griseus_ (Bennett, 1946). Later other phage specific to antibiotic-producing streptomycetes were discovered. Therefore the practical consequences of phage infection led to the investigation of ways of selectively eliminating phage, which incidentally provided information on various aspects of the streptomycete-phage systems.

In natural systems the streptomycete occupies a niche which is dependent upon the environmental conditions and nutrient availability, and also the presence of other competing microbes, as shown by the study of _Streptomyces albus_ (Hagen _et al._, 1968). The actual niche which the streptomycete _does_ occupy as distinct to that which it _could_ occupy is known as its "realised niche" (Hutchinson, 1957). Echols (1978) stated that a phage either has no niche, _i.e._ it is continually being created and then lost, or that it does have one, being present in a predator-prey equilibrium or being of net benefit to the host bacteria. Campbell (1961) stated that

> 'the fact that a free-living organism occurs in nature at all constitutes _prima facie_ evidence that it is representative of a species which can maintain itself in some ecological niche'.

The dimensions of the phage niche will hence be determined by environmental resources and also on whether the phage is able to form

a lysogenic association with the host.

Streptomycetes and their phage can be isolated from several environments, including soil and freshwater sediments (Willoughby, 1976). However the main source of streptomycetes and their phage is the soil, where streptomycetes form the majority of the actinomycetes isolated, although this may be a reflection of the isolation methods used. The sustained persistence of phage and streptomycetes, in the same environment may be an indication that there is some balanced dynamical interaction between them. Direct observation of streptomycetes in soil has shown them to be present discontinuously distributed in micro-sites which primarily consist of organic fragments such as humic materials derived from the partial decomposition of litter and dead root debris (Mayfield et al., 1972). The relative abundance of streptomycetes will be determined by the availability of resources, environmental conditions and possibly the presence of phage although the effect of the latter is not generally understood. The typical life cycle of streptomycetes (spore - germ tube - mycelium - spore) in soil was observed to occur by Pfennig (1958). Kutzner (1981) surveyed 18 papers and found that the numbers of actinomycete propagules varied from 10^4 - 10^7 colony forming units (c.f.u) g^{-1} soil. The propagules counted would be mainly spores which were shown to be the main form of actinomycetes/streptomycetes found in soil (Lloyd, 1969). The distribution of streptomycete spores in soil is known to be heterogeneous, a reflection of their sites of production and their dispersal, (Mayfield, 1969; Mayfield et al., 1972).

Until recently the number of estimates of the phage populations within the soil environments were limited, most authors being concerned with isolating novel phage and assessing the variety of types within a soil. Reanney & Marsh (1973) stated that if every phage type occurred

in soil at 0.01% of the titres obtained in the laboratory then they would be the most numerous genetic forms present within the soil. Lanning & Williams (1982) observed streptomycete phage population sizes varied from 4.7×10^4 to 295 plaque forming units (p.f.u.) g^{-1} soil, the size being dependent on the recovery method used and the type of soil from which they were isolated. Equally the phage population at any one time may be a reflection of the activity of the host population.

It is clear, therefore, that phage do exist in the soil and can be isolated either directly or by the enrichment of the soil with a specific host. However, their exact form in soil is still unclear. Phage can exist in two forms, either as a prophage in a lysogenic association with the host or as a virion, external to the host cell. The prophage is probably protected from hostile changes in the external environment unless the host is adversely affected. Another lysogenic phenomenon is that of pseudo-lysogeny, first reported for streptomycetes by Welsch (1956). Pseudo-lysogeny differs from true lysogeny in that the phage is carried from sub-culture to sub-culture without the integration of the phage at the nuclear level which characterises as true lysogeny. True lysogeny in actinomycetes was first reported in 1956 by Alexander & McCoy, Shirling and Welsch. Many lysogenic streptomycetes have since been isolated from soil (Rangarajan, 1966; Lomovskaya et al. 1972; Dowding & Hopwood, 1973; Ogata et al., 1981) and it was suggested by Reanney & Akermann (1982) that most phage genomes probably exist in nature integrated into the DNA of their host cells.

The ecology of streptomycetes phage in soil systems has received little attention in relation to their number, distribution and their response to environmental factors and indeed Lanning & Williams (1982) stated that,

'Phage are a neglected entity. Our knowledge of the ecology of the streptomycete phage and other bacteriophage clearly lags behind that of their hosts.'

The first comprehensive study of the enumeration of streptomycete phage in soils and the effect of various environmental factors in soil was performed by Williams & Lanning (1985). They indicated that phage were widespread and more numerous in soil than previously realised. Free phage particles tolerated a wide range of environmental conditions but were very sensitive below pH 5.0. Sykes (1977) showed that phage could not be isolated from soils of less than pH 5.5, even from the acidic horizons when potential acidophilic streptomycete hosts were available

One of the major problems in studying the streptomycete-phage system *in vivo* is the complex nature of the environment involved, namely the soil. The soil as an environment is by no means homogeneous gross differences being implicit in the recognition of different horizons. In all natural and cultivated soils there are smaller scale variations resulting from the intrusion of plant roots, adsorption and leaching of soluble nutrients, and the unequal distribution of particulate matter. There is highly localized variation in the concentration of solutes and gases in the soil matrix and this leads to the formation of micro-environments, their size being commensurate with that of microbiological growth sites. Also, at the surface of soil particles and at the surface of microbes, there exists a molecular environment characterized by gradations in ion concentrations, which contributes further to the heterogeneity of the environment. Microbes influencing the structure of the soil by the production of polysaccharides are major agents of aggregation and can lengthen or block the pores. Microbial cells can also hold the soil particles together by mechanical binding. The soil is then a labyrinth of interconnecting pores, mainly

mineral in nature where the pores are either water or gas-filled. The gas:water ratio is important in determining the type of microbial activity which will occur. The water phase of the soil, in particular that held in by gravitational force, carries the dissolved compounds of various types and forms, such as organic compounds, inorganic ions and dispersed colloidal particles, and serves as liquid nutrient medium for the soil microbes.

In microbial terms the soil is regarded as being a nutrient-poor environment for the chemo-organotrophic microbes with nutrients being available only in a discontinuous distribution both in time and space (Williams, 1985). This leads to the appearance of selected sites within the soil where the concentration of energy substrates is relatively high. Associated with these sites are zymogenous bacteria (Winogradsky, 1924) which exist mainly in a resting phase with brief periods of activity in the presence of suitable nutrients. Streptomycetes can therefore be classed as zymogenous since they are thought to exist for the most part as spores and exhibit brief spates of growth and sporulation when suitable nutrients are available. The source of these nutrients can be the organic fragments previously mentioned; and also fungal hyphae which have been shown to lead to significant increases in streptomycete colonies upon their addition to soil (Williams & Robinson, 1981). The heterogeneous nature of the soil by affecting the growth of the streptomycete could alone lead to a variety of streptomycete-phage interactions within a soil system.

Such interactions can be studied by examining the increase and decreases of the host and phage. However this does not indicate the nature of the interaction within individual sites in a soil system, it reflects only the balance within the soil as a whole. There are

also numerous other factors which can affect population levels of the host other than that of the hostile action of the phage and hence the size of the phage population. Therefore, it is difficult to make anything more than generalised statements on the nature and extent of the interaction occurring between the streptomycetes and their phage. To minimize this difficulty, the nature of the interaction and the factors which directly affect it can be assessed by:-

a) Observing the interaction in simulated soil systems and simplified laboratory situations;

b) Using the data obtained from the above experiments in, and together with simulation models to obtain predictions of events which may occur in the soil, both in the short term but more particularly in relation to the long-term dynamics of the streptomycete-phage system. Individual aspects of the interaction can be assessed, as can environmental factors, by their effect on the individual parameters and their subsequent effect on the interaction. The significance of particular phenomena, such as lysogeny, genetic changes and colloidal adsorption may also be assessed.

Models are conceptual frameworks which enable understanding of the general population dynamics of the system being studied to be achieved. They are usually in the form of an equation or equations which can be illustrated by graphs, and need to be general, realistic, precise and simple. Berthet (1977) states that

> 'the simulation model constitutes a very powerful tool in the study of population dynamics, mainly because it obliges us to clarify our ideas. Moreover modelling obliges us to formulate a unifying concept which should take into account all knowledge extracted from the literature as well as from preceding experience.'

Smith (1982) examining some modelling of soil microbiology stated that

'Success depends first on including enough detail to encompass the fundamentals, and secondly on recognizing what can be learned from simulation and what cannot.'

The aim here was to explore the area of modelling the interaction of streptomycetes and phage, although it was recognized that this would probably not be totally satisfactory since this was a first foray into this area of microbial ecology. The model selected was the Nicholson-Bailey model (Nicholson & Bailey, 1935) used to describe a similar interaction, that of the insect and its parasitoid. The model comprises a pair of coupled difference equations and in its basic form is:-

$$H_{t+1} = H_t \exp(r(1-H_t/K)-aP_t)$$

$$P_{t+1} = cH_t(1-\exp(-aP_t))$$

where

H_{t+1} & H_t = the number of insects at time t & t+1

P_{t+1} & P_t = the number of parasitoids at time t & t+1

r = the intrinsic rate of increase of the host

K = carrying capacity of the environment

c = number of parasitoids produced per host infected

a = searching efficiency

The parameters in the insect-parasitoid model can be readily equated to those in the streptomycete-phage system, the parallels being shown in Table 1. Furthermore it clearly highlights the biological descriptions and experimental work required for its application. The two systems 'match up' well because of the similarity in the action of the attacking entities, the parasitoid and the phage. Both infect a host and prevent its further growth, and delay the death of the host until they are ready to be released and can support themselves.

PARAMETER	DESCRIPTION FOR INSECT-PARASITOID INTERACTION	DESCRIPTION FOR STREPTOMYCETE-PHAGE INTERACTION
H_{t+1} & H_t	Number of insects at time t and t+1	Number of streptomycete infectable units at time t and t+1
P_{t+1} & P_t	Number of parasitoids at time t and t+1	Number of phage at time t and t+1
r	Intrinsic rate of increase of insects	Intrinsic rate of increase of streptomycete
K	Carrying capacity of the environoment	Carrying capacity of the environment
c	Number of parasitoids produced per host infected	Phage burst size
a	Searching efficiency	Infection efficiency

Table 1:- Parameter description for the insect-parasitoid and streptomycete-phage interactions

The aim of this work was to obtain more information on the streptomycete-phage interaction in the soil. Individual aspects of the streptomycete-phage interaction were examined for defined conditions which were as near to soil-like conditions as possible. The quantitative results of these investigations were used in a simple model in an attempt to simulate the interaction of streptomycetes and their phage in a soil system, to indicate the possible outcomes of interactions within the soil, and which aspects of the interaction are of prime importance in the final outcome.

CHAPTER 2

PARAMETER INVESTIGATION AND ASSESSMENT OF THE STREPTOMYCETE - PHAGE INTERACTION

I. INTRODUCTION

As already stated, little quantitative information is available on the interaction of streptomycete and their phages in the soil environment or on some of the individual aspects of the interaction, *e.g.* streptomycete growth in soil, the carrying capacity of the soil environment. The survival of streptomycete phage and spores has received some attention, as has the germination of spores in soil (Lloyd, 1969; Mayfield, 1969; Mayfield *et al.*, 1972; Sykes, 1977). Therefore it was necessary to assess some of the parameters within the Nicholson-Bailey model as applied to the streptomycete-phage system. These assessments were made either in the soil environment or in soil-like model systems.

The model parameters can be divided into two groups, those which deal with the availability of the phage or streptomycetes and those which describe the extent of the interaction. Those dealing with the former are:-

a) Parameter "c":- representing the quantity of phage produced per host when a successful infection occurs.

b) Parameter "r":- representing the growth of the streptomycete in the soil and termed the intrinsic rate of increase. This is a population parameter.

c) Parameter "K":- the carrying capacity of the environment, reflecting the maximum number of streptomycetes which the soil environment will support.

The actual interaction and the extent to which it occurs is described by parameter "a", which is in this instance termed the "infection efficiency".

Therefore estimates were made of the individual parameters described above in soil, or as near soil-like conditions as possible, for streptomycetes, MX1, MX8 and ISP 5069, although some investigations were performed only on MX1 and MX8. In addition to the parameters described, phage survival was investigated with a view to including a new parameter in the model which reflected the loss of phage from the environment assumed to be due to inactivation by adverse conditions. These investigations provided not only numerical assessments of the parameters, but also more information on the behaviour of streptomycetes and their phages in soil and in laboratory cultures.

II. GENERAL MATERIALS AND METHODS

This section gives details of techniques used routinely throughout this study.

A) GENERAL HOST CULTURE TECHNIQUES

Three streptomycetes were used in this study, details of which are given in Table 2.

i) PROPAGATION AND HARVESTING

All streptomycetes were grown on media giving optimal growth and sporulation:-

a) ISP 5069 (*S. lavendulae*) on oatmeal agar (Shirling & Gottlieb, 1966).

b) MX1 (*S. albidoflavus*) on yeast-malt extract agar (Pridham *et al.*, 1956-1957).

c) MX8 (*S. albidoflavus*) on Bennett's agar (Williams *et al.*, 1983).

They were incubated at 25°C for 7 - 14 days until heavy sporulation occurred.

Spores were harvested by scraping the surface of two plates into 10ml of sterile 10% v/v glycerol. The suspension was agitated vigorously with sterile glass beads for 10 mins, to break up spore chains and to separate spores from mycelium and mycelial fragments. The crude spore suspension was filtered through sterile glass wool to remove mycelial debris. Finally, it was passed through a 10µm teflon membrane filter to obtain a pure spore suspension. Before use, the suspensions were shaken to reduce the possibility of clumping. The spore suspensions were enumerated using a Thoma counting slide and were stored at -20°C, the glycerol acting as a cryoprotectant (Wellington and Williams, 1978).

CULTURE	NAME	CORRESPONDING PHAGE	ORIGIN OF HOST AND PHAGE
ISP 5069	*Streptomyces lavendulae*	ø85	Culture collection
MX1	*Streptomyces albidoflavus*	ømx1	Soil isolate*
MX8	*Streptomyces albidoflavus*	ømx8	Soil isolate*

Table 2:- Details of the streptomycetes used throughout this study

* Supplied by S. Lanning

B) GENERAL PHAGE CULTURE TECHNIQUES

The phages used in this study are listed in Table 2 with their corresponding host.

i) PREPARATION OF PHAGE STOCKS

High titre stocks were obtained using the confluent plate lysate method.

Host spores were inoculated onto a peptone-yeast calcium agar plate (PYCa) (Bradley et al., 1961). Approximately 1ml of the corresponding phage was placed in the centre of the plate and incubated overnight. The clear zone of lysis was removed and placed in 5ml of PYCa broth, which was shaken gently and allowed to stand for at least 2h at 4°C. The broth was centrifuged at 1,200g for 15 min. The supernatant was membrane-filtered and the filtrate stored at 4°C for further use.

ii) MEMBRANE-FILTRATION

Large volumes (20ml) were membrane-filtered through sterile millipore vacuum filtration units with filters of 47mm diameter and 0.45μm pore size.

Small volumes (20ml) were filtered through Swinnex filter units with filters also of 0.45μm pore size.

iii) PHAGE ASSAY

Phage assays were performed by the usual double layer method (Adams, 1959).

Serial ten-fold dilutions of the phage to be assayed were prepared in PYCa broth. 0.1ml of dilutions to be assayed were added to 2.5ml of 0.6% (w/w) PYCa agar together with 0.1ml of host spore suspension. The inoculated soft agar was poured over a basal layer of PYCa agar and allowed to set. All plates were incubated at 25°C for 24 - 48 hr depending on the requirements of the phage and host plated.

III. INVESTIGATION AND ENUMERATION OF THE EFFECT OF TEMPERATURE ON PHAGE REPLICATION CYCLES, WITH PARTICULAR REFERENCE TO THE EFFECT ON BURST SIZE

i) Introduction

In a soil system, apart from immigration, the only source of phage will be replication, either from lytic phage or the reversion of a phage in a lysogenic association to the lytic cycle.

In the simple Nicholson-Bailey model the only source of phage considered is that produced through successful infection of the host. The size of the pool of phage available is therefore dependent on the number of phage produced per infected host - the burst size. This is denoted in the model as parameter "c".

Once a successful adsorption and entry into the host cell has been effected, a period of time elapses known as the latent period. This is the time interval between infection and the final release of complete phage progeny from the host cell. During the latent period new phage components are synthesized and assembled. The interval between infection and assembly of new phage particles is the eclipse period. Once phage are assembled, a short period of maturation occurs which is followed by lysis of the host cell and the release of the phage particles.

The number of phage progeny has been shown to vary between *Streptomyces* species, (Alexander & McCoy, 1956; Sykes, 1977; Lomovskaya *et al.*, 1980). To obtain a numerical assessment of the contribution to phage numbers by a single lytic infection of the host and to assess the other parameters of the replication cycle, one step growth experiments were performed. The one step growth experiment was first developed by Ellis & Delbruck (1939) for use with unicellular

bacteria and it enables the infective properties of a phage to be investigated. With this technique burst size and latent periods can be numerically assessed. Apart from characterising the phage, it enables one to determine the effect of changes in environmental conditions on the infectious properties of a phage and hence its likely success under a given set of conditions.

Use of a one step experiment with actinomycetes presents some problems because of the mycelial nature of these organisms. The network nature of the mycelium prevents the exact determination of the number of phage adsorbed, and thus it is difficult to quantitatively assess the number of phage produced per unit length of mycelium. This problem can be minimized by using newly germinated spores; as Gilmour, Noller and Watkins (1959) stated,

> "the newly germinated spore is the closest facsimile to the unicellular condition".

Use of this growth stage also minimizes clumping which can lead to an artificially high burst size (Dowding, 1973).

Generally mean temperatures occurring in soil are much lower than those usually used in laboratory studies (25°C). Therefore, in order to obtain a more reasonable measurement of the replication cycle parameters of the phage in soil, the effects of temperature which were similar to those occurring in soil were investigated.

ii) Method

Host spores were added to 30ml PYCa broth (pH7) to give a final concentration of 5×10^7 colony forming units (c.f.u.) ml^{-1}. Germination was allowed to proceed at the relevant temperature until the emerging germ tubes were 3 - 8x the length of the original spore. The number of spores at the required stage of germination was determined with the use of a Thoma counting slide. Then potassium cyanide was added to give a final concentration of $10^{-4}M$, to temporarily poison the host by acting as a respiratory inhibitor and hence allow synchronous infection.

Phage were added to give a m.o.i. of 0.01pfu $germling^{-1}$ and adsorption was allowed to proceed for 30 min. A 1ml sample was removed from the adsorption vessel and filtered through a membrane filter of 0.45µm pore size. The filter was washed with sterile PYCa broth (pH7) to remove any remaining free phage. It was then carefully suspended in a flask containing 50ml of fresh PYCa broth (pH7), previously equilibrated to the relevant temperature which was placed on an orbital shaker at the required temperature and 75 rpm. Samples were removed at timed intervals and appropriate dilutions were made and assayed for plaque-forming ability by the double layer method. This procedure was applied to each streptomycete-phage system at temperatures of 12°C, 15°C and 27°C.

iii) Results

Data obtained from the one-step growth experiments are presented in Figs 1, 2 and 3, and a summary of the replication cycles parameters are shown in Table 3. The p.f.u. ml^{-1} are plotted on a logarithmic scale as a function of time, thus the rising portion of the curve is linear. It is possible to determine the following replication cycle parameters from the one-step growth curves:-

a) Minimum latent period - the minimum length of time after adsorption of the phage to the cell for the release of complete phage particles. The intersection of the rising portion of the curve with the base count of p.f.u. ml^{-1} (i.e. infected hosts) provides a suitable measurement.

b) Rise period - the time period over which cell lysis takes place, which can also be used as an indication of the amount of variation in the latent periods. This parameter is indicated by the time period from the start of the rising portion of the curve to its end (i.e. to the beginning of the upper plateau).

c) Burst size - the average of the number of phages released per infected spore. This is calculated by the number of phage particles finally released (i.e. the upper plateau) divided by the number of infected spores (i.e. the lower plateau).

Fig. 1:- One step growth curves of Ø85 at 12°, 15° and 27°C

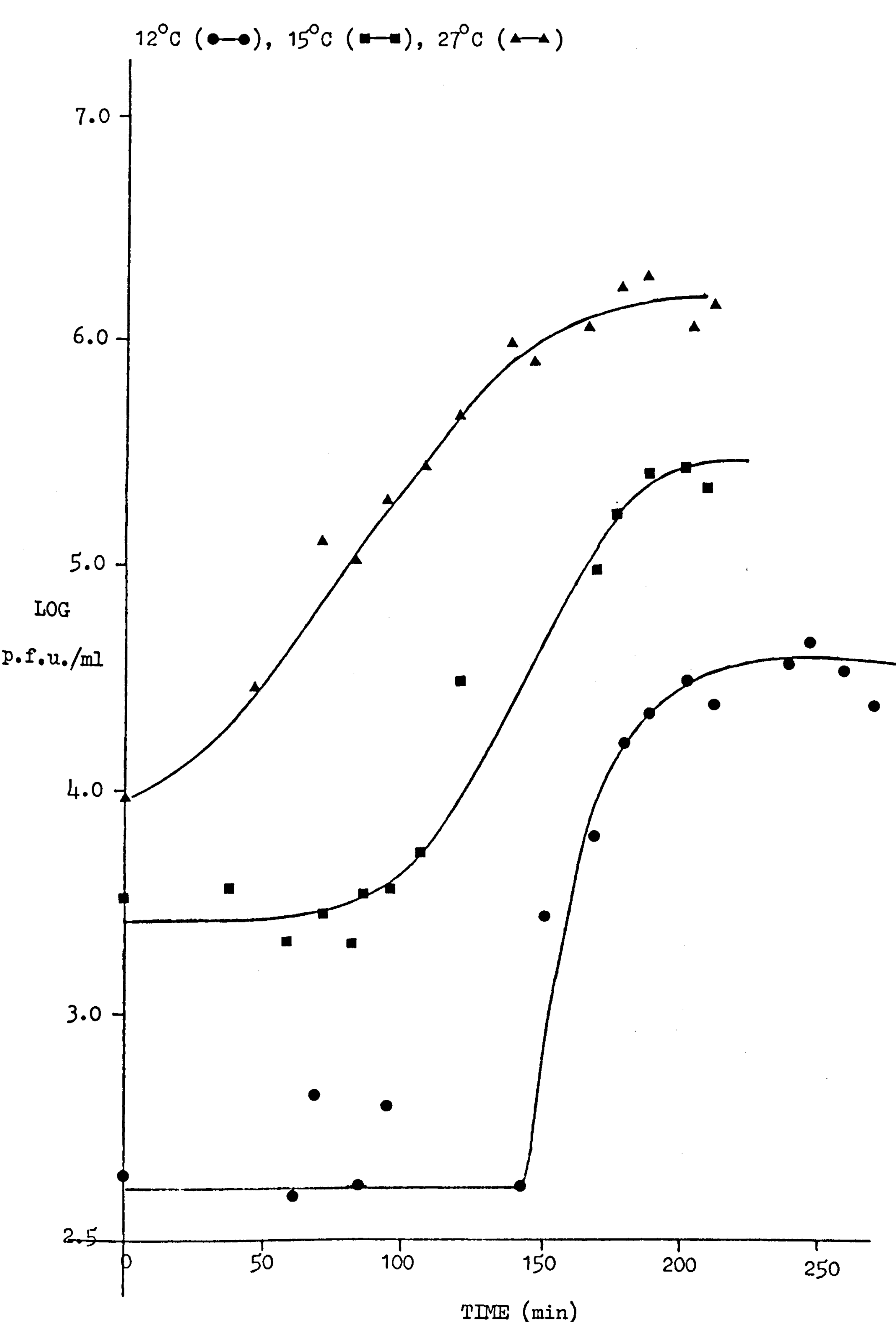


Fig. 2:- One step growth curves of Ømx1 at 12°, 15° and 27°C

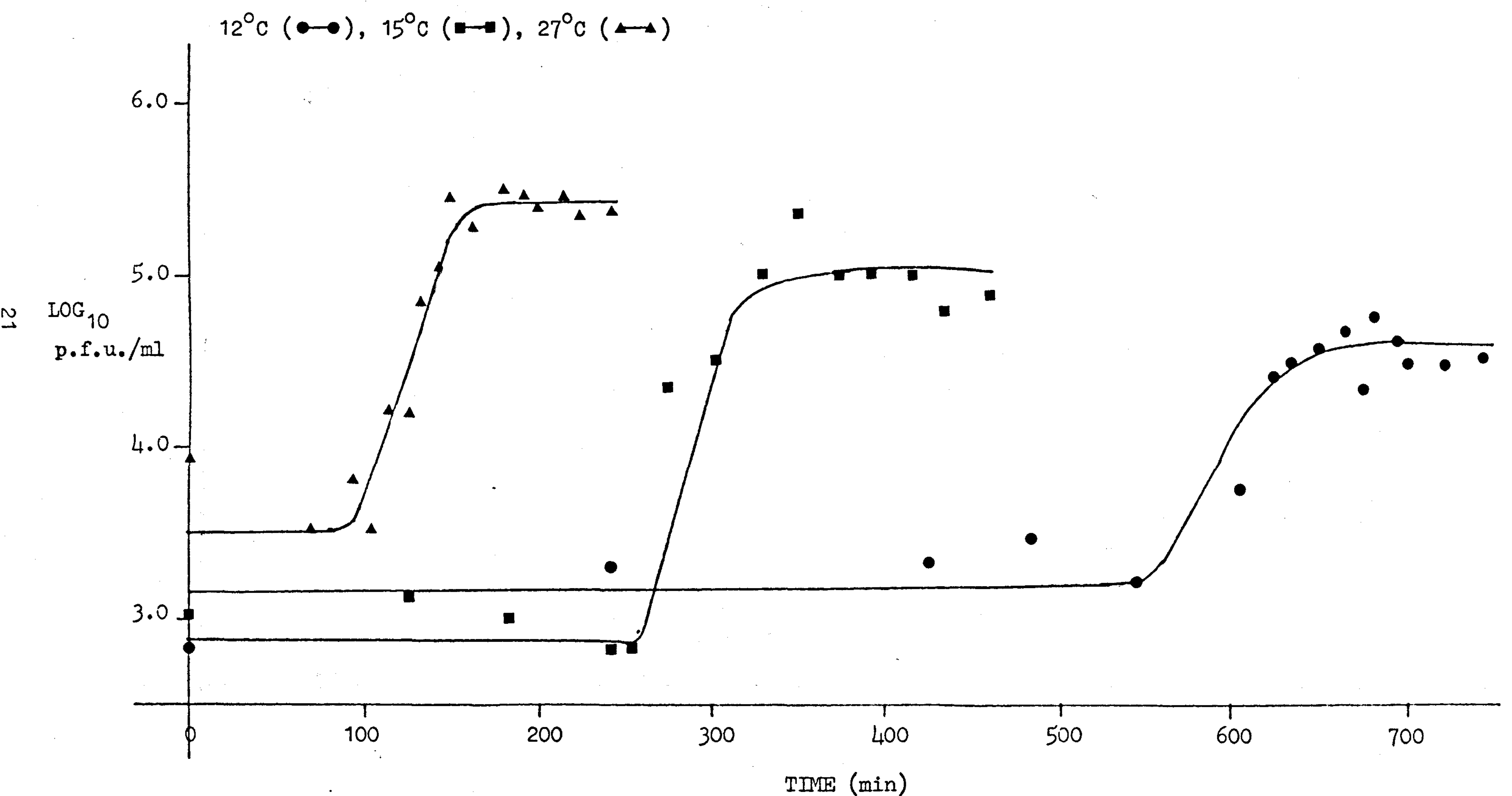
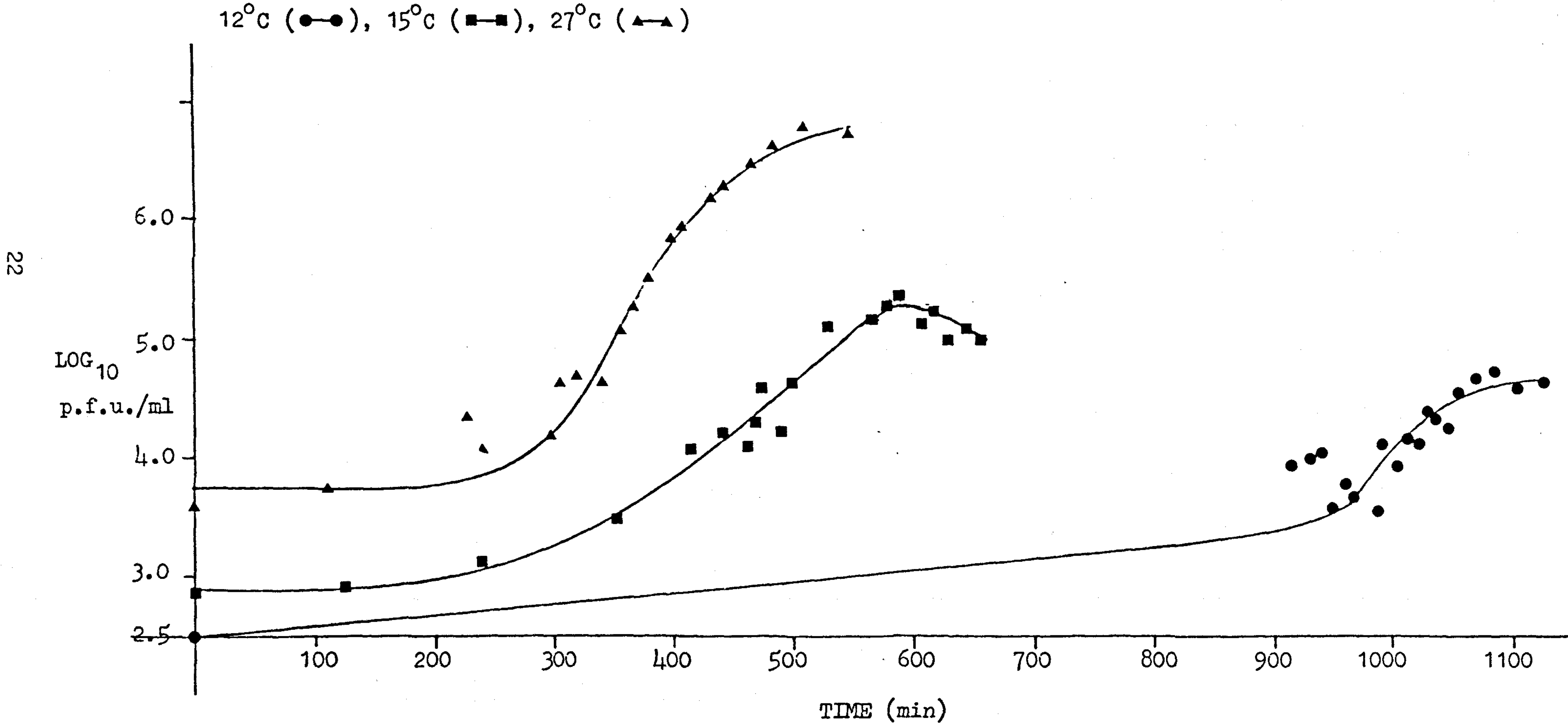


Fig. 3:- One step growth curves of Ømx8 at 12°, 15° and 27°C



TEMPERATURE	HOST AND PHAGE								
	ISP 5069+Ø85			MX1+Ømx1			MX8+Ømx8		
	LATENT PERIOD (min)	BURST SIZE (p.f.u./spore)	RISE PERIOD (min)	LATENT PERIOD (min)	BURST SIZE (p.f.u./spore)	RISE PERIOD (min)	LATENT PERIOD (min)	BURST SIZE (p.f.u./spore)	RISE PERIOD (min)
27°C	60	398	142	60	148	93	300	336	200
15°C	70	224	110	250	100	100	440	66	135
12°C	120	100	80	540	19	110	900	13	170

Table 3:- Parameters of the one step growth curves of Ø85, Ømx1 and Ømx8

iv) Results and discussion

The parameters of the streptomycete phage replication cycles are known to vary between species and with respect to the phage used. Table 4 presents a selection of various workers' findings. However, not all of the variation observed can be directly related to differences between the species, as some reflects differences between methods and so care must be taken when comparing the results of different workers'. However, streptomycetes generally appear to need at least twice the time as the other bacteria shown in the table for replication. The parameters for the three systems investigated here fall into the region of long latent periods and large burst sizes.

Variation within a species is illustrated by comparison of parameters for phages mx1 and mx8 both of which infect S. albidoflavus although they lysed different strains. The replication parameters are different but reflect the same trends with respect to temperature. The effect of temperature on the overall aspects of the replication cycle can be clearly seen from the results in Table 3. Lowered temperatures result in a generally decreased burst size and an increased latent period. In contrast, rise periods show no obvious response to temperature. The rise period is a reflection of the variation in the latent periods in the phage because all the hosts are infected synchronously, and thus it is unaffected by the temperatures studied.

Burst size is an average measurement of a range of burst sizes that occur during one one-step growth experiment. Delbrück (1945) showed that for Escherichia coli B and virus alpha, the range can extend from below 20 to over 2000, with a broad maximum around 180. The large variations in burst sizes observed here was not matched by that for the latent periods, and it is probable that cell lysis was not dependent upon the attainment of a threshold number of phage.

HOST	PHAGE	BURST SIZE (p.f.u./cell)	LATENT PD (min)	RISE PERIOD (min)	AUTHOR(S)
i) Streptomycetes					
Streptomyces griseus	W-2a	226	90		Alexander & McCoy (1956)
	B, W-1a, C-131	118-340	120		
	W-1, W-5	125-168	160		
	W-3	108	180		
Streptomyces venezuelae	MSP2		180	90	Bradley & Ritzi (1967)
Streptomyces coelicolor	VPII	30-35	40	30	Dowding (1973)
Streptomyces griseus	MSP2	500	120	60	Ritzi, Bradley & Jones (1968)
Streptomyces venezuelae	MSP2	500-5000	75-90	90-180	Jones & Bradley (1965)
Streptomyces venezuelae	MSP8	60	95	80	Kolstad & Bradley (1967)
Streptomyces ceolicolor	ACP13cl	10-50	50	20	Lomovskaya et al. (1980)
	VP12	20-100	40-50	15-35	
Streptomyces fradiae	FP4	75	40	30	
Streptomyces virginiae	S1	164	95	80	
Streptomyces coelicolor A3(2)	ØC31	20-30	40	60	Novikova et al. (1973)
Streptomyces chrysomallus	Ø17	2000	40	10	Wilde (1966)
Streptomyces sp. F6	F6	15.5	70	35	Sykes (1977)
F13	F13	6.6	76	15	
SW72	F13	29.1	78	22	
H1	F13	11.5	75	26	

Table 4

HOST	PHAGE	BURST SIZE (p.f.u./cell)	LATENT PD (min)	RISE PERIOD (min)	AUTHOR(S)
ii) Other bacteria					
Bacillus stearothermophillus	TP84	20	22-24		Saunders & Campbell (1966)
Escherichia coli B	alpha delta gamma	180 300 135	13 21 21		Delbrück (1945)
Escherichia coli	Anti. E. coli	60	30		Ellis & Delbrück (1939)
Pseudomonas geniculata	PX14	300-350			Olsen (1967)
Shigella sonnei	T3 T4 T7	104 7 11.5	17±0.5 24.5±0.5 20±0.5	13 12-13 14-15	Barry & Goebel (1951)
Vibrio cholerea	ø2	120	38	17	Maiti & Chaudhuri (1939)

Table 4 cont:- One-step growth parameters of Streptomyces and other bacteria

The data presented here indicate that as temperature decreases, so does the burst size. Both phage mx1 and mx8 are more affected than ø85 by the decrease in temperature. Previous reports of the effect of temperature on burst size for a range of bacteria vary. Bentzon, Maaløe & Rasch (1952) working with E. coli B and phage T4r also showed a decrease in burst size with temperature. However, both Ellis & Delbrück (1939) and Seeley & Primrose (1980) observed no difference in burst size as temperature decreased. The latter, using LT phage, did, however, show that at a higher temperature, 35°C, than that at which the phage is usually plated, the burst size fell to less than 2 p.f.u./cell.

Indications that the temperature at which the host is grown can interact with the temperature at which infection is allowed to proceed at were provided by Olsen (1967) with Pseudomonas geniculata and the psychrophilic phage Px14 (Table 5). Therefore, in order to obtain a true reflection of the replication cycle in soil, the host must be grown and infection performed at temperatures equivalent to those occurring in the soil.

The minimum latent period is a reproducible characteristic of a given phage-host system with, as already shown by the rise period, the precise time of lysis of individual cells being far from identical. As temperature decreases, the minimum latent period is extended. The work of both Ellis & Delbrück (1939) and Seeley & Primrose (1980) confirms this trend, the latter suggesting that the effect was similar to that of temperature on the generation time of bacteria. The time taken for generation of the streptomycete spores to the required stage was similarly affected, - as temperature decreased the generation time increased. The increased germination time indicated a slower metabolism, and hence as the phage takes

TEMPERATURE AT WHICH BACTERIA GROWN	TEMPERATURE FOR PHAGE GROWTH	BURST SIZE (p.f.u./cell)
25°C	25°C	100-150
3.5°C	25°C	100-150
25°C	3.5°C	10-40
3.5°C	3.5°C	300-350

Table 5:- Data to demonstrate the effect of bacterial growth temperature on the burst size of the Pseudomonas geniculata - PX14 phage system

(From Olsen, 1967)

over the host metabolism, a longer time is required for the synthesis of new phage particles.

Temperature, therefore, had a profound effect on the growth characteristics of the phage-host systems examined. There were substantial differences between the cycles at standard laboratory temperatures and those expected to occur in the soil.

Kuroda & Bradley (1967), Ritzi et al. (1968) and Lomovskaya et al. (1972) have all performed temperature shift experiments on streptomycete phage at elevated temperatures to examine at what stage temperature has an effect. Their results indicate that the temperature sensitive effects occur after the injection of the phage DNA and that the temperature sensitive step occurred within 30 mins after injection of the phage DNA. Kuroda & Bradley (1967) demonstrated another temperature-sensitive step which occurred in the middle of the burst cycle.

Maaøle (1950) studying the effects of lowered temperatures on E. coli B showed that the latent period could be subdivided on the basis of the temperature coefficients of two processes which in turn control the time of lysis. DeLisle & Levin (1972) working with Pseudomonas putrefaciens and a psychrophilic phage demonstrated that the phage had a requirement for a period at 2°C in order for it to be produced or restriction to occur.

What then do the results obtained mean in terms of the natural environment? As stated, temperatures experienced by phage in temperate soils (2-15°C) are much lower than those routinely used in the laboratory and the optimum ones required for maximum phage production. It can be assumed, therefore, that a restriction on the phage pool in the soil is caused by sub-optimal temperatures.

Variation in temperatures is common both within and between

soils of different types. Okafar (1966) suggested that an average temperature for a temperate soil was 10°C and for a tropical soil was 29°C. Temperature is also affected by soil colour, and density of vegetation. Temperature variations within a soil have been shown to occur by Wilkins & Harris (1947). Average monthly temperatures at the surface of a forest soil varied from 2-19°C, whilst at 7.5cm depth the range was 4-14°C. As the optimum temperatures for the production of phage isolated from soil are higher than those bulk temperatures, it seems incongruous that these temperatures do not occur in the soil. It may be possible, however, that within this heterogenous environment temperatures closer to the optimum for phage production occur in sites of concentrated microbial activity.

The temperature fluctuations within a single soil could result in a single streptomycete-phage system undergoing replication cycles with varying parameters, i.e. one at 10°C and one at 12°C. Higher temperatures lead to increased host growth and therefore more opportunities for phage replication cycles and production of phage. Thus these variations in temperature within a soil could contribute to the variation of phage numbers with time and probably with space, leading to heterogeneous distribution of phage within soil.

In this study only the effect of temperature on phage productivity was investigated, but clearly other factors can influence the number of phage produced, and also other cycle parameters. Ritzi, Bradley & Jones (1969) showed that the age of the host and therefore its physiological state can alter the burst size, with a 10 fold increase occurring between germinated (4 h old) and well germinated spores (16 h old). Rosner & Gutstein (1980) demonstrated a latent period four times longer in germinating spores than in fully developed mycelium. Webb *et al.* (1982), studying *Bacillus subtilus* and phage

SP82, indicated that burst size at least can be a reflection of the growth rate of the host, with a logarithmic relationship between the two.

The composition of media can affect phage productivity, either causing an increase or a decrease in the burst size. Gold (1959), working with S. griseus, showed that Ca^{2+} is required not only for phage adsorption (a common phenomenon) but also for a short time after injection of DNA. Barry & Goebel (1951), Gilmour, Noller & Watkins (1959) and Kolstad & Bradley (1967) also showed that media composition is important and can affect the number of phage produced.

Also ratios of bacteria to phage have been shown to affect phage production. Shigella sonnei + phage T4 gives optimum production at low multiple infection, whilst with phage T7 best yields are at high bacteria to phage ratios (i.e. conditions of single infection), (Barry & Goebel, 1951).

It seems likely therefore, that the nutrient status and composition of the natural environment could affect the extent of phage productivity. Nutrient status also affects the host growth and as shown this in itself can alter the number of phage produced per cell.

Therefore, it has been shown here that both the latent period and burst size of the streptomycete-phage systems investigated are influenced by a decrease of temperature to those levels generally occurring in soils. The latent period increased and the burst size decreased as temperature decreases, while the rise period remained unaffected.

IV. AN INVESTIGATION OF THE ABILITY OF PHAGE TO SURVIVE IN THE SOIL ENVIRONMENT

i) Introduction

There is much evidence to support the view that streptomycetes are present in the soil for the greater time as spores (Mayfield et al., 1972; Lloyd, 1969). As spores they are unavailable for phage infection due to the inability of the phage to adsorb to the spore wall (Dowding, 1972; Novikova et al., 1973; Sykes, 1977). It has been suggested that this is due to the composition of the wall, which has been shown to be different from that of mycelial walls (DeJong & McCoy, 1966). Germination of the spores and further growth in soil occurs only in microsites where sufficient nutrients are available, therefore soil streptomycetes are unavailable to the phage for the major part of their cycle.

Between cycles of replication in the soil, phage exist either in an extracellular state, a virion, or can seek refuge in the host in a lysogenic association. If present as virions, the phages will be exposed to fluctuations in the environmental conditions, such as changes in temperature and pH. In a lysogenic association some protection against these fluctuations may be provided by the host.

The distribution of phage and streptomycetes is probably heterogeneous both in time and space, although there is no available literature to indicate how the streptomycetes and phage are distributed in relation to one another. Therefore there is no reason to suppose that all phage are in close proximity to their sensitive host, or that spore germination and growth lead to immediate infection. It would seem, therefore, that if the phage is present in the soil as a virion

it will have to spend considerable lengths of time inactive in the soil.

An underlying assumption of this application of the Nicholson-Bailey model is that phage are in an extracellular state and no parameter is included for the phage which do not survive between cycles of replication. It is therefore necessary to discover whether the phage studied can survive in a natural environment as a virion, and if not to obtain some measure of the losses which occur in order to adapt the model to include phage loss. Therefore, a study was made of the ability of the three streptomycete phage to survive for a protracted time in a natural environment, a Freshfield mature sand-dune soil, and to consider the reasons for any apparent losses of viability.

ii) Method

Weighed samples (4g) of air-dried and sieved unsterile soil were added to vials. Half the samples were sterilised by autoclaving twice at 15 p.s.i. for 15 mins. Both sterile and non-sterile soil samples were inoculated with the relevant phage suspension to give a final water content of 5.1% (w/w). The samples were stored at 4°C.

Three replicate samples were removed at 0, 1, 2 and 3 weeks, and phage recovery was affected by the method of Lanning & Williams (1982). 10ml of PYCa broth + 0.1% (w/v) egg albumen were added to each vial. Egg albumen was used as it promotes the desorption of phage particles from clay minerals. The soil suspensions were shaken for 30 mins on a Griffin flask shaker and then allowed to stand for 16h at 4°C. Samples were diluted and 1ml aliquots plated on a basal layer of PYCa agar. The basal layer was then overlayed with 2.5ml of 0.6% PYCa agar seeded with host spores. Three replicate plates were prepared from each sample and incubated at 25°C for 24 - 48 h, depending on the requirements of the phage and host plated.

iii) Results

Fig 4 present the phage survival data obtained together with fitted regression lines. Table 6 presents the half lives of the phage as calculated from the regression lines.

Fig. 4

a) Ømx8

Sterile soil (R^2 = 93)

p.f.u. RECOVERED g^{-1} SOIL ($x10^6$)

0 2 4

0 1 2 3

TIME (weeks)

Non-sterile soil (R^2 = 66.75)

p.f.u. RECOVERED g^{-1} SOIL ($x10^5$)

0 2 4

0 1 2 3

TIME (weeks)

b) Ømx1

Sterile soil (R^2 = 59.8)

p.f.u. RECOVERED g^{-1}SOIL ($x10^6$)

0 1 2

0 1 2 3

TIME (weeks)

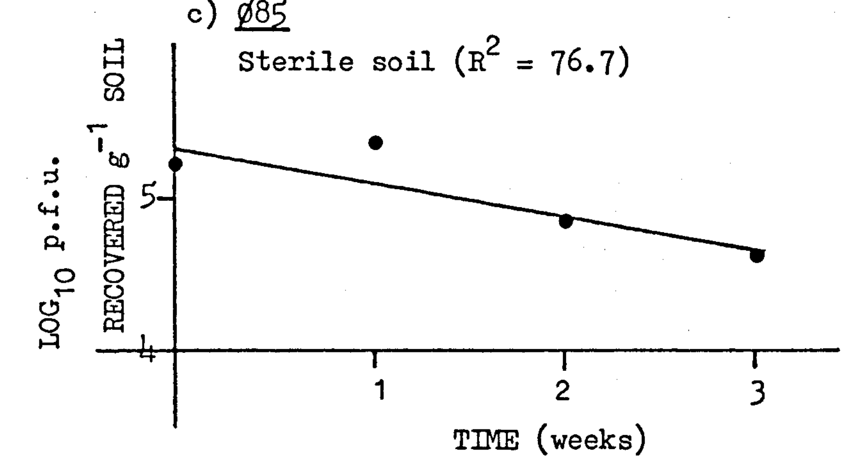
Non-sterile soil (R^2 = 65.6)

p.f.u. RECOVERED g^{-1} SOIL ($x10^6$)

0 2 4

0 1 2 3

TIME (weeks)



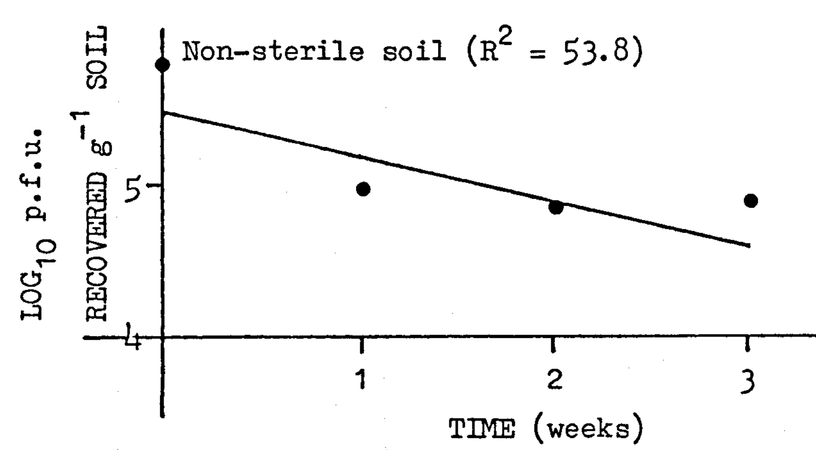


Fig. 4 cont:- Survival of Ømx1, Ømx8 and Ø85 in Freshfield mature sand-dune soil

PHAGE TYPE	SOIL STERILITY	
	STERILE	NON-STERILE
Ømx1	2.7	2.2
Ømx8	1.5	4.7
Ø85	1.2	0.9

Table 6:- Time (wks) taken for half phage population to be lost for Ømx1, Ømx8 and Ø85

iv) Results and discussion

Fig 4 presents the survival data obtained for the three phage Ømx1, Ømx8 and Ø85. Ømx1 shows a steady loss over the three week experimental period. The data for Ømx8 is more difficult to interpret, in sterile soil there is a steady loss of phage until week 3 when a sudden increase in phage number occurs. This may be due to an experimental error, and data calculations were made on the first part of the data. Ømx8 in non-sterile soil shows an increase within the first week. This again may be due to experimental error, or due to phage relication in an indigenous host, although at 4°C replication would not be expected to occur. Ø85 shows losses in both sterile and non-sterile soil. Table 6 presents the time taken for half the phage population to be lost. Although there appear to be differences between the phage, statistical comparison of the slopes from which these values were derived indicates that the slopes are not significantly different.

The losses determined may be somewhat ambiguous in so far as some losses may be due to ineffective recovery of phage from the soil. There is much evidence to show that phage are adsorbed to colloidal and organic particles within the soil (Stotzky, 1967; Marshall, 1974; Sykes & Williams, 1978). Both phage and clays carry a net negative charge under most conditions, and the adsorption of phage onto clay colloids is dependent on the presence, concentration and valency of associated cations. The direct attachment of phage particles to positively charged edge sites on clays has been sited as a possible mechanism of adsorption (Sykes & Williams, 1978). Phage also adsorb to suspended particles in aquatic environments (Bitton & Mitchell, 1974; Moore et al., 1975; Stagg et al., 1977). Lanning & Williams, (1982) demonstrated that egg albumen when used to extract phage facilitated a greater recovery from sterile sand (83%) than the other

protein additives, gelatin (66.7%) and bovine albumin (67%); it can also prevent surface inactivation of the phage on shaking. They also demonstrated that soil type can affect recovery. Use of radioactively labelled virions can help to distinguish between viral inactivation and incomplete recovery of soil bound virions (Yeager & O'Brien, 1979).

A low temperature, 4°C, was utilised because the survival of the phage was investigated and not its ability to replicate; therefore it was necessary to try and prevent any growth of hosts which may be susceptible to the phage concerned, although this may not have been achieved for Ømx8 in non-sterile soil.

Although some of the losses are probably due to incomplete phage recovery and phage aggregation, the results obtained indicate that the phage will be lost completely from the soil in approximately 8 weeks, unless a suitable host is encountered. The phage studied will therefore probably be able to persist for sufficient time, under the same conditions to infect new hosts and replicate.

The soil is a heterogeneous environment, and virions can be subject to a variety of microenvironments, so what conditions affect phage in the soil and how do they inactivate them or make them unavailable for infection?

Phage loss in an adverse environment is usually caused by the destruction of the integrity of the protein coat so inactivating the phage genome. Alternatively it can affect the attachment organ of the phage, either by altering it in an irreversible manner or making it unavailable for use, thereby preventing adsorption to a susceptible host and the subsequent cycle of replication. Tsutsaena et al. (1982) investigated the effect of freezing on the survival of Escherichia coli phage. Salt concentrations altered with the nature of the water molecules, causing the equivalent of hypotonic shock during melting

and hypertonic shock during freezing.

There is little information on the effect of environmental conditions on phage survival in soils as most authors have been primarily interested in isolating phage from soil, and the effect of varied conditions were only important in as much as they could effect the preservation of the isolated phage. Plant pathogens, like phage, have a similar need for a susceptible host and if no host is available they are present in the soil as inert bodies. Work by several authors has shown that these organisms show a greater decline in non-sterile rather than sterile soil (Lee, 1920 & Fulton 1920, Pseudomonas citrii; Dickey, 1961, Agrobacterium tumefaciens). Increased temperatures and increased pH both decrease the numbers of pathogens in soil. P. citrii disappeared quickly in soil and therefore needed to find a refuge in which it could overwinter, whereas A. tumefaciens could survive for long periods without the presence of an actively growing host.

Virus survival is important in aquatic environments such as sewage systems because of their adverse affect on man (T2, Berry & Noton, 1976; T7, Niemi, 1976; MS2, Tyler & Beswick, 1976; Poliovirus, Young & Sharp, 1977; Poliovirus 1, Labelle & Gerba, 1980; Coliphage, Seeley & Primrose, 1980). Berry & Noton (1976) demonstrated that T2 survival is independent of salinity but that temperature increase leads to phage loss. The major factor affecting phage loss was biological in nature and more than one factor was involved with the loss of the phage, the factors affecting loss being dependent on the phage used. Biological dependence was also shown to affect polio virus (LSc) and echovirus 1 (Faruk) with survival being better in sterile rather than non-sterile seawater (Labelle & Gerba, 1980). Tyler & Beswick (1976) reported phage concentration effects, up to 10^5 p.f.u. ml^{-1} inactivation decreased and above it increased. Water type can also affect the survival of

coliphage T7, its survival being better in clean water (Niemi, 1976).

Studies of streptomycete phages have dealt with individual components of environmental conditions such as temperature, pH and soil moisture content. Much work has been performed Sykes (1977), Sykes & Williams (1978) and Williams & Lanning (1985). Sykes (1977) investigated streptomycete phage survival in different soil types and horizons. Some of the phage losses observed were attributed to adsorption to colloids. Acidic peat soil and acidic horizons showed almost total phage loss, whereas the more alkaline lower C horizons supported phage survival. Garden soil exhibited less than a 10 fold decrease in phage numbers over 200h and in saline soil a 100 fold drop in phage titre was observed.

Obviously, as shown above, pH has a considerable effect as proteins are very susceptible to changes in pH; acidic pH can cause protein denaturation. Acidophilic phage are uncommon, although adicophilic streptomycetes do occur and therefore potential hosts are available. Sykes, Lanning & Williams (1981) have shown that phage will infect and replicate in acidophilic hosts providing the pH is not at or below those which inactivate them. Williams & Lanning (1985) attempted to isolate phage from four acidic soils with both acidophilic and neutrophilic hosts and isolated two phage for acidophilic hosts only. Further work considered phage recovered from soils at pH 3.6, 4.6 and 8.0 after 16h; phage used included ∅85, ∅mx1 and ∅mx8. Recovery increased as the pH increased with only one phage (∅mx8) recovered at pH 3.6. In nutrient broth it was possible to recover phage at pH 2.5 and at pH 4 all were recovered. Alexander & McCoy (1956) demonstrated that the loss was more pronounced below pH 6 for a range of *Streptomyces griseus* phages.

Temperature effects on survival cannot be performed in natural

undisturbed environments since increased temperatures can lead to increased microbial (host) activity and so increased opportunities for phage replication. Temperature effects are therefore usually investigated in broth and here again there are some difficulties as media composition can affect phage survival (Gilmour et al., 1959; Gold, 1959; Roslycky et al., 1963; Bradley & Lee, 1964). All work indicates that as temperature increases phage survival decreases, e.g. Kuroda & Bradley (1967) with *Streptomyces aureofaciens* phage, Williams & Lanning (1985) with a range of *Streptomyces* phages, and Cheo (1980) with *E. coli* B + T phage series. Roslycky et al. (1963) working with *Agrobacterium radiobacter* phages demonstrated that no appreciable loss occurred at 4°C, but that at 25°C decreases in titre were considerable, the rate of decrease depending on the phage used. This effect was not related to the morphological features of mesophilic and psychrophilic phage (Olsen et al., 1968). Psychrophilic phage were more thermolabile but the morphological features were similarly distributed amoung both groups of phage. Sakaki & Oshima (1975) demonstrated that *Thermus thermophilus* HB8 and its phage were stable at high temperatures and that thermostability was dependent on a factor in solution and that the phage had no special properties for survival at high temperatures. Seeley & Primrose (1980) recognised three types of coliphage on the basis of the effect of temperature on e.o.p. These were high temperature phage plated above or at 25°C, low temperature phage at or below 30°C, and mid temperature phage in the range 15-42°C. Stability of phage found in soil and other mesophilic environments is then greatest at low temperatures.

Moisture content of the soil was shown by Williams & Lanning (1985) to have little effect on streptomycete phage stability except for high moisture holding capacities (MHC) in non-sterile soils which resulted

in a ten-fold decrease in phage numbers. Duboise et al. (1978) indicated that at 15% MHC survival of enteric viruses was somewhat better than at 25% MHC. The most dramatic loss was seen when the soil was allowed to dry, therefore losses may be more profound at the surface of a soil where heating and drying are most intense.

Other factors in a soil which can affect phage survival are antiphage substances produced by actinomycetes themselves. Gause et al. (1957) tested 1,000 actinomycetes and found that approximately half had an inhibitory action on actinophages. Hanson & Elbe (1949) isolated several antiphage agent-producing organisms from soil for Staphylococcus aureus, the most potent originating from an Aspergillus species. An anti-actinophage substance has been isolated from Actinomyces globisporus by Kuraishi et al. (1970). The addition of herbicides at practical concentrations has been shown to exert no significant effect on the activity of two phage for Streptomyces chroysomallus (Roslycky, 1982).

The heterogeneity of most soils then suggests that virions will encounter a diversity of physicochemical conditions, so is there any evidence of ways in which adverse conditions can be avoided or minimised?

Much work has been performed on the effect of colloids in the soil or as suspended solids in aquatic environments. Sykes & Williams (1978) found that increasing concentrations of montmorillonite protected phage from losses due to experimental manipulation. However, the pH stabilities of kaolin-adsorbed phage differed to that of free phage. Adsorbed phage were more susceptible to pH because the pH is lower at the colloidal surface because the hydrogen ion concentration is increased. Therefore colloid adsorbed phage may be more susceptible to pH changes but are afforded some protection from other conditions. Bitton & Mitchell (1974) demonstrated that T7 phage adsorbed to

montmorillonite or E. coli cells in seawater were relatively resistant to inactivation. Other work demonstrating the beneficial affects of suspended particulate matter on phage has been performed by Berry & Noton (1976). They showed that kaolinite increases the survival of T2 possibly by adsorbing toxic substances and preventing their interaction with the virus. Stagg et al. (1977) demonstrated that MS-2 phage adsorbed to bentonite clay was more resistant to hypochlorus acid than free phage and Sobsey et al. (1980) indicated that survival in settled sewage solids was better than in soil-free controls. Aggregation for poliovirus 1 has been postulated as a major part of the mechanism for its survival in water (Young & Sharp, 1977). Aggregation has also been reported for phage MS2 in seawater (Tyler & Beswick, 1976).

Two other alternatives for protection could be lysogeny and polyvalency. Lysogeny as previously mentioned could provide protection from at least some of the adverse factors providing the host itself is resistant to the adverse condition. Polyvalency* can offer alternative hosts to a phage which cannot form a lysogenic association. Thus the phage could infect several different hosts so increasing the overall phage numbers and lessening the time exposed to the adverse extracellular conditions. Phage will still be available when more favourable conditions occur. Sykes (1977) has shown that a soil phage attacked both neutrophilic and acidophilic hosts, and ∅mx1 has been shown to infect at least two different streptomycetes (S. Lanning, personal communication).

It is obvious then that the ability of a phage to survive in the soil environment is dependent on the interaction of several factors which can be either beneficial or detrimental. The survival of three phage, ∅85, ∅mx1 and ∅mx8, has been investigated in a single mature

* Polyvalency - ability to infect more than one host species.

sand-dune soil. It has been shown that phage numbers do decline over a long time period, but that there is no difference between the survival of the three phage in sterile or non-sterile soil. It is likely, therefore, that sufficient virions of these three phage, Ømx1, Ømx8 and Ø85, will be able to survive in the soil and be ready for replication when a suitable streptomycete becomes available.

V. AN INVESTIGATION OF THE GROWTH RATES, AND AN EVALUATION OF THE INTRINSIC RATE OF INCREASE, OF TWO STREPTOMYCES ALBIDOFLAVUS STRAINS MX1 AND MX8

i) Introduction

Actinomycete and therefore streptomycete growth is unique amongst bacteria in that under favourable conditions a spore will germinate and grow vegetatively to produce a large substrate mycelial network. Aerial mycelium is produced which ultimately segments into spore chains; vegetative mycelium can also segment to produce arthrospores. Any attempt to measure the growth rates of streptomycetes is therefore complicated by their mycelial nature. Qualitative investigations of streptomycete growth have been undertaken by several authors (Vernon, 1955; Pfennig,1958; Lloyd, 1969; Wildermuth, 1970; Sharples & Williams, 1976; Zhuravleva et al., 1982).

Growth of mycelial organisms, in particular fungi, has been quantitatively studied by several authors (e.g. Trinci, 1971a; Trinci, 1971b; Trinci, 1974; Prosser, 1979; Allan & Prosser, 1983). Allan & Prosser (1983) investigating the early filamentous growth of *Streptomyces coelicolor* A3(2) found that for this streptomycete at least, the germ tubes extended linearly rather than exponentially as generally thought, with the branches also extending linearly. Bergter & Reisenberg (1982) indicated that exponential growth of *Streptomyces hygroscopicus* germ tubes developed into individual hyphae growing at a constant rate, and that the linear growth of hyphae and branching resulted in an overall exponential growth of the whole mycelium. Prosser (1979) reviewed models of individual aspects of the growth of mycelial organisms, such as spore germination, branching patterns and hyphal tip growth. He stated that

> 'the integration of models representing the different levels and aspects of mycelial growth has not yet been achieved'.

To this end he included a model previously presented by Prosser & Trinci (1979) which attempted to relate cytological events within fungal hyphae to colony growth kinetics.

Previous estimates of streptomycete growth rates have been made on the basis of optical densities and weight measurements. Flowers & Williams (1977) recorded maximum specific growth rates of up to $0.61h^{-1}$ and a doubling time of 1.12h. Mayfield et al. (1972) estimated the mean growth rate of germ tubes in natural soil to be $0.8\mu m\ h^{-1}$ and the mean generation time to be up to 15.2 days. These indicate that any estimate of growth rates in a soil must be performed in as near soil-like conditions as possible.

In the Nicholson-Bailey model the streptomycete growth rate is represented by parameter "r" and is the intrinsic rate of increase. The intrinsic rate of increase is that growth rate which would be achieved in the environment if there were no limiting factors, i.e. the streptomycete was growing exponentially. The Nicholson-Bailey model deals only with infectable units, in this case the parts of the streptomycete which are in the correct condition for phage infection. Therefore the aim of this work is to obtain a growth rate measurement which includes infectable units. Since phage regulate the host's metabolism it would be unprofitable to infect parts of the host which would be unproductive, and therefore they attack points of actively growing host. Streptomycete growth, as for other mycelial organisms, has been shown to occur by tip extension (Gottlieb, 1953; Brana et al., 1982) and therefore the points of phage attack (infectable units) are likely to be the mycelium tips and the germ tubes.

An estimate of "r" for a prescribed set of conditions can be obtained by use of matrix methods entailing the classification of stage-specific groups (Lefkovitch, 1967). For the streptomycete system these

stage-specific groups have been classified as spores, germ tubes and mycelial tips representing the transition stages within the streptomycete life cycle (Fig. 5). It is therefore necessary to obtain measurements of the transition from one stage to the next, and to include any detrimental factors such as spore loss, and any productive factors such as the number of spores produced per streptomycete colony. To this end, three aspects of streptomycete life cycle were investigated:-

a) Spore germination and subsequent growth in soil - to provide estimates of spore germination and therefore germ tube production, and number of mycelial tips produced per germinated spore.

b) Spore production - to determine the number of spores per colony and to investigate the effect of colony density on spore production.

c) Spore survival - to determine if spore survival is affected by the conditions used and if so what extent.

Therefore each aspect was investigated separately for *S.albidoflavus* strains MX1 and MX8 and the results obtained incorporated within a transition matrix from which the intrinsic rate of increase was determined.

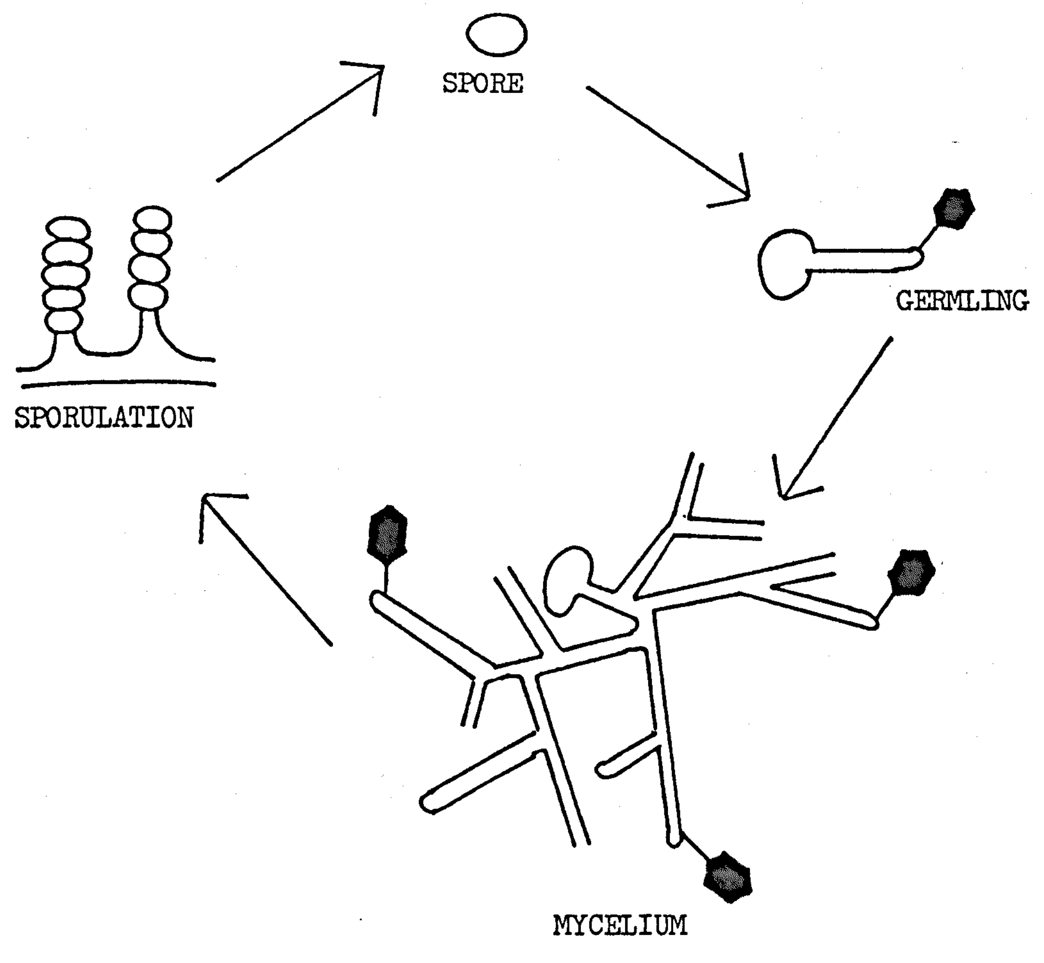


Fig. 5:- Transition stages in the streptomycete life cycle

A) Spore germination and subsequent growth in soil

a) Introduction

All stages of the streptomycete life cycle, spores, germinated spores and mycelium, have been seen, by direct observation, to occur in soil (Pfennig, 1958; Lloyd, 1969; Mayfield et al., 1972; Ruddick & Williams, 1972). Ruddick & Williams (1972) noted that growth was mostly in association with organic fragments. Unfortunately apart from spore germination, no attempts have been made to quantify the other stages of streptomycete growth in soil. As already indicated, phage only infect points of active growth and in the streptomycetes these are germ tubes and mycelial tips.

In determining the instrinsic rate of increase by matrix methods it was necessary to obtain a quantitative assessment of the proportion of spores germinating in a set time period and the number of germ tubes and mycelial tips produced by these spores. A comparison between the quantitative behaviour of the two streptomycete strains used, MX1 and MX8, in sterile and non-sterile soil was carried out.

b) Method

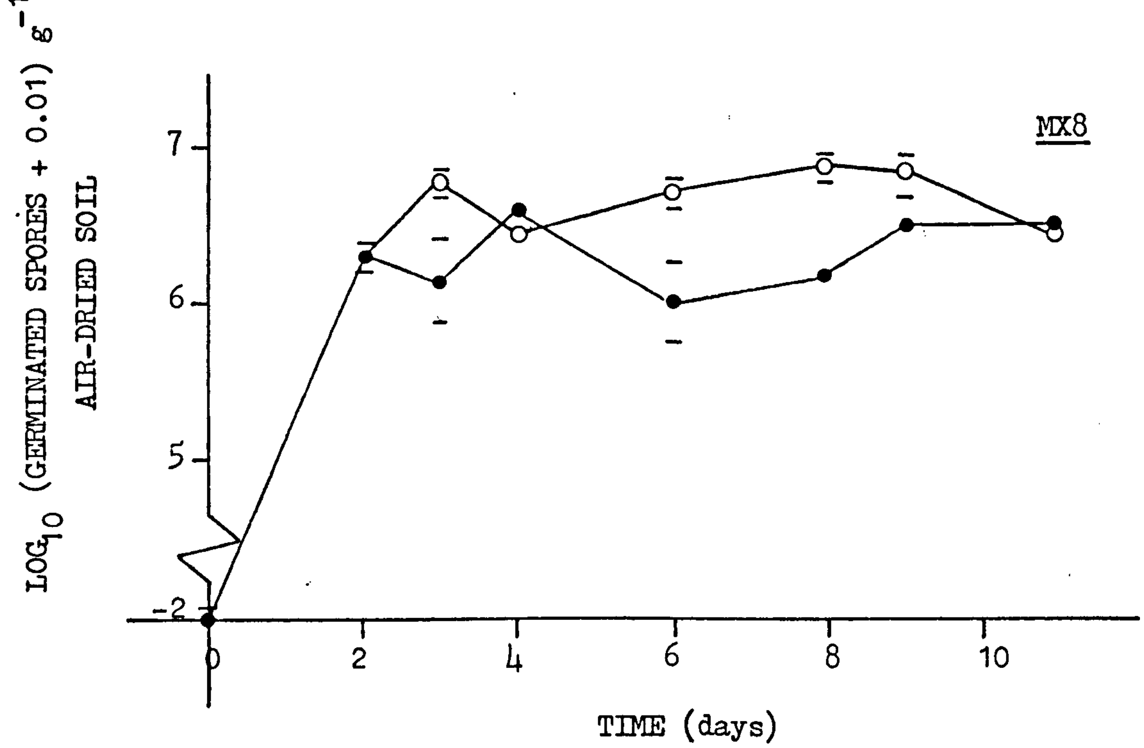
4g samples of a Freshfield mature sand-dune soil (pH7.6) were placed in sterile vials. Sterile soil was prepared by sterilising the samples twice at 15p.s.i. for 15 min. The soil samples, both sterile and non-sterile, were inoculated with spore suspensions to a final moisture content of 5.1% (w/w); three replicates were prepared for each sampling time along with control. The soil was incubated at 15°C and samples taken at predetermined intervals. 5ml of sterile water was added to each vial and shaken of 30 min on a Griffin arm-shaker. The soil supernatants were examined microscopically using a Thoma counting slide and numerically assessed for spores, germ tubes and mycelial tips.

c) Results

Figs 6, 7 and 8 represent the spore germination, the production of germ tubes, and the production of mycelial tips observed for MX1 and MX8. Fig 9 represents the total number of infectable units observed, i.e. the total number of mycelial tips and germ tubes found at each sampling time. Table 7 shows the proportion of spores which had germinated by the end of the eleventh day of the experimental period in both sterile and non-sterile soil. Table 8 presents the average number of mycelial tips produced per germinated spore.

Fig. 6:- Number of germinated spores for MX1 and MX8 in sterile and non-sterile mature sand-dune soil

Sterile soil (o—o), Non-sterile soil (●—●)



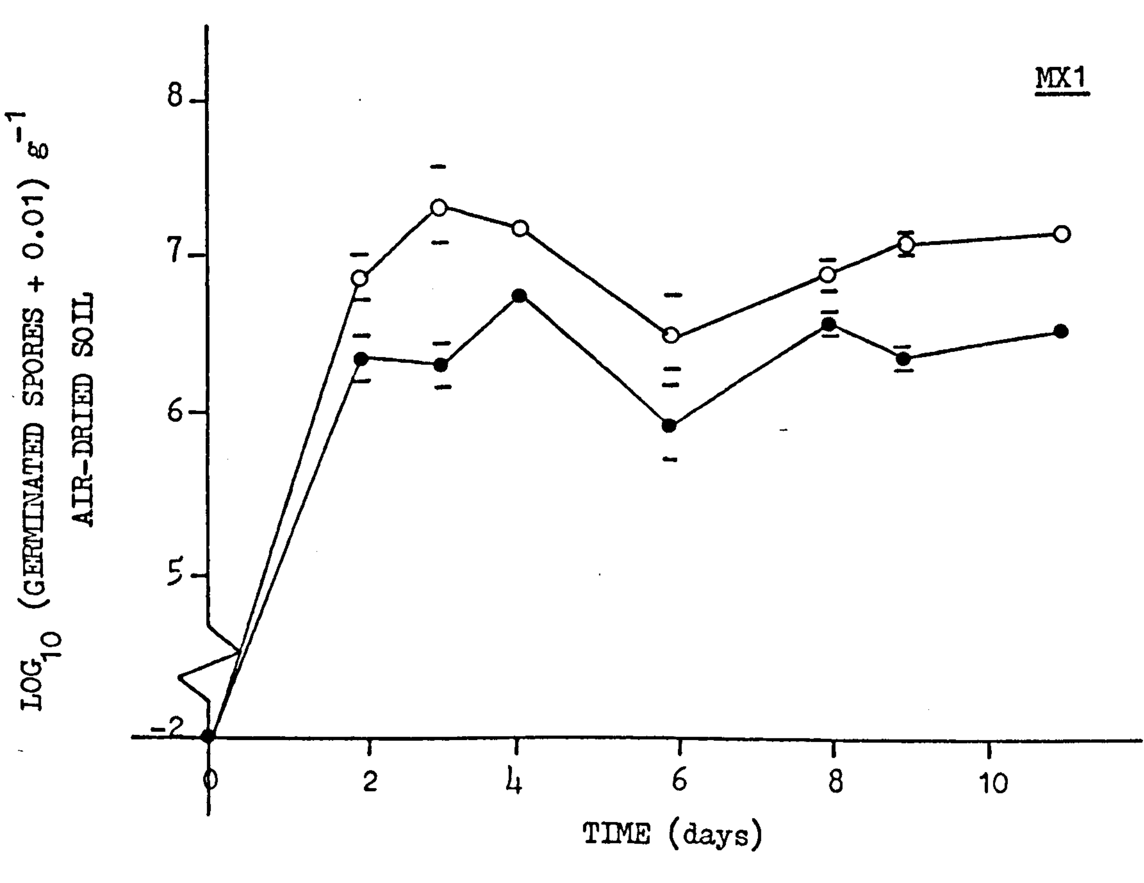
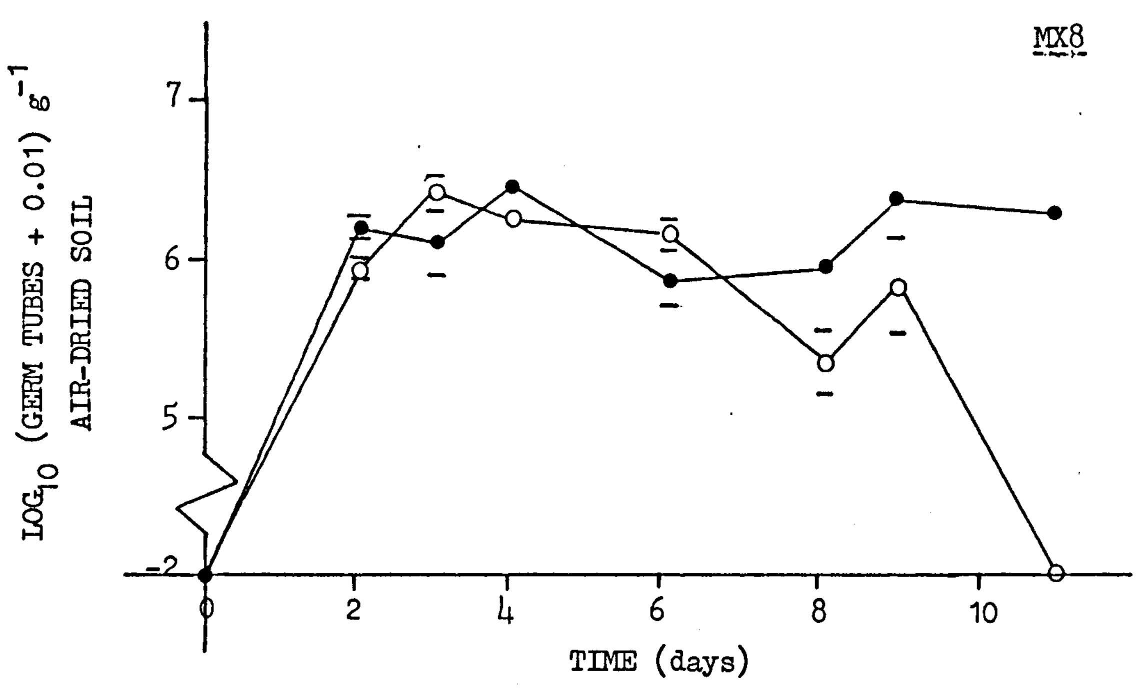


Fig. 7:- Germ tube production for MX1 and MX8 in sterile and non-sterile mature sand-dune soil

Sterile soil (o—o), Non-sterile soil (●—●)



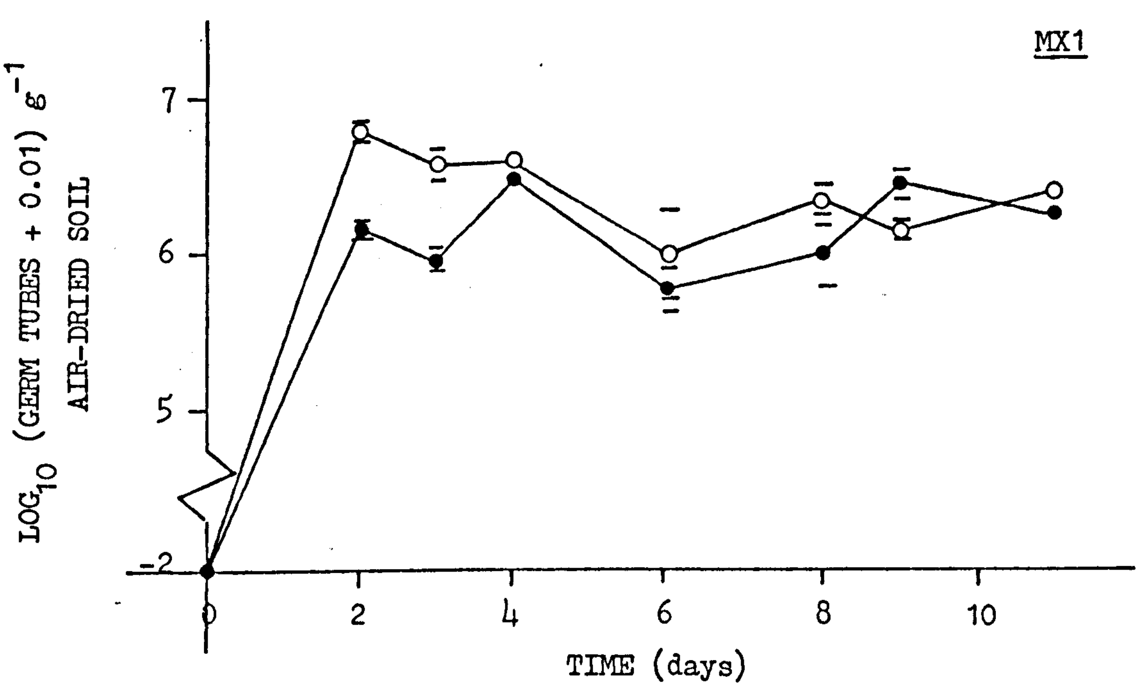
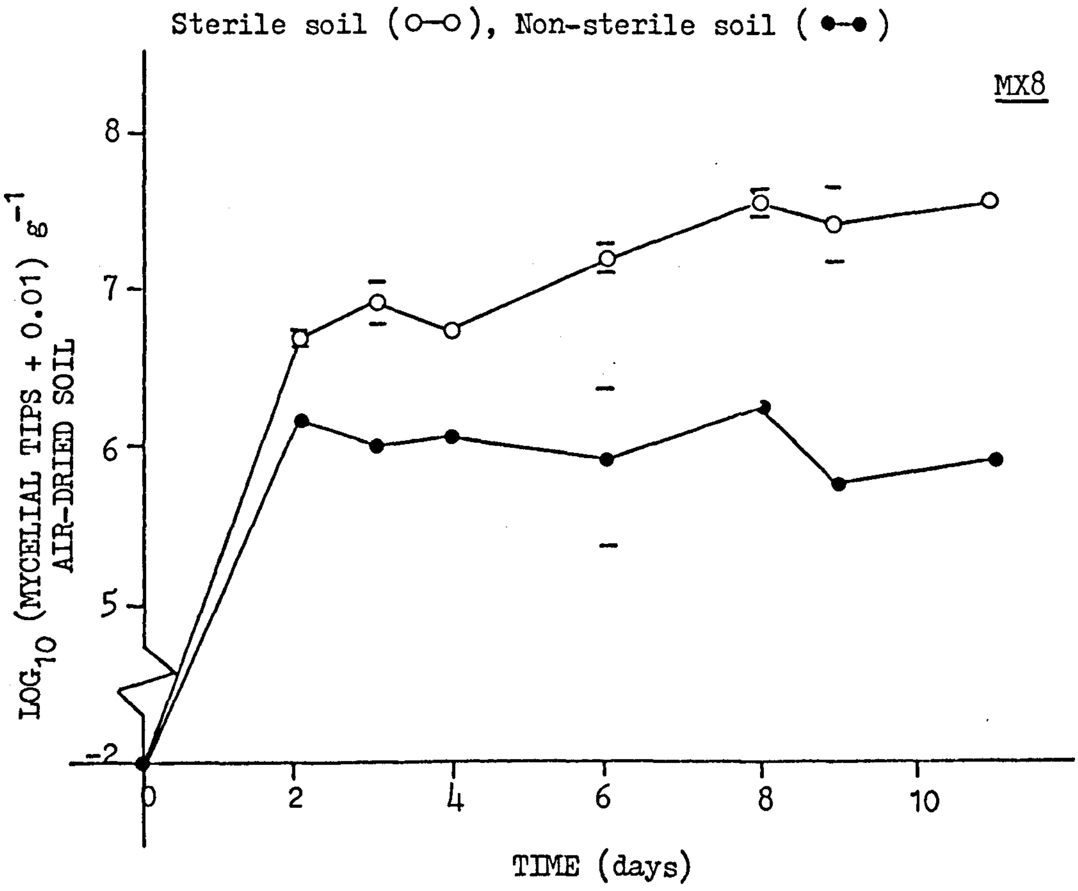


Fig. 8:- Mycelium tip production in sterile and non-sterile mature sand-dune soil

Sterile soil (o—o), Non-sterile soil (●—●)



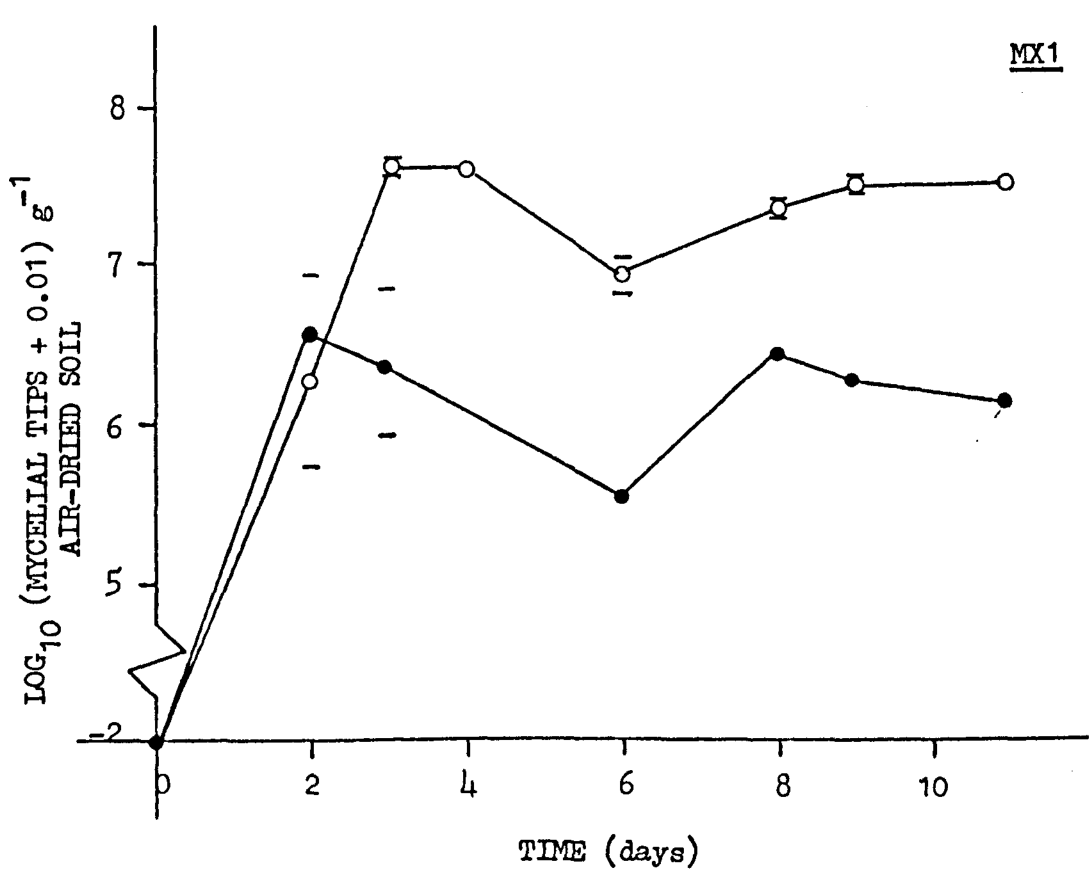
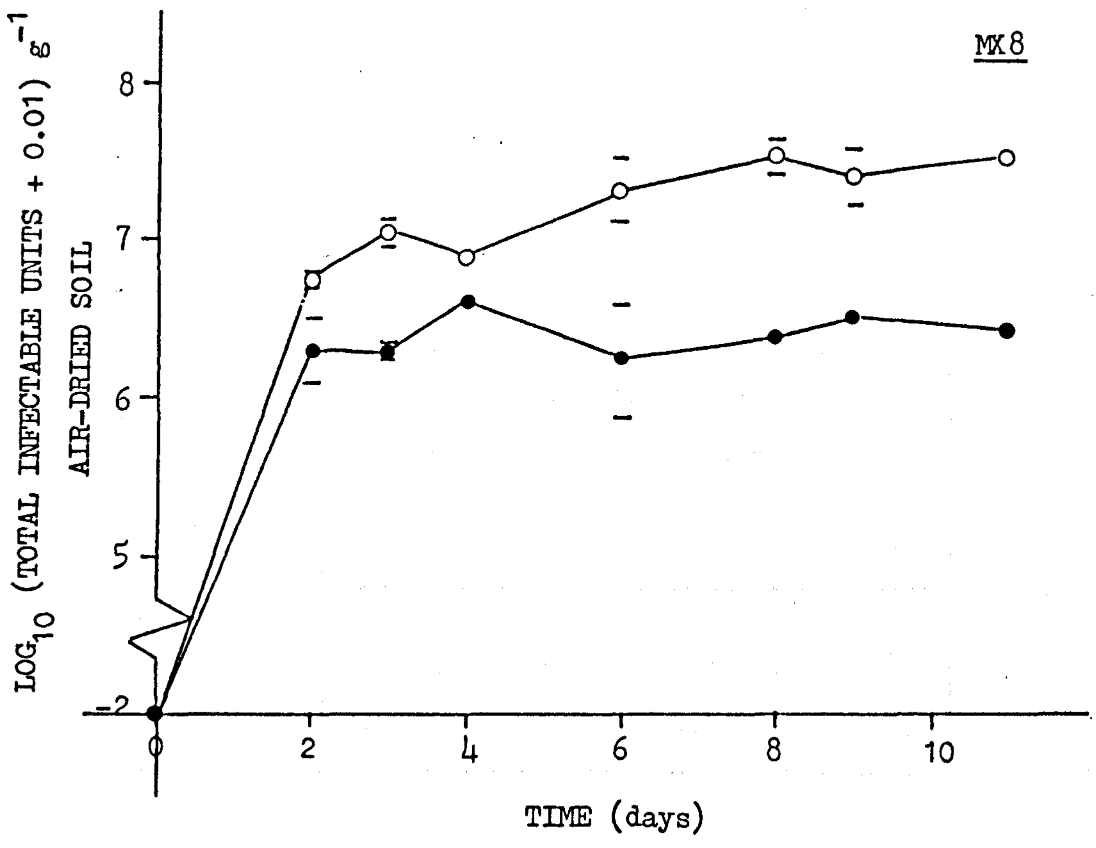
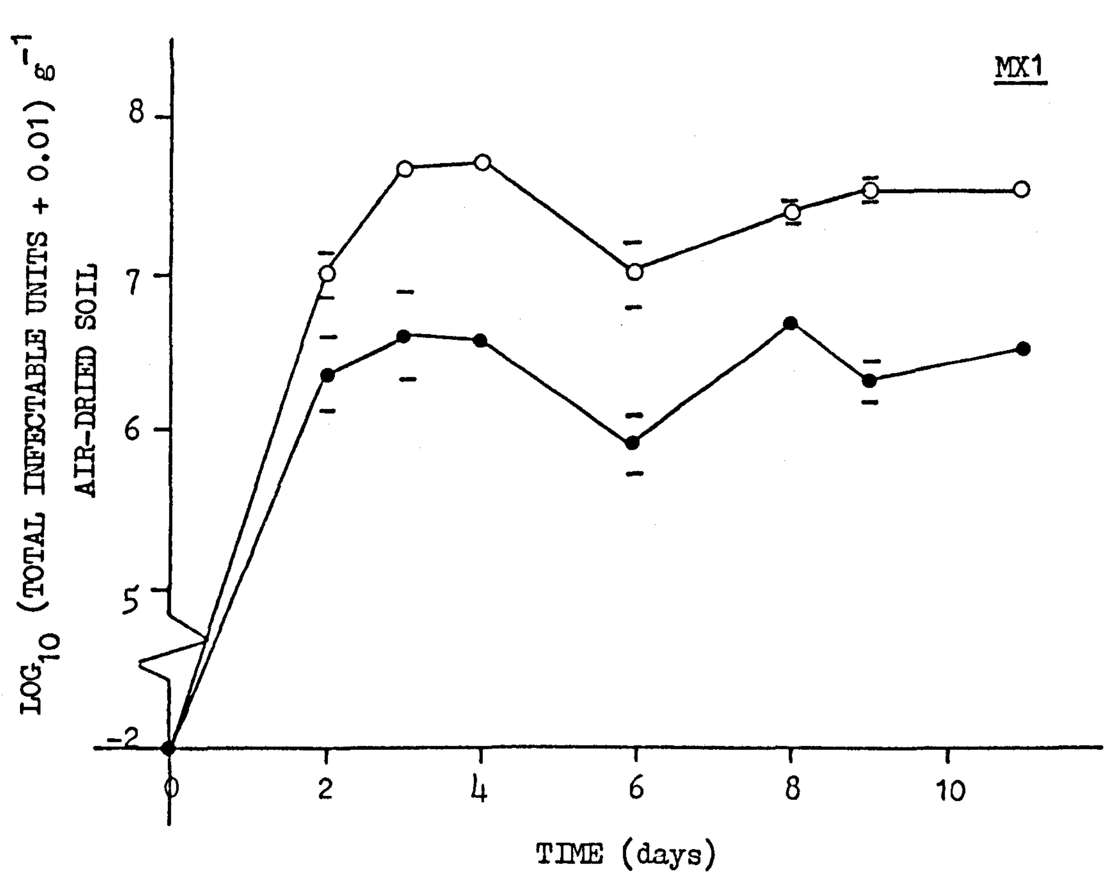


Fig. 9:- Total infectable unit production (germ tubes + mycelial tips) in sterile and non-sterile soil

Sterile soil (o—o), Non-sterile soil (●—●)





HOST	SOIL STERILITY	
	STERILE	NON-STERILE
MX1	0.549 ± 0.007	0.134 ± 0.006
MX8	0.3375 ± 0.0625	0.1125 ± 0.0125

Table 7:- Proportion of both MX1 and MX8 spores inoculated into sterile and non-sterile soil which had germinated after 11 days (± standard error)

HOST	SOIL STERILITY	
	STERILE	NON-STERILE
MX1	3.452 ± 0.109	1.518 ± 0.161
MX8	4.559 ± 0.007	1.605 ± 0.145

Table 8:- The average number of mycelial tips produced per germinated spore after 11 days for MX1 and MX8 in sterile and non-sterile soil

(± standard error)

d) Results and Discussion

Figs 6, 7, 8 and 9 all indicate that there is a difference between the growth of the two streptomycete strains in sterile and non-sterile (natural) soil. However, both strains show a similar production, MX1 being slightly greater than MX8. Sterile soil always affords a greater opportunity for streptomycete growth. Soil sterilization is difficult to achieve without altering the chemical and physical integrity of the soil. Kale & Raghu (1982) demonstrated that steam sterilization, as used here, is more effective than chemical treatments or gamma irradiation, but it is well known that this method causes a greater destruction within the soil than other methods. On sterilization dead microbes and the disruption of organic fragments contribute new chemicals to the soil making available previously unusable nutrient sources. An alteration in the amounts of extractable nitrogen, phosphorus and sulphur also occurs. All these changes lead to the establishment of more favourable conditions with respect to exogenous nutrients for growth, both of streptomycetes and other bacteria.

The proportion of spores germinating is much greater in sterile than non-sterile soil, (Table 7) but the difference is only significant at the 20% probability level. Lloyd (1969) and Mayfield et al. (1972) noted that spores added to non-sterile soil germinated to a much lesser extent than those added to sterile or nutrient-amended natural soil. Mayfield et al. (1972) demonstrated a maximum level of germination in non-sterile soil of 8.6% compared to 13.4% here; the difference is due to species and strain variation, different soils, and the fact that germination here was assessed over eleven days rather than 48 hours. Lloyd (1969) stated that the inhibition of germination was associated with soil micro-organisms, with a deficiency of the exogenous nutrients required for germination being imposed by microbial activity in the soil.

This is supported by the fact that amendment of natural soil with a 1% glucose solution, and the sterilization of soil, led to an increase in germination to 40.2% and 35.4% respectively (Mayfield et al., 1972) Visual examination of germinating spores showed that growth is mainly associated with organic fragments (Mayfield et al., 1972). Fig 6, which represents the number of germinated spores, and is a cumulative representation, indicates that within two days of inoculation germination was common and that within the following nine days this spurt did not recur. A plateau appears to have been reached which may include some new germination, but this is very small if indeed it does occur. There appears, therefore to be a synchrony in germination in a fraction of the spore population at the start of the propagation cycle and any spore which does not germinate immediately is unlikely to do so.

Germ tubes are thought to be produced from a hole in the spore wall (DeJong & McCoy, 1966). A germ tube is classed as such until it branches. More than one germ tube per spore can be produced; Allan & Prosser (1983) observed up to four germ tubes per spore for Streptomyces coelicolor A3(2). Germ tube production was greater in sterile than non-sterile soil. The Streptomyces albidoflavus strain MX8 germ tubes produced were observed to decrease in number at the end of the eleven day period, indicating that the germinated spores which move on to the mycelial stage are not being replaced by fresh germination. The number of germ tubes observed at any one time may reflect those spores which have just germinated or more likely it represents those germ tubes which have not developed into mycelium between the sampling times.

Mycelial tip production (Fig 8 and Table 8) indicates, as in all other cases, that sterile soil is more amenable to growth. The numerical difference observed between the two soil sterilities is more pronounced for mycelial tip production than for germ tube production,

indicating that many more mycelial tips are produced per spore in sterile soil than non-sterile soil. All the graphs show that, except for MX8 in sterile soil, a plateau of mycelium tip numbers has been reached by 11 days. That the number of mycelial tips observed here is less than would be expected is borne out by Allan & Prosser (1983) who observed up to ninety branches on one branch system of a colony on solid medium. However, after 11 days, at which up to 22 tips had been seen on a few colonies, it became too difficult to count. The plateau may then be caused by the inability to remove streptomycetes which have formed an extensive mycelium. Formation of mycelial networks is not a prerequisite for sporulation. Lloyd (1969) indicated that pre-germinated spores added to soil grew for only a short period and then sporulated leaving mainly conidia in the soil as the hyphae disappeared. Conidia added to the soil, in most cases either produced a germ tube which gave rise to a terminal sporophore or was fragmented with the formation of arthrospores. Fragmentation of vegetative mycelium in soil was also noted by Pfennig (1958).

As shown in Fig 9 the total number of infectable units (i.e. mycelial tips and germ tubes) present in the soil is greater in sterile soil and mirrors the numerical behaviour of mycelial tips, as at all times the mycelial tips were more in evidence than germ tubes. Therefore it appears that the most likely sites for phage infection are mycelial tips, at least after two days of growth.

There are several assumptions which are inherent in the method used here. They are that the spores added are representative of the whole population of streptomycetes, that those spores extracted mirror exactly the whole population, and that there is no difference in the extraction ability between spores, germinated spores and mycelium. Unless direct observation of soil, which would be difficult to perform, is used instead

of extraction procedures, it seems unlikely that these assumptions can be substantiated. Two other factors which must be borne in mind are that the spores are inoculated into an environment to which they are not native and culture grown spores have been shown to exhibit different survival abilites to soil-borne conidia (Lloyd, 1969). The number of spores inoculated was slightly larger than that found in the indigenous population in the soil i.e. 10^4-10^7 g^{-1} of soil, (Kutzner, 1981) and are therefore in addition to the indigenous streptomycetes which would have been present; however controls indicated that little natural streptomycete growth occurred.

All the results obtained are in agreement with the conclusion of Mayfield et al. (1972) that streptomycete growth in soil is in the order of days and weeks and not hours as observed in laboratory conditions. After eleven days no sporulation of the mycelium had occurred, there being no increase in the numbers of spores extracted from the soil and there being no visual signs of sporulation on the mycelium extracted. Under optimal conditions in the laboratory, sporulation would be expected to occur within 10-14 days of inoculation, but the lowered temperature would account for the difference. Jensen (1943b) found that the vegetative mycelium of actinomycetes developed most rapidly at 28°C to 37°C, but that at 15°C the rate of growth was slower but eventually reached the same level.

Unfortunately only one set of soil conditions was studied, i.e. pH 7.6, moisture content 5.1% (w/w) in a sandy soil which exhibits good drainage. Mayfield (1969) examined the effect of pH, temperature, soil moisture content and gas concentration on Streptomyces sp. F1, the degree of the effect being determined by the amount of radial growth achieved by colonies inoculated into sterile soil. pH was found to negate growth at a pH less that 5.5 and show a maximum at pH 7. Waksman (1922)

demonstrated that streptomycetes had an acid-limiting reaction at pH 5.2. Temperature limitation occurred at both low and high levels, i.e. less than 10°C and at greater than 35°C with the optimum growth at 25°C. Moisture content and gas concentration affects were interrelated in that high moisture content can result in water-filled pores in which the rate of oxygen diffusion is much lower than that of carbon dioxide. Streptomyces sp. Fl was shown to be sensitive to the high ratios of CO_2 to O_2 but not to low levels of oxygen (Mayfield, 1969). Stotzky & Goos (1956) also showed that actinomycetes were sensitive to low concentrations of CO_2 to O_2. The high ratios of carbon dioxide to oxygen can also be caused by microbial activity. Therefore there are several factors which can affect streptomycete productivity in the soil environment.

In conclusion, a study has been performed to obtain a numerical assessment of the germination ability and, germ tube and mycelium tip production of the two strains of S. albidoflavus, MX1 and MX8, in sterile and non-sterile soil. Sterile soil was more supportive to streptomycete growth in both cases, but germ tube production was less enhanced than mycelial tip production. A spurt of germination and tip production occurred within two days of inoculation into the soil, however a significant steady increase in the number of infectable units with time was seen only with strain MX8 in sterile soil.

B) Spore production

a) Introduction

There appears to be no literature available on the quantitative nature of spore production, whereas qualitatively there is an abundance. Many authors have examined the morphological and biochemical mechanisms of spore production for individual Streptomyces species (Vernon, 1955; Wildermuth, 1970; Kendrick & Ensign, 1983). Spores are produced at times of nutrient depletion and can be of various morphological types. They are produced on aerial hyphae, which originate as simple branches of substrate mycelium, and these aerial hyphae are enveloped by a fibrous sheath. Spore production is affected by the almost simultaneous growth of a number of closely spaced sporulation septae, followed by the rounding up of the newly formed spores and thickening of the spore wall. At the climax of sporulation the aerial mycelium shows two trends, one towards spore formation and the other to lysis of non-sporulating hyphae (Chater & Merrick, 1979; Kendrick & Ensign, 1983).

There appears to have been no attempt to quantify the spore production in a streptomycete colony. Therefore an investigation was performed to determine whether spore production is a function of colony density, and if so to obtain a description of this density-dependent function for use in the determination of the intrinsic rate of increase.

b) Method

Spore suspensions of MX1 and MX8 were inoculated onto plates of minimal media agar (pH 7). The inoculum size was varied to obtain a range of final colony densities. Three replicate plates of each density were prepared. The minimal media agar contained DL-asparagine, 0.5g; K_2HPO_4, 0.5g; KOH, 0.3g; $MgSO_4.7H_2O$, 0.2g; $FeSO_4.7H_2O$, 0.01g; glucose, 10g; Oxoid agar, 15g; distilled water, 1000ml. The plates were surface spread and incubated at 25°C until sporulation had occurred. The number of colonies on the plates was assessed. Spores were harvested by scraping the surface of each plate into 10ml of sterile 10% (w/v) glycerol in vials. The plate was flooded with a further 5ml of 10% (w/v) glycerol and any further spores collected. Sterile glass beads were added to the vials and they were shaken vigorously for 2 min to detach spores from mycelium and to break up spore chains. The number of viable spores produced per colony was assessed by plating dilutions of the spore suspensions on minimal media agar plates. These were incubated at 25°C for 10-14 days and then the colonies produced enumerated.

A selection of spore suspensions were microscopically enumerated using a Thoma counting slide to determine the total number of spores produced per colony.

c) Results

The number of viable spores produced per colony at varying plate densities of original spores are shown in Fig 10 for MX1 and Fig 11 for MX8. In each case the regression line of y on x is fitted and Table 9 shows the equation of these lines obtained by use of the statistical package MINITAB. Figs 12 and 13 present a comparison of the total number of spores produced per colony against the number of viable spores produced per colony.

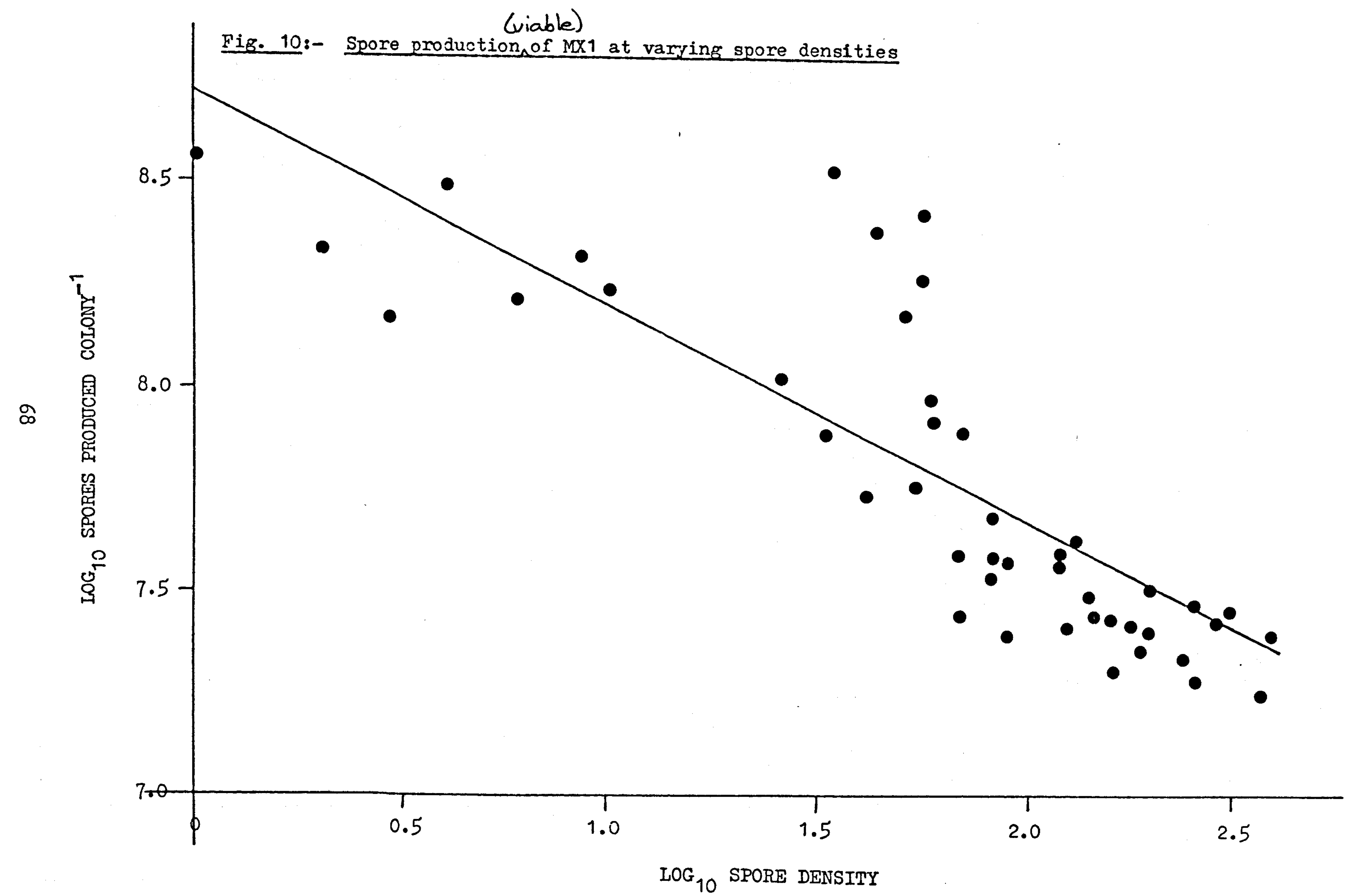


Fig. 10:- Spore production (viable) of MX1 at varying spore densities

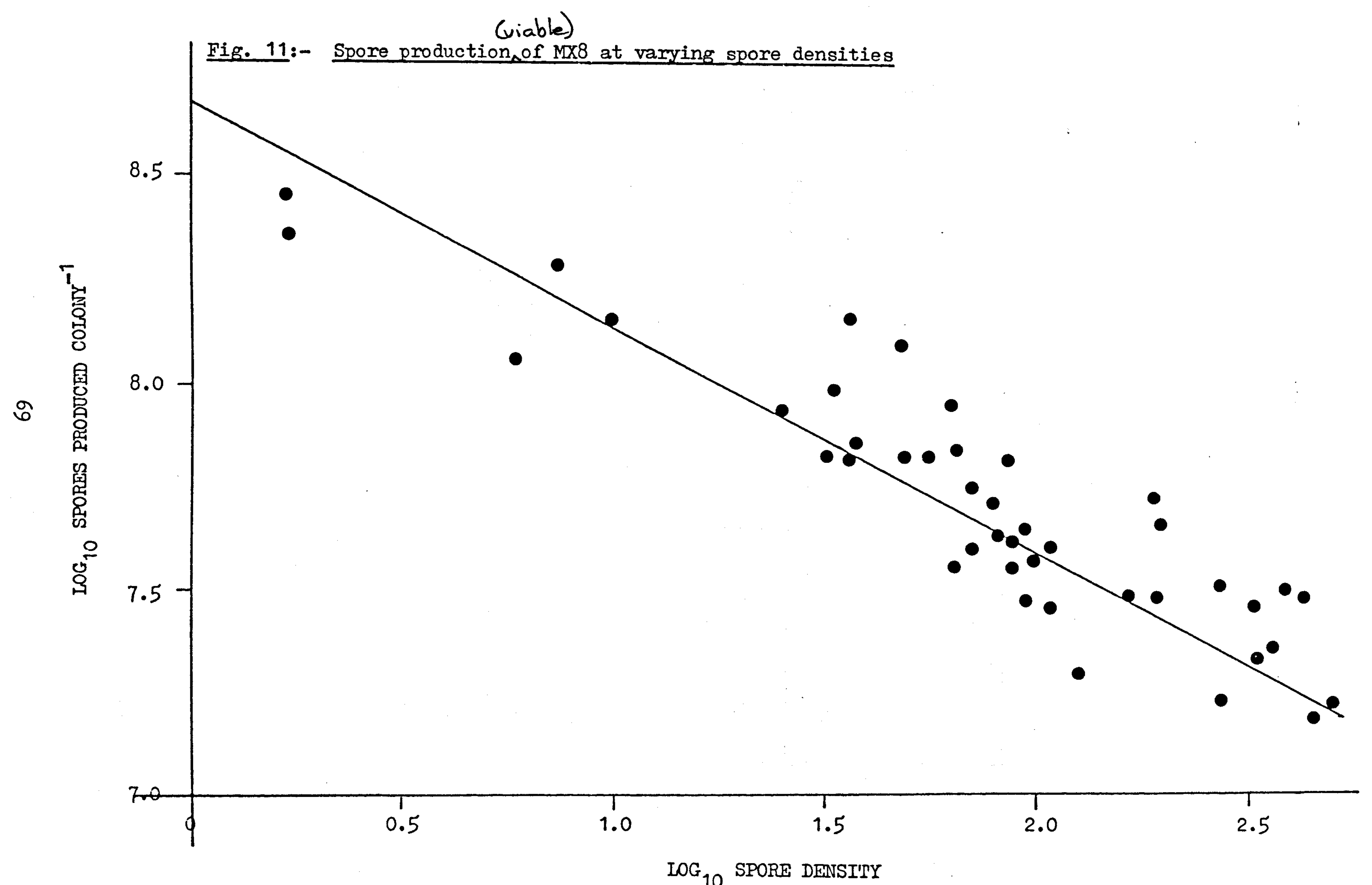


Fig. 11:- Spore production (viable) of MX8 at varying spore densities

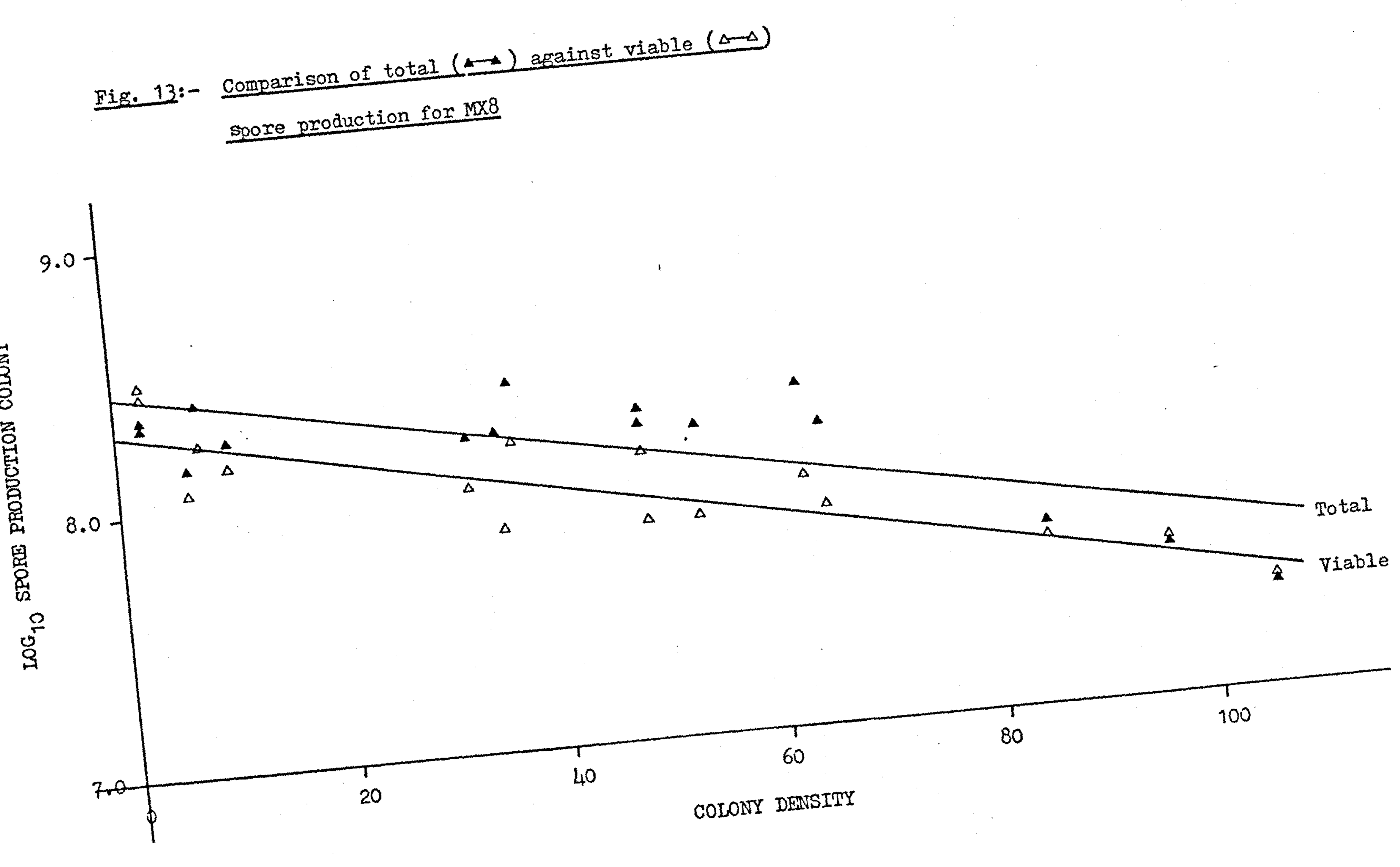
HOST	DENSITY-DEPENDENT FUNCTION	F-RATIO	R^2
MX1	lgy = 8.72 - 0.518 lgx	72.17	67.3
MX8	lgy = 8.64 - 0.507 lgx	99.57	77.4

Table 9:- Density-dependent functions for MX1 and MX8 spore production

(x = colony density; y = spores produced colony^{-1})

Fig. 12:- Comparison of total (▲—▲) against viable (△—△) spore production for MX1

Fig. 13:- Comparison of total (▲—▲) against viable (△—△) spore production for MX8



d) Results and discussion

The Figs 10 and 11 show that viable spore production is a density-dependent function in relation to colony density, the number of viable spores per colony decreasing as colony density increases. The fitted regression lines (Table 9) indicate that MX1 produces more spores than MX8 and that it is a slightly more negatively density response, however, the density-dependent function is similar for both strains. Close examination of the Figs. indicates that at single figure low densities the spore production is relatively unaffected, however as the colony density increases the density effect becomes evident.

A density-dependent effect would be expected because as colonies are more densely packed they would become smaller in diameter. Smaller colonies have a less extensive mycelial network, less aerial mycelium and therefore afford fewer opportunities for spore production. It was observed that the smaller colonies were present on the plates with the more densely packed colonies. Plates with up to ten colonies had a range of colony size from 2-5mm (MX1) and 2-4mm (MX8), whilst those with over seventy colonies showed the presence of several colonies of 1mm and others at the lower end of the ranges indicated.

The observed density-dependent effect on viable spore production may be due to two possible effects, either direct physical interference or competition for limited nutrients. A direct physical interference would be caused by two adjacent colonies physically preventing maximum growth as seen when colonies are densely packed together. The nutritional effect is akin to the physical effect in that the increased number of colonies leads to a decreased nutrient availability per colony, and therefore they are more likely to spore when the colony is smaller. It is impossible to distinguish here between the two effects and the density-dependent effect is likely to be caused by both.

Figs 12 and 13 compare the total number of spores produced, determined by a visual count of a small selection of spore suspensions, with the number of viable spores produced. The total counts are as expected higher than those of the viable counts. The two fitted regression lines for total spore production and viable production, are almost parallel indicating a similar density-dependence relationship for the two counts for both MX1 and MX8. Some of the observed differences may be attributed to spore clumping leading to interference between spores and to losses during plating, as well as to inviability of some spores thereby leading to a lower viable spore production. There is no difference between the inviability of spores from a large colony compared to those from a small colony. In the soil environment smaller colonies would be formed by streptomycetes which are slow growers, either inherently or because of the low temperatures, and also due to competition from other microbes for exogenous nutrients.

The extrapolation from data derived on a plate to the soil environment is fraught with difficulties, and can only be used to indicate what might happen in a natural environment. Streptomycete spores in soil are usually found concentrated in localized regions in association with organic fragments which provide a limited nutrient pool (Mayfield, 1969; Mayfield <u>et al</u>., 1972). Movement through the soil can be achieved by soil water percolation and attachment to soil arthropods, (Ruddick & Williams, 1972), both of which will lead to a heterogeneous distribution of spores and therefore spore densities. In this experiment attempts were made to reproduce soil nutrient conditions by the use of minimal media as a limited nutrient environment for a standard length of time. The standard time prevented secondary growth of the colonies and spore germination in the colony centre. Temperature and competition, which can be interrelated as increased temperatures can lead to increased growth and

therefore to increased competition, were not simulated, but they can have an affect on spore growth. Although temperature, as well as most of the other soil parameters, varies in soil, it is unlikely, except possibly at the surface, to reach 25°C; more standard temperatures are 10-15°C. At lower temperatures growth is much slower and this could lead to more probable exposure to adverse environmental conditions and therefore early sporulation. In the presence of faster growing microbes at these lower temperatures, sporulation could occur earlier leading to a spore production. It does then appear to be feasible that the density-dependent function of spore production seen in the experiment performed will occur in the natural environment.

Therefore, the sporing abilities of two streptomycetes, MX1 and MX8, at different colony densities were shown to be density-dependent functions. MX1 initially produced more spores per colony than did MX8 but showed a more pronounced density-dependent effect. The function of spore production in relation to colony density has been numerically determined for both MX1 and MX8.

C. Spore survival

a) Introduction

The ability of spores to survive in soil is of utmost importance to any streptomycete since they form an integral part of the streptomycete life cycle. Studies using maceration techniques and direct observation of soil have demonstrated that the main form of streptomycetes in soil is spores (Lloyd, 1969; Mayfield et al., 1972)

Mayfield et al. (1972) suggested that mean generation times in soil were in terms of days or weeks rather than hours as experienced in the laboratory under optimal conditions. It is generally thought that these long generation times reflect periods of inactivity interspersed with brief periods of relatively fast growth which leads to spore production, rather than continuous slow growth. This type of growth is typical of zymogenous bacteria (Windogradsky, 1924).

Spores are usually formed in response to a decrease in nutrient availability and individual nutrient effects have been demonstrated, such as phosphate starvation (Kendrick & Ensign, 1983) and carbon starvation (Chen & Alexander, 1972).

Spores are the resting stage of the streptomycete life cycle and must then be able to persist in the soil for extended periods of time which correspond to the interval between the discontinuous and irregular propagation cycles. They have to be able to remain viable during fluctuations in environmental conditions and be able to germinate as soon as sufficient exogenous nutrients are available and other factors are suitable.

A study was made of the three streptomycetes (ISP 5069, MX1 and MX8) to examine their ability to survive as spores between cycles of replication in a Freshfield mature sand-dune soil, both sterile and non-sterile.

b) Method

Soil collected from mature sand-dunes was air-dried to constant weight. The soil was then sieved through a 1mm mesh and 4g samples were placed into sterile vials. Half the vials were sterilized by autoclaving twice at 15 p.s.i. for 15 min. Streptomycete spore suspensions were added to the soil, both sterile and non-sterile, to give a final water content of 5.1% (w/w). Three replicates were prepared for each streptomycete at each sampling time. Control soils with no additional spores were also prepared. Soil samples were stored at 4°C in order to minimise growth and three replicate vials were removed at 0, 1, 2 and 3 weeks. Spore recovery was affected by the addition of 20ml of distilled water to the vials, followed by vigorous shaking for 30 min on a Griffin arm shaker. Appropriate dilutions of the soil suspensions were prepared and plated on starch-casein media by the surface spread method of inoculation (Vickers, personal communication). The starch-casein agar plates prepared for non-sterile soil suspensions also contained nystatin and cycloheximide, each at 50 μg ml^{-1} to help prevent fungal and bacterial contamination. Three replicate plates of each soil suspension were prepared and incubated at 25°C. They were checked regularly for fungal and bacterial contamination, and after 10-14 days the streptomycete colonies were enumerated.

c) Results

Fig 14 presents the survival data obtained together with fitted regression lines. Table 10 presents the half lives of the streptomycetes as calculated from the regression lines.

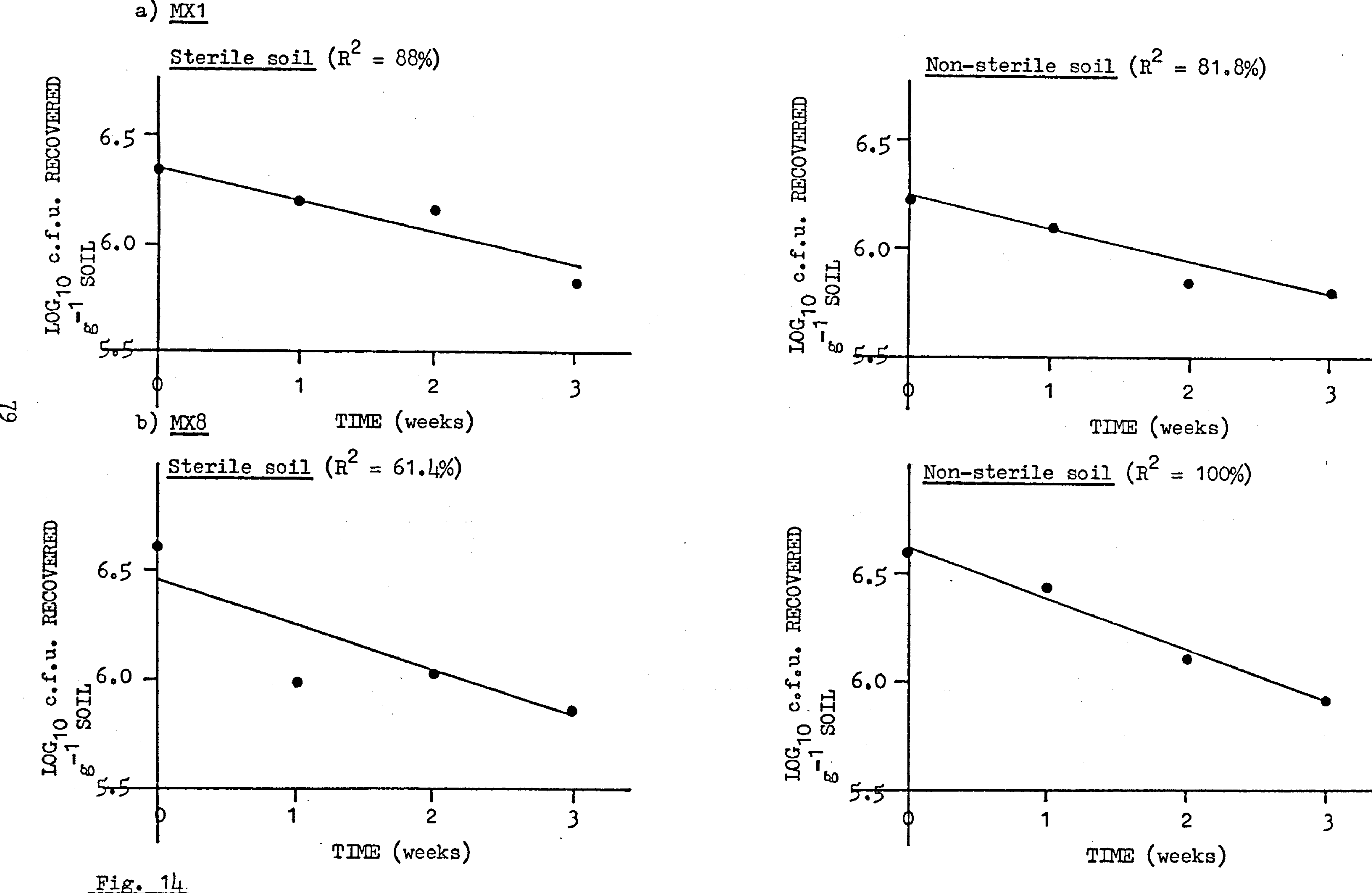
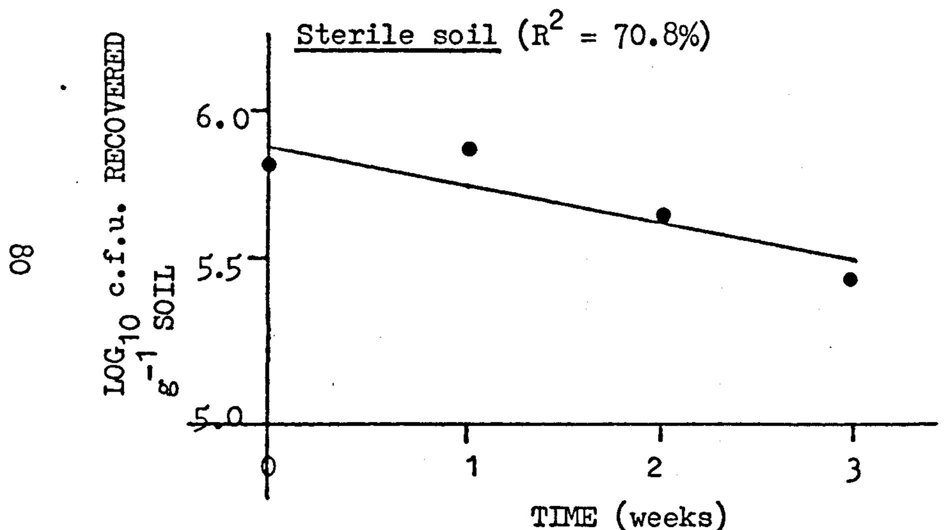


Fig. 14

c) ISP 5069



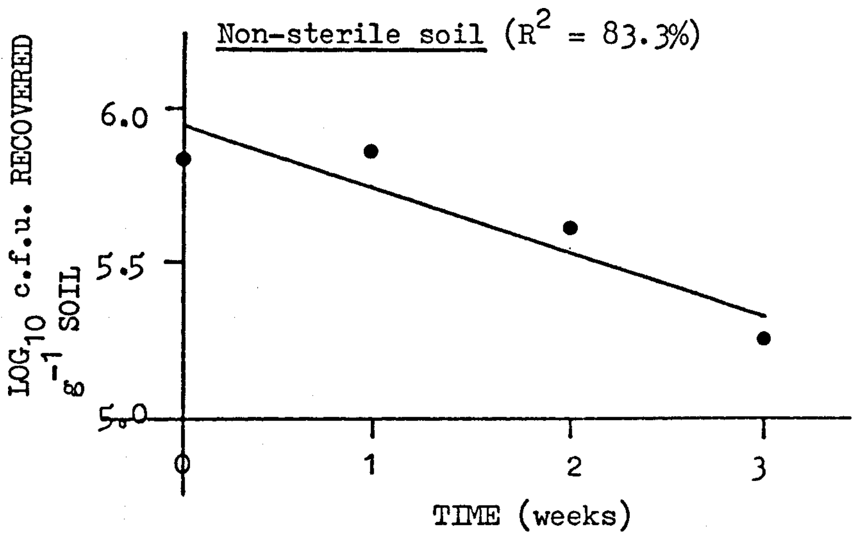


Fig. 14 cont:- Survival of MX1, MX8 and ISP 5069 spores in Freshfield mature sand-dune soil

HOST	SOIL STERILITY	
	STERILE	NON-STERILE
MX1	2.4	2.3
MX8	1.7	1.7
069	2.9	2.1

Table 10:- Time (wks) taken for half spore population to be lost for MX1, MX8 and 069

d) Results and discussion

Fig 14 presents the survival data obtained for the three streptomycetes examined, MX1, MX8 and ISP 5069. All show an apparently constant death rate over the three week experimental period. Table 10 shows the time taken for half the streptomycete populations to be lost from the soil. Statistical analysis of the slopes of the graphs from which the half lives were calculated indicated that there is no significant difference between losses in sterile and non-sterile soil, and that there is no significant difference between the losses of the three streptomycetes.

As already noted when streptomycete phage loss was investigated, the losses may reflect incomplete recovery of the spores. Measurements of the efficiency of recovery of the spores resulted in losses of MX1 - 31.8%, MX8 - 43.5%, 069 - 34.2% of spores probably due to incomplete recovery, therefore the losses are probably overestimates and many more of the spores will survive. Spore clumping which is known to occur may contribute to apparent spore loss, as some colonies may result from two or more spores giving rise to only one colony. Ruddick & Williams (1972) demonstrated that the ability to remove spores from sterile sand whilst still in their sheath was related to morphology of the sporing apparatus and the hydrophobicity of the spores. The highest recovery for streptomycetes was 48.8%, whilst for individual spores of Micromonospora it was 55.7%. Strongly hydrophobic spores were the most difficult to recover.

Adsorption of spores to clay colloids and organic fragments can also prevent spore recovery. Adsorption of spores to clay colloids has been shown by Ruddick & Williams (1972) who examined adsorption of spores onto exchange resins, and sand and clay particles. Actinomycetes spores carry a net negative surface charge at and above

pH 2.5 and therefore they will behave as negatively charged particles in the soil. Adsorption to sand particles was negligible, sand lacks positive charges, except in the presence of high valency cations, e.g. Fe^{3+}, whilst with kaolin it appeared to occur at all pH levels from 2-10, therefore making recovery more difficult.

Work by Mayfield et al., (1972) and Flowers (1974) investigated the survival of streptomycete spores in soil. Mayfield (1969) and Mayfield et al. (1972) examined the effects of moisture content, pH and temperature on Streptomyces sp. Fl spore survival in a Freshfield soil, within the A (pH 4.1) and C (pH 7.0) horizons. The greatest losses were caused by acidity and drought conditions, with acidity having the greatest effect; low temperatures had little effect over a six month period. The survival of both added and indigenous spores in the two horizons was investigated and in both horizons the spores persisted for 24 weeks although the behaviour of indigenous spores was different to added spores in each horizon. In the A horizon the added spores underwent a rapid decrease in numbers slowing to a steady decline and in sterile soil all the spores were killed; in the C horizon the initial rapid decline was not observed. Temperature and moisture had little effect except at the highest temperatures and lowest moisture tensions. Survival was largely dependent on pH, the acidic pH having the greatest effect on the indigenous population even at pH 4.1. The added spores showed less ability to survive than the indigenous spore population in both cases. This observation was borne out by Lloyd (1969) who found that culture grown conidia inoculated into garden soil had an accelerated mortality rate, whereas natural soil-borne conidia initially decreased rapidly and then the decline lessened.

Flowers (1974) investigated the ability of both acidophilic and neutrophilic streptomycete spores and mycelium to survive at low pH.

Both sets of spores showed similar pH survival values and there was little tolerance of pH values to acid to permit growth of the streptomycetes. The acidophilic streptomycete mycelium could survive at pHs above its upper limit for growth.

Temperature effects are difficult to study as moderately high temperatures will permit germination of spores. However it is well known that high temperatures can cause spore death. Although Hirsch & Ensign (1976) have used high temperatures (45°-60°C) to negate the effect of the lag period of germination, exposure to these high temperatures was limited to a few minutes only. Survival at very low temperatures e.g. -20°C, is demonstrated by storage in glycerol of spore suspensions although the glycerol does act as a cryo-protectant (Wellington & Williams, 1978).

The effects of adding herbicides to a pine forest soil have been studied by Kristufek & Blumauerova (1983a, 1983b). Labutricil 25(LAB25) affected actinomycete numbers (in particular streptomycetes) one year after application and the effect correlated with the percolation of the herbicide down the horizons. The work was performed on the total actinomycete soil population but as spores make up much of this population it will affect them as well. Different sensitivities were seen on different media, suggesting a protective effect of essential nutrients, and that soil could itself act as a protecting factor that influences the survival of streptomycetes after herbicide treatment.

Spore-forming bacteria other than streptomycetes, have been shown to be better survivors than non-spore formers (Stotzky et al., 1969; Chen & Alexander, 1972). Chen & Alexander (1972) working with Bacillus and actinomycetes demonstrated that in carbon-free solutions their numbers increased with time along with certain non-spore forming

bacteria. They, as did Stotzky et al. (1969), suggested that many, if not all, survivors probably owed their persistance to resistant structures, maintaining a low endogenous respiratory rate or accumulation of reserve polymers which are subsequently used. Resistant populations were shown to contain poly-B-hydroxybutyrate and that it was used rapidly in cells deprived of exogenous sources of carbon. However not all species contain the same reserve compounds, as Arthrobacter crystallopoietes contained a glycogen-like substance, and Aerobacter aerogenes and Streptococcus mitis contained glycogen. Stotzky et al. (1969) showed a similar increase in other organisms capable of forming spores, fungi and actinomycetes, or existing at low metabolic levels during soil storage. The increase in actinomycetes was sufficiently large to result in the increase of the total microflora despite large reductions in bacteria.

Casida & Liu (1974) studying Arthrobacter globiformis, a non-spore forming pleiomorphic bacterium, showed two cell types, cocci which were present at the late stationary phase and rods predominant in the log phase. The coccoid stage is more able to survive than are the rods, and are thought to predominate in soil. They are also resistant to phage until outgrowths form. Another non-spore forming bacterium, Pseudomonas solanacearum was shown by Moffett et al. (1983) to be more sensitive to soil drying than dry soil conditions, and they suggested that this particular organism was afforded some protection by adsorption to clay colloids. Amy et al. (1983) investigated internal cell changes of a marine vibrio caused by starvation; the cells decreased their DNA and protein whilst RNA increased to 2.5 x the minimal level. The cells also used much of their storage products and became microcells which survived for at least 6 weeks. Thus the ability to persist for long periods of time in natural environments is likely to depend on the

ability to form resistant structures, such as streptomycete spores, but other methods of survival of starvation are known.

The literature and the results obtained here indicate that the survival capacity of streptomycete spores is sufficient for persistence between their production, either on aerial mycelium or by fragmentation of the mycelium to give arthrospores, and the arrival of a sufficient supply of exogenous nutrients. However the degree of persistence will be dependent on the environmental conditions, in particular pH. The heterogeneous nature of the soil environment will enable the replenishment of the spore population because some microsites will be favourable for streptomycete growth.

The survival of three streptomycetes, MX1, MX8 and ISP 5069 has been studied in a sandy soil over a three week period. It has been shown that there is a gradual decline in spore viability but that this decline is independent of soil sterility. MX8 showed a greater spore mortality than either MX1 or ISP 5069. The measurements of spore loss are over-estimates due to the incomplete recovery of spores from the soil. It is likely that many spores will be able to survive in the soil until conditions amenable for growth occur.

ii) Results and discussion of the use of matrical modelling for obtaining an evaluation of the intrinsic rate of increase of two streptomycete systems, MX1 and MX8

The use of matrix modelling to determine both finite and intrinsic rates of increase was pioneered by Leslie (1945). It is often used in plant and animal ecology, e.g. Gregoire-Wibe & Snider (1977), but no record has been found of its application to microbial ecology. The approach involves the classification of the population studied into age or stage-specific groups and the repeated multiplication of these groups by the transition probabilities of movement from one group to another. It provides a simulation of the growth of an organism in an environment such that, after an initial period of fluctuation the age structure settles down to a stable age distribution for a specific environment. This distribution will be indefinitely maintained providing transitions remain constant. Obviously this is not achieved in the natural environment because of the constant fluctuations in all conditions that determine a given transition probability. The matrix model is therefore a stage-specific model of a population growing exponentially if there are no density-dependent factors operating.

Practically, the model consists of the multiplication of a vector, which describes the numbers of organisms at each specified stage, by a transition matrix, which contains the probability of an organism moving from one stage to another. Repeated pre-multiplication of the population vector by the same transition matrix gives rise to a stable age distribution which is achieved irrespective of the intial population vector. The stable age distribution is dependent on the components of the transition matrix determined from experimental work. The ratios of the stages are constant from one generation to the next when the population is growing at a constant rate. This growth rate is reflective

of the transition matrix and therefore the environment upon which the probabilites were obtained, and it expresses a fundamental characteristic of the population. The growth rate is the net reproductive rate or finite rate of increase per unit time, "R". This is related to the intrinsic rate of increase, "r", by the equation:-

$r = \ln R$ where r = the intrinsic rate of increase.

R = the finite rate of increase.

As previously indicated, the streptomycete systems investigated were divided into the three specific stages of spores, germ tubes and mycelial tips. This three-tier classification was used instead of a two-tier classification consisting of spores and infected units (germ tubes and mycelial tips) because of the necessity to relate spore production to germ tubes. The three-tier system is consistent with a third order transition matrix which contains the probabilites of passing from spores to germ tubes and thence to mycelial tips. The biology of the system indicates that the probability values are included at the matrical positions shown at Table 11. The transition probabilities are shown in Table 12. The proportion of spores which do not germinate and survive (a) is based on calculations involving the probability of spores surviving the eleven day period and those which do survive not germinating possibly due to lack of exogenous nutrients. The data for the combination of the two factors was derived from experimental work. The probability of spores germinating (b) was derived directly from the germination of spores in both sterile and non-sterile soil. All germ tubes were assumed to give rise to mycelial tips and therefore the probability at (c) would be 1. However more than one mycelial tip arises from a germ tube and therefore the probability of proceeding from the germ tube to mycelial tip stage is multiplied by the average number of mycelial tips

$$\begin{bmatrix} a & o & d \\ b & o & o \\ o & c & o \end{bmatrix}$$

MATRICAL POSITION	DESCRIPTION
a	Probability of spore not germinating and surviving
b	Probability of spore germinating
c	Probability of a germ tube continuing to the mycelial tip stage
d	Number of spores produced

Table 11:- Description of the positions used in the transition matrix

	MX1		MX8	
	SOIL STERILITY			
	STERILE	NON-STERILE	STERILE	NON-STERILE
PROBABILITY OF SPORE NOT GERMINATING	0.3035 ± 0.007	0.575 ± 0.004	0.3505 ± 0.0335	0.489 ± 0.007
PROBABILITY OF SPORE GERMINATING	0.05438 ± 0.02	0.134 ± 0.006	0.1527 ± 0.122	0.1125 ± 0.0125
AVERAGE NUMBER OF MYCELIAL TIPS PRODUCED PER SPORE	3.4515 ± 0.109	1.518 ± 0.161	4.5585 ± 0.007	1.605 ± 0.145
SPORE PRODUCTION:- EQUATION OF LINE MAXIMUM NUMBER OF SPORES PRODUCED	$\log_{10}y = 8.72 - 0.518 \log_{10}x$ 1.592×10^8 spores $(\text{colony})^{-1}$		$\log_{10}y = 8.64 - 0.507 \log_{10}x$ 1.358×10^8 spores $(\text{colony})^{-1}$	

Table 12:- Data used in the transition matrices for the determination of the intrinsic rate of increase of MX1 and MX8 ($\pm$ standard errors)

observed to have been produced, and this is therefore a multiplication factor rather than a probability factor. Spore production (d), which has been shown to be density dependent, is utilized in two ways. In any population a stable age distribution will be achieved although in some cases it may take a considerable time to do so. If a density-dependent function is introduced, in this case that of spore production and it does not lead to the regulation of the population such that R=1, _i.e._ the population is only maintaining itself, then the inference is that at least one of the probability values in the transition matrix is too large or too small. Therefore initially spore production in the transition matrix was used in its density-dependent form to examine the validity of the transition probabilites. For both MX1 and MX8 in sterile soil a stable number distribution of R=1 was not observed thus indicating that some further control needs to be exerted over the transition matrix. There are two ways of determining the amount of control needed, either by a systematic search which involves the random alteration of the transition probabilites, or by calculating theoretically what the alterations should be by mathematically expanding the transition matrix and examining its determinant. It may be that more than one factor needs to be altered. Here the second option was utilized. In this particular case only the proportion of spores germinating needs to be reduced to 5% (MX1) and 15% (MX8). Once the matrices had been examined and any alterations made (d) was set to a constant, the constant being the maximum number of spores produced by a single colony. All other positions possess a probability of 0, since in the simplified streptomycete life cycle adopted here no other transitions are feasible biologically. By definition spores did not give rise to mycelial tips and neither did mycelial tips produce germ tubes. It is assumed that in each cycle, germ tubes did not remain as germ tubes or if they did they became

inviable, and that on sporulation all mycelial tips produce spores or are lysed.

The instrinsic rate of increase, "r", for each of the S.albidoflavus strains, MX1 and MX8, are shown in Table 13. They indicate that MX1 grows at a slightly faster rate than MX8, and that the rates are slightly greater in non-sterile soil, but in both cases the rates are not statistically different. They reflect the increase in the numbers of spores, germ tubes and mycelial tips in the soil within a single time period of 11 days; therefore one would expect MX1 to have increased its number of spores, germ tubes and mycelial tips by 304 after each 11 day period. The difference between the intrinsic rates of increase in sterile and non-sterile soil, although not significantly different, reflects the necessary reduction of the proportion of spores germinating in sterile soil in order to support the larger mycelial tip production. This may indicate that some spores germinate but do not continue to the next stage either because of lack of nutrients or due to competition. However, if the experimental time period had been extended, non-sterile soil may have supported a larger increase in the proportion of spores germinating. It is possible that because the initial germination does not utilize exogenous nutrients that more spores germinate than is necessary, and therefore if conditions are favourable more spores will exploit these favourable conditions. The stable age distributions achieved in both cases indicated that at least 99% of the population in the soil was present as spores, and that less mycelial tips were present than germ tubes. Spores are thought to be the major form of streptomycetes in soil and therefore this distribution may not be as disproportionate as it appears. The large proportion of streptomycetes present as spores reflects the large spore production attributed to such a small number of tips. A more realistic representation of mycelial tip production in

HOST	SOIL STERILITY	
	STERILE	NON-STERILE
MX1	5.718 ± 0.124	5.762 ± 0.052
MX8	5.583 ± 0	5.669 ± 0.007

Table 13:- The intrinsic rate of increase for MX1 and MX8 in both sterile and non-sterile soil (± standard error)

relation to spore production may bring about an alteration in the final distributions at each stage. The decreased number of mycelial tips may be an indication that the full life cycle has not been performed because if it had, one would have expected the number of mycelial tips to outstrip those of germ tubes.

There are several other assumptions made in the matrix method for the determination of the intrinsic rate of increase. These are that:-

a) A germinated spore gives rise to only one germ tube. Spores can give rise to more than one germ tube and indeed on occasions this occurred.

b) The spore production observed in a plate experiment mirrors that in the soil.

c) As previously mentioned, the specific stages are all recovered with the same efficiency and therefore the extracted population reflects the behaviour of the population in the soil.

d) Finally, matrix modelling deals with overlapping generations, but with pulsed recruitment. It appears that from the data gathered, spore germination and germ tube production show an initial surge of growth followed by a slow increase in the mycelial tip production. Although the stages are not discrete, they are not continuous.

Several restraints for the measurement of "r" for the two systems are imposed as previously mentioned. The number of mycelial tips on which these measurements of "r" are based is a gross underestimate, it will be unlikely that a streptomycete colony within the soil will produce only 3 mycelial tips and that these give rise to 10^8 spores. However any increase in the mycelial tips which would more correctly reflect the growth of streptomycetes would lead to an increase in the value of "r". This has been shown by the performance of runs with increased mycelial tip production leading to increased rates to 6.01 (MX1) and 5.997 (MX8),

both in non-sterile soil. This illustrates an advantage of the use of this type of model, in that hypothetical changes in environmental conditions leading to numerical changes in the streptomycete growth can be investigated. Additionally the time periods for the rates of growth were only eleven days, therefore assuming that the whole of the streptomycete life cycle has been completed in this time period. However this obviously results in an underestimate of the intrinsic rate of increase, as a longer time period would allow increased mycelial tip production which has been shown to result in increased "r" values.

Therefore matrix modelling has been applied to obtain an estimate of the intrinsic rate of increase of MX1 and MX8. The increase was measured in terms of those sites which are susceptible to phage infection, i.e. germ tubes and mycelial tips and spores. Values of "r" were obtained which were shown to be underestimates of the growth in the soil due to the limited time period of the experiment resulting in an underestimate of the number of mycelial tips produced per spore.

SECTION VI AN ASSESSMENT OF THE CARRYING CAPACITY OF A SOIL FOR STREPTOMYCES ALBIDOFLAVUS STRAINS MX1 AND MX8

i) Introduction

When a population, be it bacterial or another type of organism, is growing in a natural environment there is always at least one limiting factor, such as nutrients, pH or competing organisms. The density of the population will increase, probably exponentially, but it cannot do so indefinitely. Eventually the limiting factors make their effect felt, reducing the fecundity and survival of the population and thus reducing the rate of increase of the population until it ceases to grow. This stationary phase is not the result of intrinsic ageing of the cells, at least of unicellular bacteria, because transfer of exponentially growing cells to fresh medium results in continued exponential growth of the cells and this can be performed indefinitely (Sistrom, 1962). For streptomycetes, of course, introduction of a limiting factor results in sporulation. This maximum limit to which a population can expand is usually known as the carrying capacity of the environment and is represented by the parameter "K". It describes the upper limit to which a population can expand, any population below it will increase in size and any population above it will decrease in size. Units of measurement of carrying capacity are dependent on its definition with respect to the system being studied, e.g. kg ha^{-1}, cells ml^{-1}. It is also dependent on the aims of the investigation. Thus in ecological studies of game ranching in Africa, it is the maximum possible density of animals that can be sustained in the absence of harvesting, whereas to the economist, it is the density of animals that will allow maximal sustained harvesting (Krebs, 1978).

The Nicholson-Bailey model deals with infectable units, which have already been defined for streptomycetes as germ tubes and mycelial

tips. Therefore, the carrying capacity must be measured and defined in terms of these. Carrying capacity is then either the maximum number of mycelial tips and germ tubes observed or the calculated maximum number of mycelial tips, assuming that all germinated spores produce a mycelial network which can be supported by the soil environment. Spores germinate with the production of a germ tube which then continues to form a mycelial network. It is therefore more logical to assume that all the germ tubes observed would continue to form mycelium thereby making the calculated carrying capacity based on mycelial tip production a more realistic estimate of that within the soil environment.

Experimental data for calculating carrying capacities, i.e. the observed number of germ tubes and mycelial tips in a soil environment, and the number of germinated spores, can be obtained from work previously performed in Section V, Part A. This investigated the germination of streptomycete spores and their subsequent growth, the data being used to determine the intrinsic rate of increase. Therefore two estimates of the carrying capacity of a mature Freshfield sand dune soil for MX1 and MX8 were obtained, one being based on the observed number of germ tubes and mycelial tips and the other on the assumption that all germ tubes give rise to a mycelial network.

ii) Results

Tables 14 and 15 present the calculated carrying capacities derived by two methods of a Freshfield mature sand dune soil for both MX1 and MX8.

Method 1:- Table 14 presents the carrying capacities for the two strains based upon the assumption that all the observed germ tubes will give rise to a mycelial network, where the number of mycelial tips within these networks is equivalent to the average number of mycelial tips observed to be produced per germinated spore.

i.e. $K = Tg \times M$ where Tg = number of germinated spores

M = average number of mycelial tips produced per spore

Tg and M being obtained from Fig 6 and Table 8 in Section V, Part A.

Method 2:- Table 15 presents the carrying capacities which are equivalent to the observed number of mycelial tips and germ tubes within the soil after the initial surge of streptomycete growth appeared to have occurred (Fig 9, Section V, Part A).

HOST	SOIL STERILITY	
	STERILE	NON-STERILE
MX1	1.374×10^8	8.54×10^6
MX8	3.62×10^7	6.39×10^6

Table 14:- Carrying capacities of MX1 and MX8 in sterile and non-sterile Freshfield mature sand-dune soil determined by method 1.

(Infectable units g^{-1} soil)

HOST	SOIL STERILITY	
	STERILE	NON-STERILE
MX1	5.62×10^7	5.01×10^6
MX8	3.54×10^7	4.00×10^6

Table 15:- Carrying capacities of MX1 and MX8 in sterile and non-sterile Freshfield mature sand-dune soil determined by method 2 (Infectable units g^{-1} soil)

iii) Results and discussion

Tables 14 and 15 present the carrying capacities calculated by the two described methods. Table 14 presents the carrying capacities observed if all germinated spores are assumed to form a mycelial network with the average number of mycelial tips previously calculated (Section V, Part A). Table 15 presents the carrying capacities obtained based on method 2, i.e. the observed number of germ tubes and mycelial tips produced in the soil. Both the methods result in the carrying capacity observed in sterile soil being greater than that observed in natural soil. Sterile soil supports a much greater streptomycete population in terms of germ tubes and mycelial tips (Section V, Part A) due to increased nutrient availability caused by sterilisation and the lack of competition from other organisms for space and nutrients. MX1 shows a greater carrying capacity in both sterile and natural soil for both derivations than MX8, but the difference is not statistically significant.

The values obtained from both method 1 and 2 for the derivation of the carrying capacity for both MX1 and MX8 and in both sterile and natural soil are likely to underestimate the carrying capacity actually occurring. The maximum values of the carrying capacity derived by method 1 are based on streptomycete mycelial tip productions of 3 (MX1) and 5 (MX8) per germinated spore (Section V, Part A). As already stated these are gross underestimates of that which would occur. The values of the carrying capacity derived from method 2 are underestimates for precisely the same reason, because they are based on the same original data. Even though method 1 is an underestimate of the carrying capacity, it is less inaccurate than those values derived from method 2 and thus it is more reasonable to use those values for both soil types and for both strains as the best estimates

of the carrying capacity. Later when the model is utilized it will be possible theoretically to alter the carrying capacity and observe the effect of an increased and therefore more realistic approximation of the streptomycetes and their phage. The value of the carrying capacity will alter within different soil types and within a single soil type due to the very heterogeneous nature of the environment.

Therefore estimates of the carrying capacity of the Freshfield mature sand dune soil (pH7.6) have been calculated both for MX1 and MX8 in sterile and natural soil by two methods. It has been decided that although both of these methods give rise to underestimates of the true carrying capacity of the soil for these two strains method 1 (that based upon the assumption that all germinated spores gives rise to an average number of mycelial tips) will be used in future work as the best estimate of the carrying capacity of the soil both sterile and natural, for MX1 and MX8.

VII. AN ASSESSMENT OF THE INFECTION EFFICIENCY OF TWO STREPTOMYCETE PHAGE, Ømx1 AND Ømx8, UNDER VARIABLE DENSITIES OF BOTH HOST AND PHAGE

i) Introduction

In order for a phage to replicate it must first become adsorbed to a host cell. The adsorption of phage has attracted much attention since the initial experiments with Staphylococcus aureus and its phage by Kreuger (1931). Adsorption of phage onto host cell surfaces consists of an initial reversible stage followed by an irreversible second stage. The differences between the two stages of adsorption were emphasised by Tolmach (1957). Evidence obtained from antibody effects indicated that irreversible adsorption is prevented whereas reversible adsorption proceeds unaffected.

Reversible adsorption is brought about by random collisions of the host and phage diffusing through the media. It involves the interplay of electrostatic forces between the phage and host (Puck, Garen & Cline, 1951). If both are similarly charged there may be some difficulty in establishing close contact, and probably this is one advantage of a phage possessing a tail. It has been shown for Escherichia coli B and T6 phage that although both have large negative charges they can still reversibly adsorb (Tolmach, 1957). Puck & Tolmach (1954) suggested that attachment is through carboxyl and amino groups, the amino groups being found in the phage tail. Other factors which can be important are adsorption cofactors such as tryptophan (Anderson, 1948), and the ionic environment (Hershey et al., 1944; Walton, 1951). Reversible adsorption is relatively unaffected by temperature confirming the electrostatic nature of the bond (Puck, 1951), but can be affected

by pH (Sykes, 1977).

The alteration in the bonding which constitutes the transition from irreversible adsorption must arise from the participation in the bond of additional chemical groups, and for the T-series of phage, these have been suggested to be an increased number of carboxyl-amino groups. The physiological condition of the host has also been shown to affect adsorption (Barry & Goebel, 1951; Thompson & Shafia, 1962).

In the Nicholson-Bailey model the interaction between the host and parasitoid, the two interacting organisms for which it was originally derived was termed the searching efficiency and was represented by the parameter "a". The parasitoid searches for a suitable host. Once found, its ovipositor which bears a sense organ can determine whether the host is suitable or even whether it already contains a parasite egg. In the streptomycete-phage system the searching efficiency is analogous to a phage finding a host and completing a successful irreversible adsorption. It will therefore be termed the infection efficiency, "a". It is a constant value for a described set of conditions and should be unaffected by increases in the number of hosts as it is an independent constant.

Therefore an investigation was performed to determine the value of "a" for a particular system and to ascertain whether the system satisfies the Nicholson-Bailey assumptions that the adsorptions are randomly distributed among the available hosts, and that the number of encounters with the hosts by phage are in direct proportion to host density. This was achieved by determining the effect of varied host and phage densities on the value of "a"

ii) Method

Host spores were added to 30ml PYCa broth to give a final concentration of 5 x 10^7c.f.u.ml^{-1}. Germination was allowed to proceed at 15°C on a Griffin flask shaker at 75 rpm until the germ tubes were 3-8x the length of the original spore. Germination was assessed using a Thoma counting slide.

The adsorption vessel contained a final total volume of 20ml PYCa broth (pH7), which contained either:-

a) Germinated spores at a final concentration of 1.2 x 10^7 c.f.u. ml^{-1} and varied phage volumes, made up to 20ml with PYCa broth (pH7), for investigation of the m.o.i. effect on adsorption

b) Phage at a final concentration of 1.2 x 10^8 p.f.u. ml^{-1} and germinated spores at final concentration which were varied, made up to 20ml with PYCa broth (pH7), for investigation of the host:phage ratio effect on adsorption.

The adsorption vessel was incubated at 15°C on a Griffin flask shaker at 75 rpm. 2ml samples were removed at timed intervals and filtered through a membrane filter of 0.45μm pore size. The filtrate was appropriately diluted and assayed for residual activity, which would provide a measurement of the total number of adsorptions, i.e. reversible and irreversible. It is possible to elute non-specifically bound phage from host cells by washing and thereby obtain a measurement of irreversibly adsorbed phage. Therefore, after the initial filtration, 2ml of fresh sterile adsorption media were passed through the filter to remove any non-specifically bound phage. The filter was carefully removed from the filter case and added to vials containing 10ml PYCa broth (pH7). These vials were shaken for 30sec. and then appropriately diluted and assayed for infective centres.

For both the altered m.o.i. and the host:phage ratios a log range of ratios was investigated from 0.1 to 100 for the phage host systems of Ømx1 + MX1 and Ømx8 + MX8.

iii) Results

The proportion of phage which are adsorbed, both reversibly and irreversibly (Fig 15) and irreversibly (Fig 16) at varying m.o.i. and host:phage ratios are presented. Table 16 gives the range of "k" values (adsorption velocities) observed. These were obtained from the equation of Kreuger (1931):-

$$k = \frac{2.3}{B_t} \log \frac{P_o}{P_t} \qquad (1)$$

where P_o = the number of phage unadsorbed at the end of the time period.

P_t = the number of phage available at the start of the time period.

B_t = the bacterial concentration.

Fig 17 present the "k" values obtained at varying m.o.i. and host:phage ratios.

The parameter "a", here termed the infection efficiency, is the likelihood that a phage will effectively adsorb to a given host in its searching lifetime, which in this case is 60 min., the length of the experiment. The adsorptive behaviour of the phage must satisfy two assumptions made in the Nicholson-Bailey model:-

1) The number of infections, H_i, of H_t hosts by P_t phage is in direct proportion to host density.

   i.e. $H_i = a H_t P_t$ (2)

2) The infections are randomly distributed among the available hosts.

Assumption 1 gives a method for caluclating "a" and Fig 18 represents the infection efficiencies obtained at varying m.o.i. and host:phage ratios. Parameter "a" is dimensionless if H_t and P_t are expressed as total populations, but if expressed as numbers per unit area then "a" will be in the same units of area.

Nicholson assumed that the number of hosts not infected is given

by the zero term of the Poisson distribution, as random search is employed, and therefore the number of hosts actually infected is given by:-

$$H_i = H_t\ (1 - \exp(-aP_t)) \qquad (3)$$

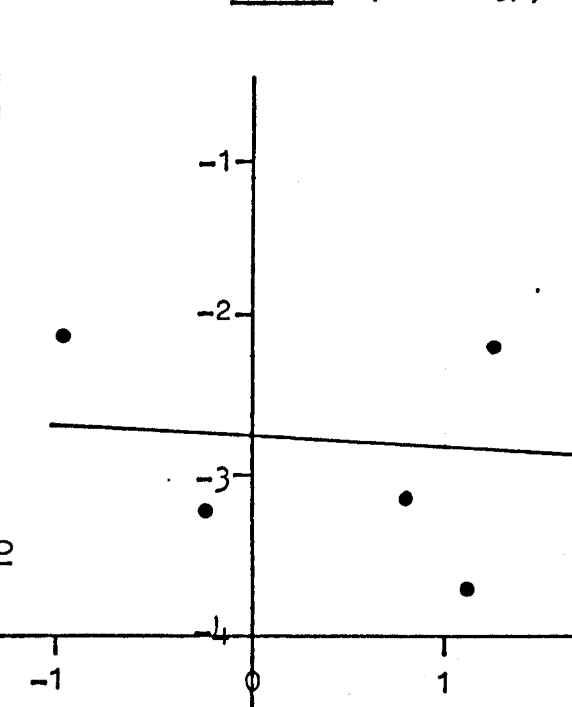
This expression serves to distribute the effective adsorptions randomly among the hosts. Table 17 gives the number of hosts calculated to be infected together with those observed to be infected.

Table 18 contains the average value of the infection efficiencies to be used in the modelling of the streptomycete-phage interaction.

Fig. 15:- Proportion of reversibly and irreversibly adsorbed phage at varied m.o.i. and host:phage for Ømx8 and Ømx1

a) Ømx8

m.o.i. (R^2 = 0.3%)



HOST:PHAGE (R^2 = 12.9%)

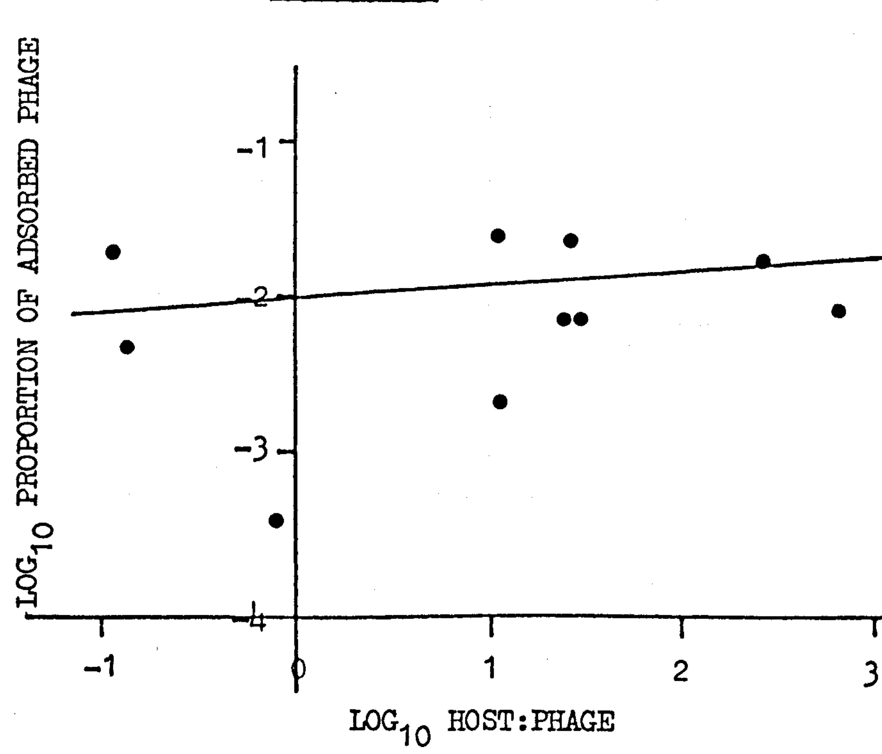


Fig. 15 cont:-

b) Ømx1

m.o.i. (R^2 = 6.99)

HOST:PHAGE (R^2 = 42.86, r = 0.653)

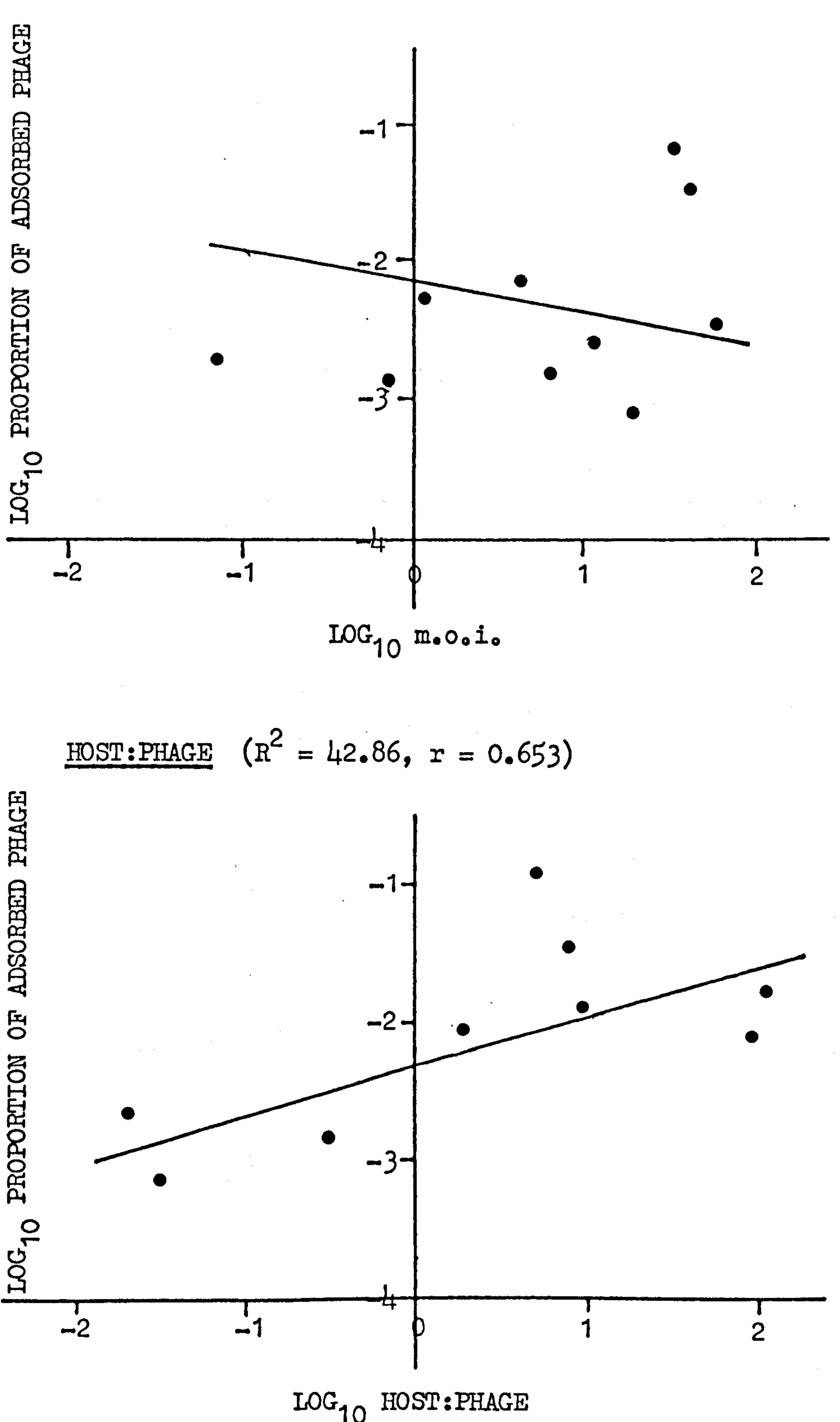
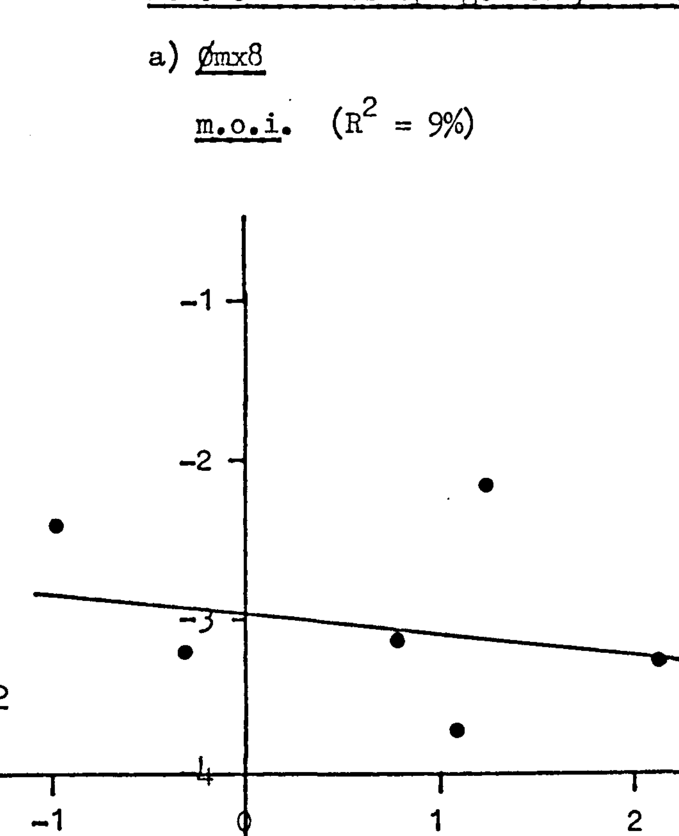


Fig. 16:- Proportion of phage irreversibly adsorbed at different m.o.i. and host:phage for Ømx8 and Ømx1

a) Ømx8

m.o.i. (R^2 = 9%)



HOST:PHAGE (R^2 = 9.4%)

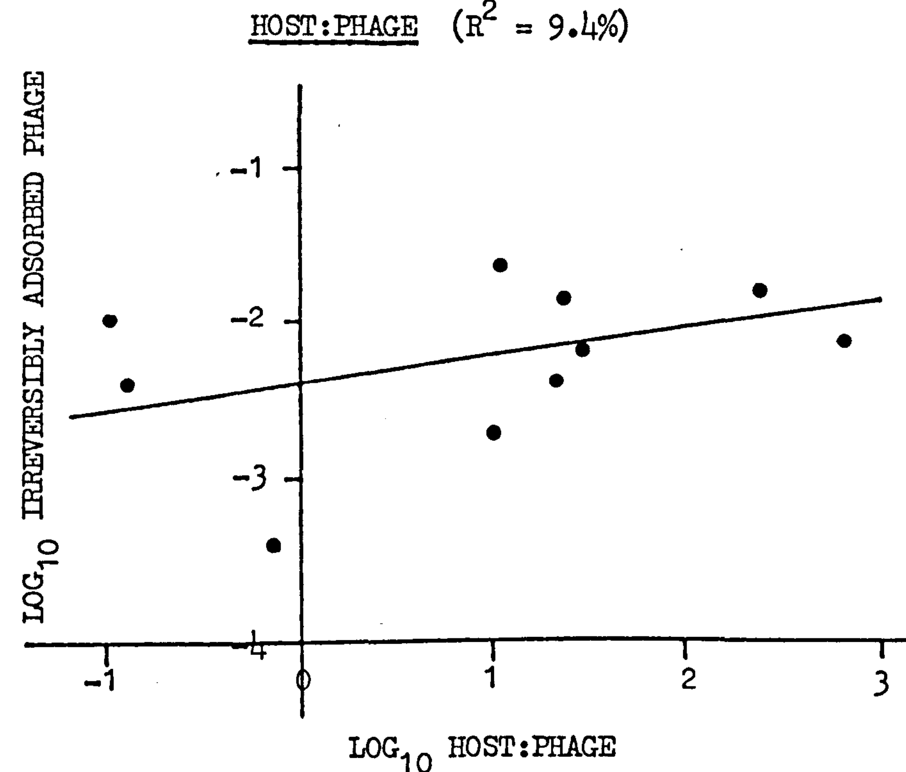
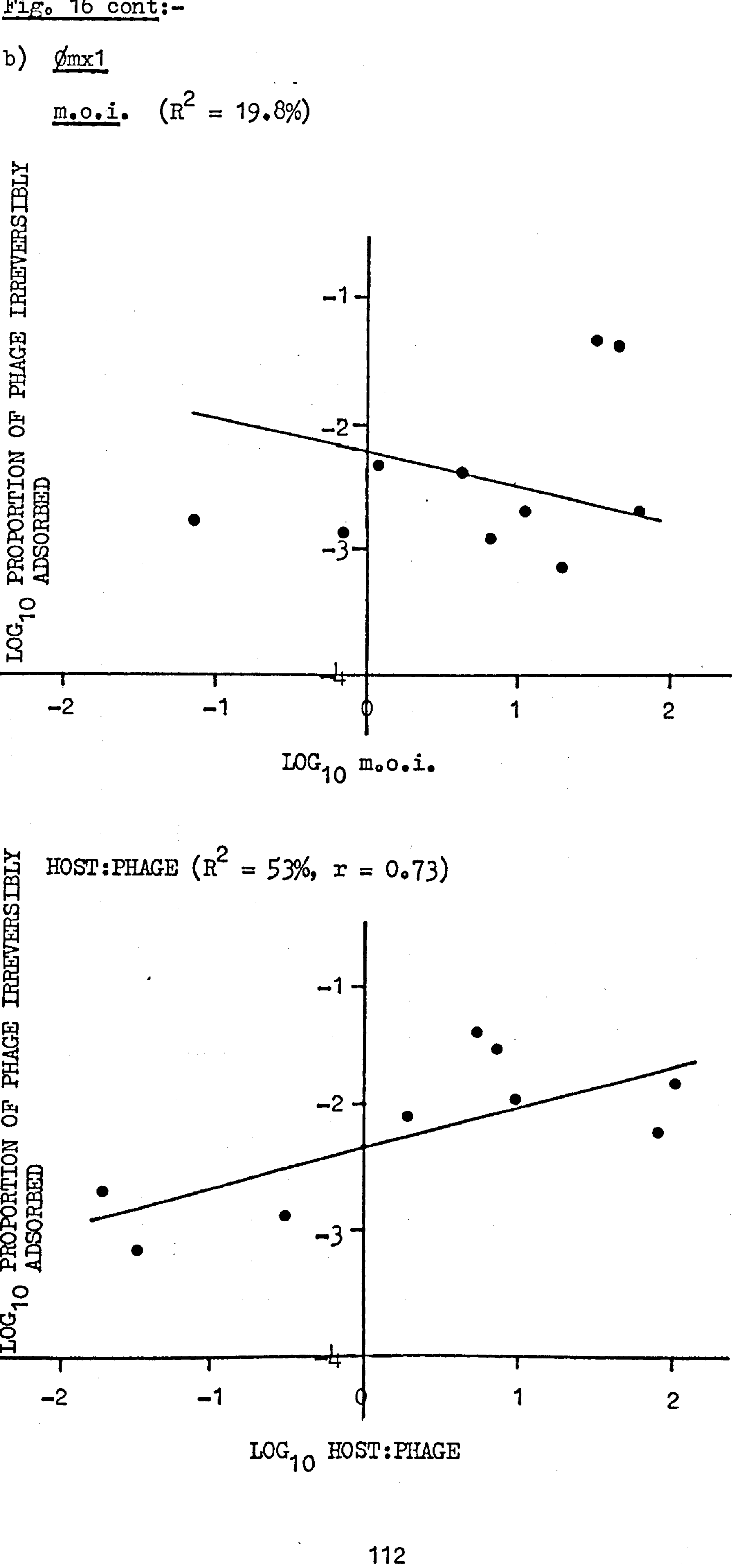


Fig. 16 cont:-

b) Ømx1

m.o.i. (R^2 = 19.8%)



EXPERIMENTAL SYSTEM	HOST + PHAGE	RANGE OF k OBSERVED (ml min^{-1})
m.o.i.	MX1 + Ømx1	$4.9328 \times 10^{-13} - 5.6696 \times 10^{-15}$
	MX8 + Ømx8	$4.7617 \times 10^{-14} - 1.3427 \times 10^{-15}$
HOST:PHAGE	MX1 + Ømx1	$1.5983 \times 10^{-12} - 4.914 \times 10^{-15}$
	MX8 + Ømx8	$6.8776 \times 10^{-12} - 5.6165 \times 10^{-15}$

Table 16:- Range of adsorption rate constants for the MX1 + Ømx1 and MX8 + Ømx8 systems under varying m.o.i. and host:phage

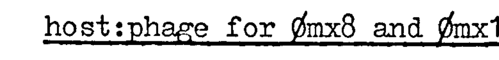
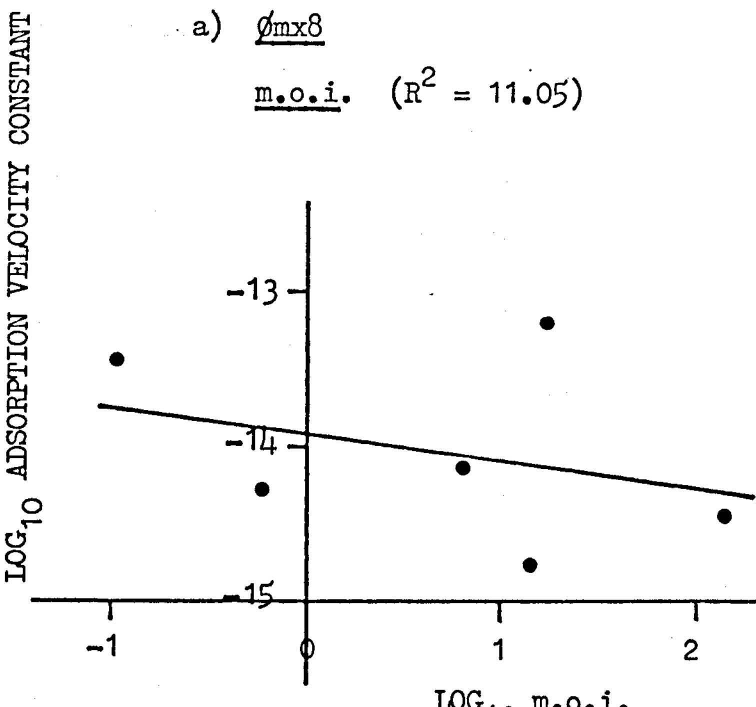


Fig. 17:- Adsorption velocity constants at varied m.o.i. and host:phage for Ømx8 and Ømx1

a) Ømx8

m.o.i. (R^2 = 11.05)



HOST:PHAGE (R^2 = 71.51, r = -0.85)

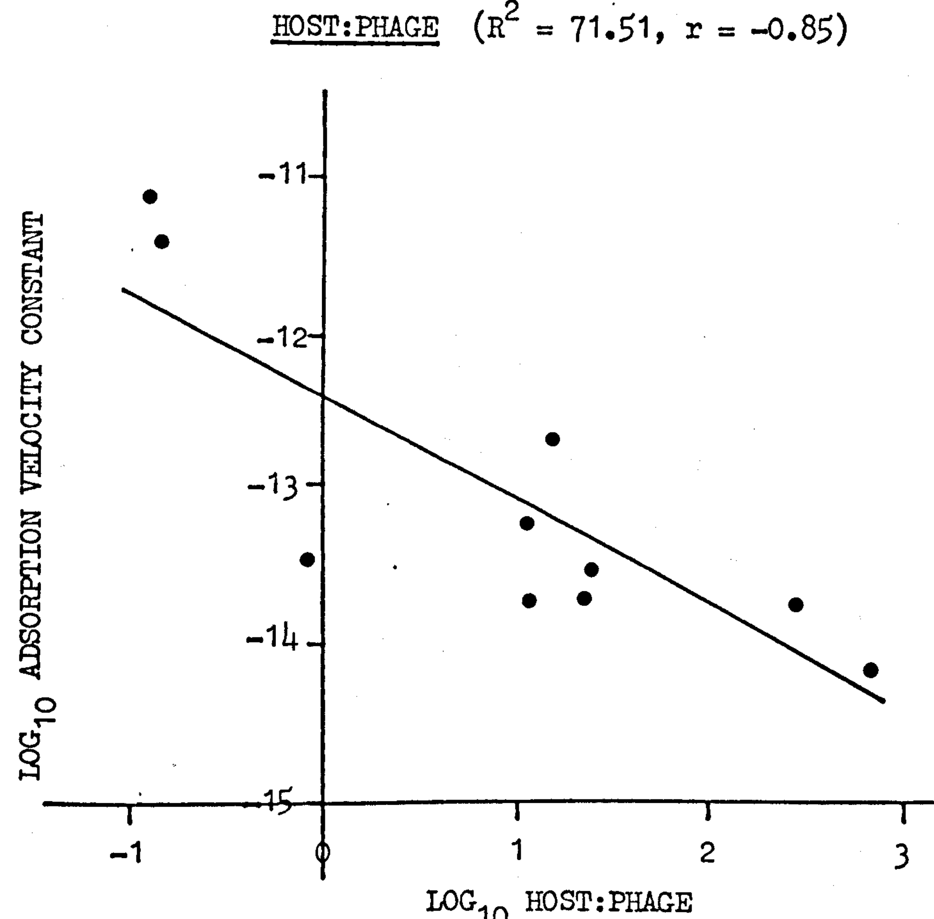


Fig. 17 cont:-

b) Ømx1

m.o.i. (R^2 = 0.3%)

HOST:PHAGE (R^2 = 74.16, r = -0.861)

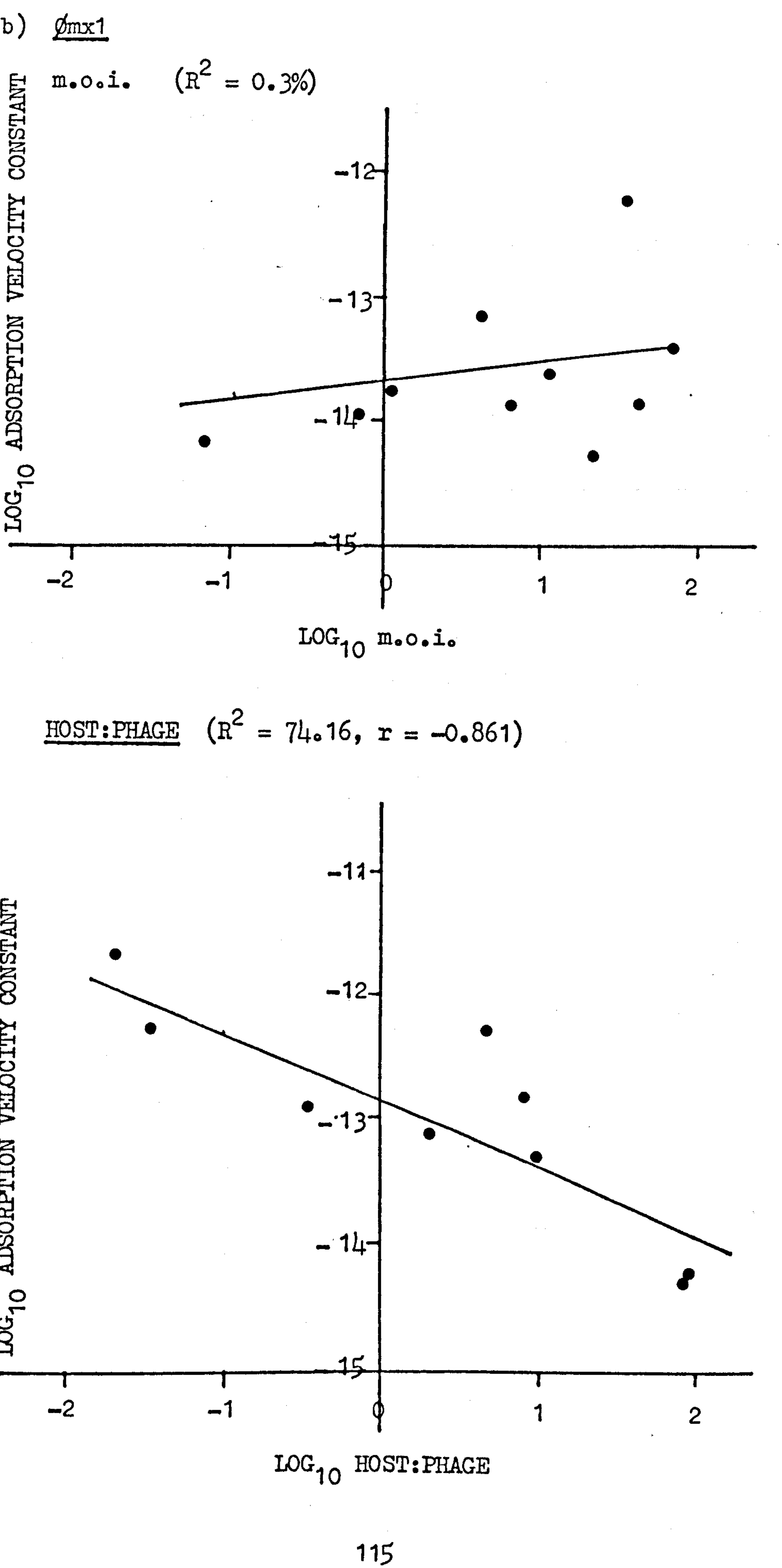
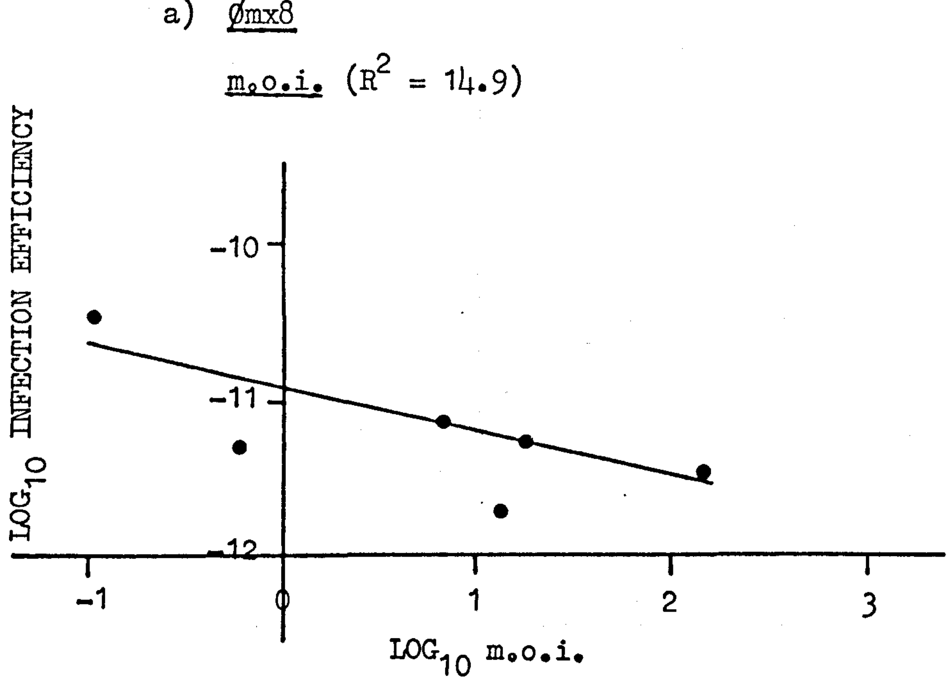


Fig. 18:- Infection efficiencies at varied m.o.i. and host:phage for Ømx8 and Ømx1

a) Ømx8

m.o.i. (R^2 = 14.9)



HOST:PHAGE (R^2 = 67.4, r = -0.821)

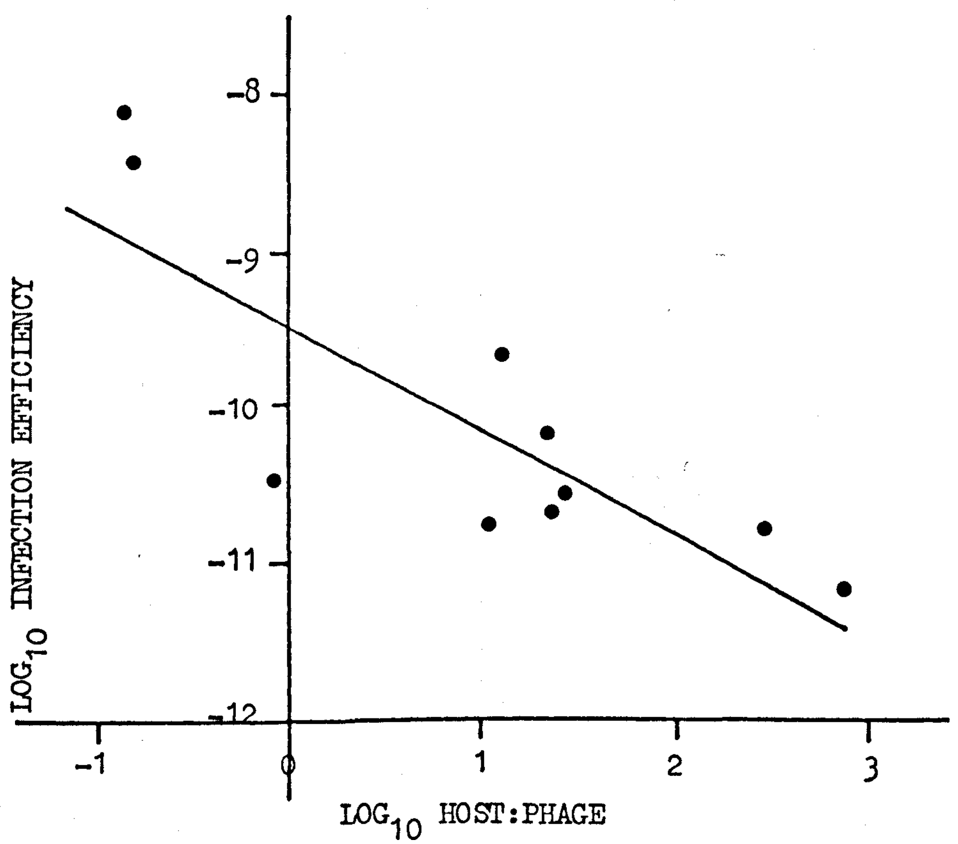
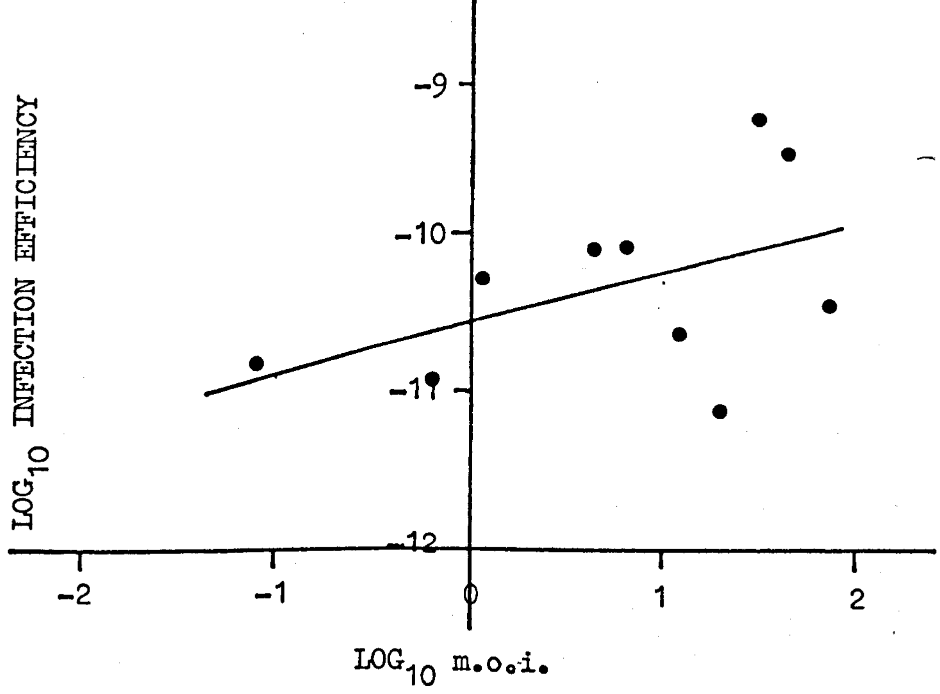


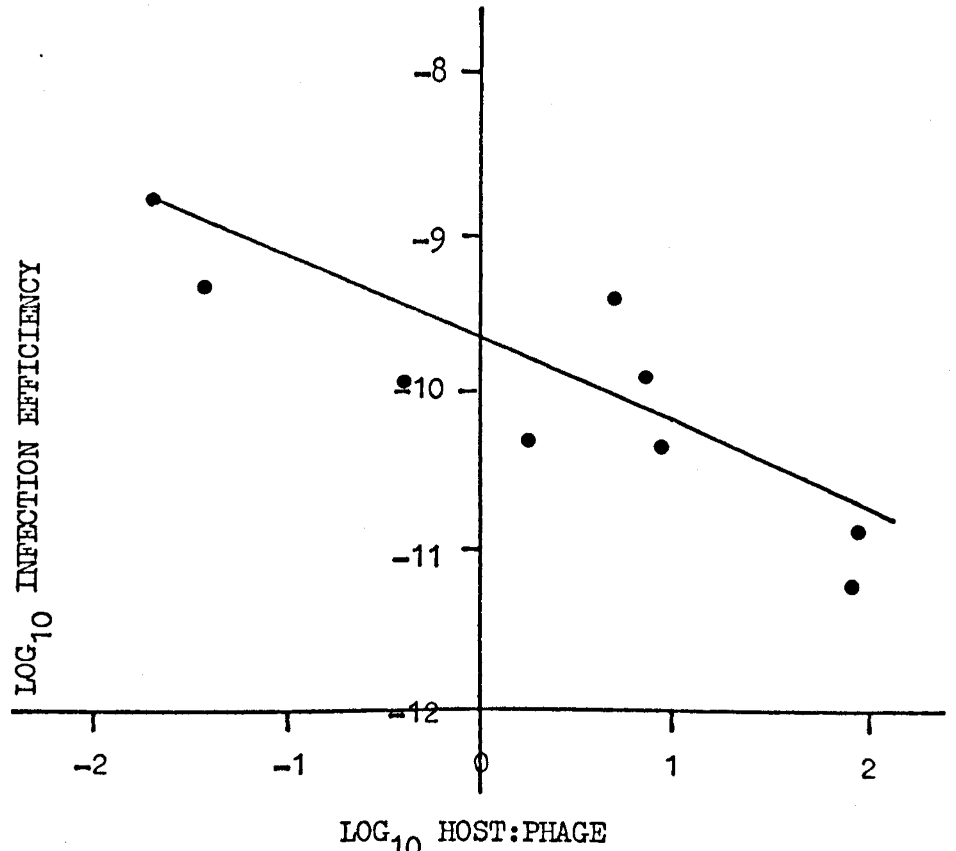
Fig. 18 cont:-

b) Ømx1

m.o.i. (R^2 = 21.54)



HOST:PHAGE (R^2 = 73.16, r = -0.855)



HOST + PHAGE	INFECTION EFFICIENCY
MX1 + ∅mx1	$1.2117 \times 10^{-10} \pm 5.97672 \times 10^{-11}$
MX8 + ∅mx8	$1.37351 \times 10^{-11} \pm 9.48285 \times 10^{-12}$

Table 17:- Infection efficiencies for the MX1 + ∅mx1 and MX8 + ∅mx8 systems (± standard error)

OBSERVED NUMBER OF INFECTIONS	CALCULATED NUMBER OF INFECTIONS
MX1 + Ømx1	
a) m.o.i. 2.6 $\times 10^8$	1.0 $\times 10^8$
1.4 $\times 10^8$	7.6 $\times 10^7$
2.0 $\times 10^7$	1.8 $\times 10^7$
3.4 $\times 10^6$	3.3 $\times 10^6$
3.0 $\times 10^6$	3.0 $\times 10^6$
1.9 $\times 10^6$	1.[illegible] $\times 10^6$
8.5 $\times 10^5$	8.5 $\times 10^5$
5.5 $\times 10^5$	5.5 $\times 10^5$
1.2 $\times 10^5$	1.2 $\times 10^5$
1.1 $\times 10^4$	1.1 $\times 10^4$
MX8 + Ømx8	
6.5 $\times 10^6$	6.4 $\times 10^6$
1.2 $\times 10^6$	1.1 $\times 10^6$
5.8 $\times 10^5$	5.8 $\times 10^5$
4.0 $\times 10^5$	4.0 $\times 10^5$
4.4 $\times 10^4$	4.4 $\times 10^4$
4.3 $\times 10^4$	4.3 $\times 10^4$

Table 17:- Observed and calculated infections for the MX1 + Ømx1 and MX8 + Ømx8 systems from varying host:phage and m.o.i. experiments

OBSERVED NUMBER OF INFECTIONS	CALCULATED NUMBER OF INFECTIONS
MX1 + Ømx1 b) HOST:PHAGE	
1.1×10^6	1.1×10^6
9.1×10^5	9.1×10^5
3.2×10^5	3.2×10^5
2.6×10^5	2.6×10^5
2.0×10^5	2.0×10^5
1.0×10^5	1.0×10^5
8.7×10^4	8.7×10^4
4.7×10^4	4.7×10^4
3.2×10^4	3.2×10^4
MX8 + Ømx8	
2.2×10^5	2.2×10^5
1.4×10^5	1.4×10^5
1.2×10^5	1.1×10^5
7.0×10^4	7.0×10^4
6.9×10^4	6.9×10^4
4.8×10^4	4.7×10^4
4.8×10^4	4.8×10^4
2.5×10^4	2.5×10^4
1.1×10^4	1.1×10^4
5.3×10^3	5.3×10^3

Table 17:- Continued

iv) Results and discussion

Fig 15 shows the proportion of phage which are reversibly and irreversibly adsorbed and Fig 16 those phage which are irreversibly adsorbed at varying m.o.i. (host density constant and phage density varied) and host:phage ratios (phage density constant and host density varied). There is only a small numerical difference between corresponding graphs in the two figs, indicating that most of the phage adsorbed result in successful infections. The maximum adsorption observed after 60 min, was 10% for the MX1 + Ømx1 system. It is probable that if left for longer, more phage would have been adsorbed and resulted in successful infections. The results indicate that increased host density results in more adsorption than do increases in phage density, because increased host density can afford greater opportunity for phage adsorption. Increasing phage densities can lead to interference between phage because of competition for limited adsorption sites, and to the mutual exclusion effect observed by Delbrück (1945). This results from more than one phage adsorbing to the host cell, the first phage only leading to a successful infection. The results also illustrate that for the MX1 + Ømx1 system that as the host density increases then so does the proportion of phage adsorbed. This is particularly evident at host:phage below 10. The correlation coefficient for host density and proportion of adsorbed phage is significant at the 5% probability level. The proportions of phage being dealt with are very small (0.1), a reflection of the system used, and this may result in the variation observed, and lack of any significantly correlated trend in the MX8 + Ømx8 system. In both systems alterations in phage density (m.o.i.) appeared to have no effect on the proportion adsorbed - there being no statistical correlation observed.

The effect of variation of host and phage densities on adsorption

has been investigated in several species of bacteria by many authors. Watnabe *et al*. (1982) demonstrated that when the number of *Lactobacillus casei* cells was decreased ten-fold a similar decrease was seen in the adsorption of PL-1 phage to form reversible complexes but that the rate of formation of irreversibly-bound phage remained the same, the numbers then being determined by the collision rates. Tzagloff & Pratt (1964) also found that increased host cell concentration could lead to an increase in adsorption rate. Kolstad & Bradley (1967) demonstrated with *Streptomyces venezulae* + MSP8 phage that increased phage or host numbers lead to increased infection. A comparison of attachment at two different host concentrations showed that irreversible attachment was proportional to host concentration, but that as phage concentration increased, a smaller proportion of phage was irreversibly adsorbed. *Escherichia coli* + ØT4 show a rate of adsorption which increases proportionally in relation to phage concentration, and there is a limit to the bacterial concentration above which no further increase in the adsorption rate occurs (Stent & Wollman, 1952).

The kinetics of adsorption were investigated by Kreuger (1931) and later by Schlesinger (1932), Garen & Puck (1951) and Stent & Wollman (1952). Kreuger found that with an excess of bacteria, the time course of adsorption followed first-order kinetics:-

$$\text{Rate of adsorption} = -\frac{dP}{dt} = k\,B\,P$$

where K = velocity constant

B = bacterial concentration

P = phage concentration

The solution of the above equation is (as already explained):-

$$k = \frac{2.3}{B} \log \frac{P_o}{P_t}$$

which is generally applied to phage adsorption. Schlesinger (1932), studying the nature of the rate constant "r", postulated that adsorption

is analogous to random collisions of two bodies. Adsorption was treated as the diffusion of small bodies representing the phage towards large bodies representing the host cells which were spherical with a radius of "a". Host cells were present in a concentration of "B" and all collisions led to an adsorption. This situation was represented by the von Smoluchowski coagulation theory:-

$$-\frac{dP}{dt} = 4DaBP \qquad \text{where } D = \text{the diffusion constant of the phage}$$

Implicit in the system was the assumption that the bacteria and fluid were stationary and the phage particles dimensionless. By assuming that every collision gives rise to adsorption, an estimate of the maximum possible adsorption rate can be obtained. Recently Watnabe et al.(1982) have proposed a model for the adsorption of PL-1 phage to L. casei which includes the rate constant for each step of adsorption. Three steps were proposed; one for those phage which form reversibly bound complexes but do not continue to form irreversibly bound complexes and two for those which continue to the irreversibly bound stage.

Biphasic phage adsorption has been shown by several authors. Sykes (1977) showed with several streptomycete phages that up to 95% were adsorbed at a constant rate in 20 min and at the end of this period attachment rates began to decrease. Schlesinger (1932) also demonstrated a similar biphasic adsorption with E. coli 88 and coliphage WLL, whilst Maiti & Chaudhuri (1979), working with Vibrio cholerae and phage Ø2, measured adsorption rate constants falling from 1×10^{-9} ml min^{-1} to 0.085×10^{-10} ml min^{-1}, which indicated a small proportion of slow adsorbers within the phage population. In the current work, biphasic adsorption was not observed during the time period studied rather a monophasic adsorption (straight line adsorption graph) at a slow rate. Similar straight line adsorptions, which were also of a slow rate similar to those found in this work have been observed by

HOST	PHAGE	k (ADSORPTION RATE CONSTANT) (ml min^{-1})	AUTHOR(S)
Escherichia coli B	anticoli-phage	Obtained under varied cultival conditions of host 5.2×10^{-10} - 2.3×10^{-10}	Delbrück (1941)
Escherichia coli B	T1	$k_{37^oC} = 2.7\times10^{-9}$ $k_{2^oC} = 1.3\times10^{-9}$	Garen & Puck (1951)
Escherichia coli	M13	3×10^{-11}	Tzagloff & Pratt (1964)
Vibrio cholerae	Ø2	1.09×10^{-9}	Maiti & Chaudhuri (1979)
Mycobacterium smegmatis *Mycobacterium aurum*	D_{29} Sm D_{29} Au	1.9×10^{-10} 8.7×10^{-11}	David *et al*. (1980)
Streptomyces coelicolor A3(2)	ØC31	7.8×10^{-10}	Lomovskaya *et al*. (1972)
Streptomyces coelicolor A3(2)	Ø31c	8.0×10^{-10}	Novikova *et al*. (1973)
Streptomyces coelicolor A3(2)	VPII	Germinated spores:- $5.3\ 10^{-10}$ - 1.2z10^{-9} Mycelium:- 1.2×10^{-9} - 2.8×10^{-9}	Dowding (1973)
Streptomyces sps F6 F13	 F6 F13	 2.84×10^{-9} 1.06×10^{-9}	Sykes (1977)

Table 19:- Adsorption rate constants of phage to various organisms

Novikova *et al*. (1973) and Rosner & Gutstein (1980). In the latter work a straight line adsorption was observed for phage adsorption to spores whilst adsorption to mycelium was biphasic. Table 19 presents some adsorption rate constants determined for several bacteria-phage systems. The values obtained from the two systems studied here fall outside the lower end of the range (see Table 16), indicating a very slow adsorption rate and may be reflective of the low temperature used in the experiments. Fig 17 indicates that the adsorption rate constants are well correlated, although negatively, with changes in host density, indicating that adsorption rate constants do not appear to be correlated with phage density, indicating that, at least at the phage concentrations studied, phage density is not a limiting factor in adsorption.

The low adsorption rate observed is a reflection of the low temperature used, and perhaps the media composition. The first reversible adsorption is thought to be unaffected by temperature because of its electrostatic nature which requires small energies of activation. It is possible that the temperature affects the diffusion constant of the phage particles, making it more difficult for the phage to encounter the host particles. Tzagloff & Pratt (1964) reported that an increase in attachment rates at 0 - 25°C corresponded to a decrease in viscosity of water over the same temperature range. Several authors have dealt with the effect of temperature on adsorption at both the reversible and irreversible stages. Watnabe *et al*. (1982) demonstrated that the initial reversible adsorption of the *Lactobacillus* phage PL-1 was unaffected at 37°C and 0°C, but that the irreversible complex was inhibited at

$0^{o}C$. Use of their model of adsorption indicated that at lower temperatures the rate of collisions of phage with host cells was lowered and that once adsorbed, phage were unlikely to be dislodged again. Garen & Puck (1951) also demonstrated equally rapid initial attachment rates with phage T1 to E. coli B at $37^{o}C$ and $2^{o}C$, but the subsequent enzymatic step was decreased sharply as temperature decreased. Kolstad & Bradley (1967) with Streptomyces venezuelae + phage MSP8 indicated that reversible attachment was as extensive at $4^{o}C$ as at $28^{o}C$ but that irreversible attachment was more extensive at $28^{o}C$. Other work showing the same effect was performed by Jones & Bradley (1965), Stent & Wollman (1952), Puck (1951) and Anderson (1948).

Other factors which can affect adsorption of a phage to its susceptible host are the ionic composition of the media, cofactor absence of presence, pH and the physiological condition of the host. Ionic composition has received much attention from authors because of the extreme importance of adsorption in the phage replication cycle. For the systems studied here, MX1 + Ømx1 and MX8 + Ømx8 the presence of calcium ions was necessary to facilitate good adsorption. Use of nutrient broth allowed little detectable adsorption and therefore infections, but the addition of calcium ions did lead to infection. The requirement of calcium for good adsorption has been shown by many authors (Adams, 1949; Alexander & McCoy, 1956; Gold, 1959). Other ions have been shown to be required, such as manganese and magnesium ions for Streptomyces griseus actinophage S-1 (Walton, 1951). Puck et al. (1951) investigated the role of ions in the primary attachment of T-phage to E. coli B and demonstrated that the addition of some ions increases adsorption, whilst some trivalent ions, Al^{3+}, Cr^{3+}, Fe^{3+},

decreased it by causing inactivation of the phage. They postulated that the cations react to specific sites on the phages and possibly the host cell forming two complementary configurations which can unite with a high specificity. The cations can also reduce the large repulsive forces which can occur on the host and phage thereby enabling a closer contact between the two and increasing the possibility of adsorption.

The effect of pH on phage adsorption has been studied by Anderson (1948), Puck & Tolmach (1954), Fraser & Fleischmann (1974), Reese et al.(1974) and Sykes (1977). The effect of pH varied with the phage used but in general as it was lowered, the rate of overall adsorption fell, but it was shown to have little effect on the irreversible adsorption complex. Tzagloff & Pratt (1964) found that E. coli and phage M13 showed little or no change in adsorption between pH 5-8. Puch & Tolmach (1954) showed that at pH greater than 10.5 and less than 4.8 no adsorption occurred, corresponding to the regions in which the ionization of carboxyl and amino groups is prevented. Both of these groups are important in the adsorption of the T-phage series and E. coli. Sykes (1977) demonstrated that at low pH attachment of phage to acidophilic hosts was no more efficient than to neutrophilic hosts and therefore the optimum pH of the host was independent of the requirements for phage attachment.

Delbrück (1941) demonstrated that the physiological condition of the host can have a profound effect on adsorption. The adsorption rates of coliphage to E. coli B were found to be dependent on the previous cultural conditions of the host cell. Those cultured under adverse conditions had the lowest adsorption rates, possibly indicating that some change in the binding sites may have occurred. Schlesinger (1932) also obtained a slower adsorption rate onto heat-killed cells

than live resting cells, again possibly indicating changes in the binding sites. David <u>et al.</u> (1980) showed that the adsorption capacity of mycobacteria changed during incubation, adsorption improving significantly during the exponential phase of growth. Thompson & Shafia (1962) and Tzagloff & Pratt (1964) demonstrated with metabolic inhibitors that irreversible adsorption at least is dependent on cell metabolism. Thompson & Shafia (1962) also showed that the metabolic inhibitors prevented any adsorption of $\phi\mu$-4 to <u>Bacillus stearothermophillus</u> and postulated that either an active energy transfer mechanism was required in order to maintain receptor sites or that adsorption and penetration are synonomous for this phage, the energy being required for phage transfer across the membrane. Therefore for $\phi\mu$-4 at least, initial adsorption is a totally active process but for other phage it still appears to be a passive response.

Conflicting results have been obtained on adsorption of phage to spores. Dowding (1973), Novikova <u>et al.</u> (1973) and Sykes (1977) found that little or no adsorption to spores occurred and it has been suggested that specific adsorption can only occur after the outer membrane of the spores are ruptured. However, Lomovskya <u>et al.</u> (1980) demonstrated that adsorption did occur but that it did not become irreversible until five hours after germination. Wilde (1966) also reported as many as 1000 phage were adsorbed per spore of <u>Streptomyces chrysomallus</u>. The composition of the spore walls differs from that of mycelium walls by the additional presence of aspartic acid (DeJong & McCoy, 1966). Phage binding is very specific and therefore the presence of aspartic acid may cause a change in the configuration of the spore wall thereby inhibiting adsorption.

Thus adsorption is dependent on several factors any of which can either reduce or increase the amount and rate of adsorption achieved.

This must be borne in mind when extrapolating from the results obtained here from a defined liquid medium to the soil environment.

In this work the basis of the calculation of "a" is the data obtained for the effective adsorptions. The number of effective adsorptions rather than the total number of adsorptions was used for three reasons:-

1) The majority of the adsorptions observed here led to infections.
2) When calculations are made from equation (3) of the number of hosts attacked with the infection efficiency based upon the number of effective adsorptions, they closely resemble the actually observed number of attacks (see Table 17).
3) In the Nicholson-Bailey model itself, "a" is utilized in such a manner that those hosts encountered are in fact infected.

Fig 18 shows the calculated infection efficiencies at the varying m.o.i. and host:phage ratios, and indicates that there appears to be no constant infection efficiency. In fact as host density increases in the host:phage systems there appears to be a decrease in "a" and this is borne out by the correlation coefficient which is significant at the 5% level of probability. The calculated coefficients of correlation for the m.o.i. systems appear to show no correlation between m.o.i. and infections. Therefore the value of the infection efficiency appears to be independent of phage numbers but limited by host numbers.

The absence of a constant infection efficiency indicates that the first of Nicholson's assumptions that the number of infections of hosts by P_t phages is in direct proportion to host density is not satisfied. This assumption is very simplistic and does not reflect the time complexity of predator-prey interactions, a criticism which has often been levelled at this particular aspect of the Nicholson-Bailey model.

The second assumption that the number of encounters are distributed randomly among the available hosts, is borne out by the calculation of attacks based on the Poisson distribution, which closely resemble the observed values. Also the kinetics of adsorption is based on the principle of random collisions; any other interpretation would imply co-operation between phage particles to avoid areas where infections have already occurred.

At this stage in the modelling of the streptomycete-phage system a simplistic approach to the measurement of the infection efficiency for use in the model must be taken. Table 18 shows the average value of the calculated infection efficiencies derived from the m.o.i. data which will be used as a starting point for the modelling of the streptomycete-phage interaction. The infection efficiencies were utilized from the m.o.i. systems because the infection efficiency range was much more reduced than in the host:phage systems (MX1 + Ømx1:- 6.9×10^{-12} - 5.7×10^{-10}(m.o.i.), 1.77×10^{-9} - 5.8×10^{-12} (host: phage) and MX8 + Ømx8:- 3.106×10^{-11} - 7.02×10^{-12} (m.o.i.) - 3.2×10^{-9} - 5.6×10^{-12} (host:phage)). In both cases, i.e. MX1 + Ømx1 and MX8 + Ømx8 systems, the values are very small, reflecting the low temperatures used in the adsorption experiments in order to help mirror the natural environment and indicate that the likelihood of a phage infecting a host in the two systems is very small.

Direct comparison of these values to the soil situation is very difficult. The system used did not contain any solid surfaces, apart from the flask sides, which can increase the likelihood of the phage and host meeting. Therefore the estimate of the infection efficiency obtained is probably much lower than that which actually occurs, especially as it is also based on a lifespan of only 60 min. In addition it was measured under one set of conditions and as the

literature shows adsorption can be affected by changes in the external environment, either by making phage or host unavailable or by 'killing' the phage or host. Since the soil is a heterogeneous environment it is likely that there is a whole range of infection efficiencies distributed within one soil and between different soil types.

An investigation has been performed on the adsorption behaviour of two phage, Ømx1 and Ømx8, to their respective hosts, MX1 and MX8, at varying m.o.i. and host:phage ratios. It was found that for the MX1 + Ømx1 system the proportion of phage adsorbed increased with increasing host density. The MX8 + Ømx8 system appeared to show an increase in the proportion of phage adsorbed but this was not statistically well correlated with increases in host density. Alterations in phage density had no effect on the proportion of phage adsorbed in both the MX1 + Ømx1 and MX8 + Ømx8 systems. The values obtained for the adsorption velocity constant and infection efficiency were negatively correlated with increasing host density. The average value calculated for the infection efficiency indicated that the likelihood of a phage infecting a host under the conditions studied was very small.

VII. CONCLUSIONS

The individual parameters of the streptomycete-phage systems for MX1 + Ømx1 and MX8 + Ømx8, and to some extent ISP 5069 + Ø85, have been investigated. The results obtained which were used for the modelling of the streptomycete-phage systems MX1 + Ømx1 and MX8 + Ømx8 based on the Nicholson-Bailey model are shown on Table 20. Some of the data used to assess the individual parameters were derived from broth and plate experiments and may not then reflect the exact situation occurring in the soil, but this was unavoidable since some aspects of the parameters were very difficult to assess in soil conditions.

The individual results have been discussed in the relevant sections but basically reflect a system in which the host, the streptomycete, shows a large total increase in the numbers of spores, germ tubes and mycelial tips. The phage produce fewer progeny at soil-like temperatures and those phage which are produced can be subject to inactivation in the soil. The likelihood of a phage adsorbing and infecting a host is very small, but if a phage does adsorb to a host cell it is likely to continue to carry out a full replication cycle.

PARAMETER		HOST + PHAGE			
		MX1 + Ømx1		MX8 + Ømx8	
		SOIL STERILITY			
		STERILE	NON-STERILE	STERILE	NON-STERILE
PHAGE BURST SIZE (p.f.u. $cell^{-1}$)	"c" 12°C 15°C	13 66		19 100	
PHAGE SURVIVAL (losses $week^{-1}$ g^{-1} soil)	? %	18.46	21.81	32.23	10.90
INFECTION EFFICIENCY	"a"	1.2117×10^{-10}		1.37351×10^{-11}	
INTRINSIC RATE OF INCREASE	"r"	5.718	5.762	5.585	5.669
CARRYING CAPACITY (infectable units g^{-1} soil)	"K"	1.374×10^{8}	8.54×10^{6}	3.62×10^{7}	6.39×10^{6}

Table 20:- Parameter values for use in modelling the streptomycete-phage interaction

CHAPTER 3

THE INTERACTION OF STREPTOMYCETES AND THEIR PHAGE IN THE NATURAL ENVIRONMENT:- A SOIL EXPERIMENT

I. INTRODUCTION

The interaction of bacteria and phage over protracted periods of time has received attention from several authors. Batch and continuous culture have been used more frequently, possibly because they facilitate the manipulation of the environment, keep it constant and achieve a greater degree of homogeneity than in a natural environment. Example of interactions studied by such methods are *Escherichia coli* + phage (Roper & Marshall, 1974); *E.coli* + T7 and MS2 phages (Gaspar *et al.*, 1979); *Mycobacterium leprae* + mycobacteriophage D_{29} (David *et al.*, 1978); *Brevibacterium* sp. 22L + *Brevibacterium* phage (Khlebopros *et al.*, 1980); *Plectomena boryanum* + LPP-DUN1 phage (Barnett *et al.*, 1984). The interaction of indigenous populations of bacteria and phage in soil environments, and in particular *Bacillus* spp., has been investigated by Reanney & Marsh (1973), Reanney & Teh (1976) and Tan & Reanney (1976). *Arthrobacter globiformis* and its soil phage has been studied by Casida & Liu (1974).

The existence of phage and their susceptible hosts in the same environment indicates that an interaction must take place in order to sustain the existence of the phage population. It has been suggested by Reanney *et al.*, (1983), Kelly & Reanney (1978) and Reanney & Teh (1976) that the interaction of hosts and their phage can mediate genetic exchange between host cells, and Kelly & Reanney (1978) stated that

'phage can provide an extensive potential for cell to cell passage of polynucleotide for the genus Bacillus'.

Assessment of the number of hosts and phage present in the soil has received some attention, although most studies have been primarily concerned with the qualitative nature of the populations. To determine whether an interaction between a particular streptomycete and phage can occur in a soil environment the two interacting organisms can be added to a natural soil system. The numbers added of each organism must reflect those population sizes generally observed in soil, although it must be remembered that the final population sizes of each of the organisms in the soil may actually be greater than those added if indigenous populations of similar streptomycetes and phage are already present. Streptomycetes isolated from soil are enumerated in terms of colony-forming units (c.f.u.) which reflect the number of propagules present rather than the actively growing mycelium. Kutzner (1981) cited an earlier study by Flaig & Kutzner (1960) of 18 papers published between 1903 and 1956. It was found that in most soils, colony counts were between 10^4 and 10^7 c.f.u. g^{-1}, but that higher counts of 2×10^8 and 2×10^9 c.f.u. g^{-1} had been observed by Jensen (1943a) and Jagnow (1956) respectively. These estimates of the numbers of streptomycetes are based on all the species present in the soils examined. Therefore it was decided in the experiment presented here to add streptomycete populations to the soil which were equivalent to the lowest population size of the range observed by Kutzner (1981). The streptomycete populations of MX1 and MX8 were added in the form of spores; spores being the most prevalent streptomycete form observed in soil (Lloyd, 1969; Mayfield et al., 1972).

The number of phage isolated from a soil is very much dependent

on both the recovery method used and the soil from which they are recovered (Lanning & Williams, 1982). The total number of phage which they isolated ranged from 4.7×10^4 to 295 p.f.u. g^{-1}. The ranges of the two phages to be used, i.e. ∅mx1 and ∅mx8, isolated from garden, arable and cultivated soils were:-

a) ∅mx1 - 1.5×10^3 to 64.7 p.f.u. g^{-1} soil.

b) ∅mx8 - 2.8×10^3 to 416 p.f.u. g^{-1} soil.

None of the estimates was made from a sandy soil and it was arbitarily decided to add the maximum number of phage recovered. The estimates used for both the number of streptomycetes and phage do not reflect the likely heterogeneous distribution of the two organisms within a soil as shown for Streptomyces isolate F1 by Mayfield (1969).

The aim of this study was to provide information on the extent of the interaction, if any, in the two streptomycete-phage systems, and also to provide an estimate of the infection efficiency, parameter "a", with respect to this soil system. Therefore the estimate of the parameter "a" from the previous broth experiments, (Chapter 2, section VII) can be assessed to see how well it reflects soil system.

Streptomycete spores and phage (MX1 + ∅mx1 and MX8 + ∅mx8) were added to a natural Freshfield nature sand-dune soil (pH 7.6). The subsequent behaviour of the streptomycete and phage population sizes was investigated and presented graphically for each of the streptomycete-phage systems studied.

II. METHOD

Spore and phage suspensions were prepared in sterile distilled water and added to 4g of air-dried Freshfield mature sand-dune soil (pH 7.6). The final concentrations achieved varied between the streptomycete-phage systems involved and attempted to reflect an average measurement of those concentrations which had been found in soil environments. The concentrations added were:-

a) MX1 + Ømx1 system:- Spores added to give 1×10^4 spores g^{-1} air dried soil.

Phage added to give 1.5×10^3 phage g^{-1} air dried soil.

b) MX8 + Ømx8 system:- Spores added to give 1×10^4 spores g^{-1} air dried soil

Phage added to give 1.19×10^3 phage g^{-1} air dried soil.

Controls were also prepared for each system consisting of spores and phage alone added to soil at the relevant concentrations. Sterile water was added to give a final water content of 5.1% (w/w). The soil was 'mixed' in order to achieve a homogeneous distribution of spores and phage. All soils were incubated at 15°C.

At time 0 and at subsequent intervals, three replicate vials of each system and a control were removed. Phage and spore extraction was affected by the phage extraction method of Lanning & Williams (1982). 10ml of PYCa broth + 0.1% (w/v) egg albumen was added to each vial, shaken for 30 min on a Griffin flask shaker and allowed to stand for 16h at 4°C. Samples for colony-forming estimation were removed from the supernatant and plated by the surface spread method of plate innoculation (Vickers, personal communication) on starch-caesin agar with the addition of the following antibiotics:-

a) Carbenicillin - 100 $\mu g\ ml^{-1}$

b) Cycloheximide - 50 $\mu g\ ml^{-1}$

c) Ampicillin - 25 $\mu g\ ml^{-1}$

d) Cephaloridine - 25 $\mu g\ ml^{-1}$

e) Cloxacillin - 5 $\mu g\ ml^{-1}$

f) Oleandomycin - 5 $\mu g\ ml^{-1}$

g) Lincomycin - 2 $\mu g\ ml^{-1}$

h) Penicillin - 1 $\mu g\ ml^{-1}$

Ampicillin was omitted when plating the MX8 system as it inhibited its growth. The isolation medium of Williams & Davies (1965) which consisted of starch-caesin agar with the addition of nystatin (50 $\mu g\ ml^{-1}$), actidione (50 $\mu g\ ml^{-1}$), polymyxin B sulphage (5 $\mu g\ ml^{-1}$) and sodium penicillin (1 $\mu g\ ml^{-1}$) proved unable to prevent the excessive baterial growth which occurred as the experimental time period lengthened. The plates were prepared by the Vickers' method, being dried in a laminar flow cabinet for 20 min., placed in a 45°C incubator for 1h and finally dried in a laminar flow cabinet for 15-20 min. Appropriate dilutions were surface spread, dried for 15-20 min. and incubated at 25°C for 7-10 days when counts of streptomycete colonies were made. Three replicate plates were prepared for each of the replicate samples taken. Regular checks of fungal and bacterial contamination were made.

Phage extraction procedure was completed by the centrifugation of the supernatant at 1,200g for 30 min. and passage through a filter of 0.45μm pore size. Phage activity was assessed by the plating of suitable dilutions of the filtrate by the double layer method. Three replicate plates were prepared for each sample and incubated at 25°C for 24-48h when plaques were counted.

III. RESULTS

Figs. 19 and 20 present the average number of streptomycete colony forming units and phage extracted for both the MX1 + Ømx1 and MX8 + Ømx8 systems from the Freshfield sand-dune soil over a protracted period of time. Fig. 21 presents the observed alteration in numbers of both the phage and streptomycete controls together with the corresponding plot from the streptomycete-phage interaction systems.

Table 21 presents the number of hosts calculated to have been infected, based on both the phage production and the number of hosts lost.

Table 22 gives the calculated infection efficiencies, i.e. values of "a", which were based on the interactions observed to occur in the soil examined. The infection efficiencies were calculated from the Nicholson equation:-

$$a = \frac{H_i}{H_t P_t}$$

where H_i = the number of hosts infected.

H_t = the initial number of hosts present.

P_t = the initial number of phage present.

The number of hosts infected was derived from:-

$$H_i = \frac{P_t - P_o}{c}$$

where P_t = the upper number of phage recovered after the interaction in the soil.

P_o = the initial number of phage recovered.

c = the burst size.

The burst size was obtained from the broth experiments previously performed (Chapter 2, section III) at 12°C and 15°C. Table 22 also gives the infection efficiencies derived from broth experiments (Chapter 2, section VI).

Fig. 19:- Numbers of phage (p.f.u.) and streptomycetes (c.f.u.) recovered from a Freshfield nature sand-dune soil following the addition of MX1 + Ømx1

p.f.u. (▲—▲), c.f.u. (●—●)

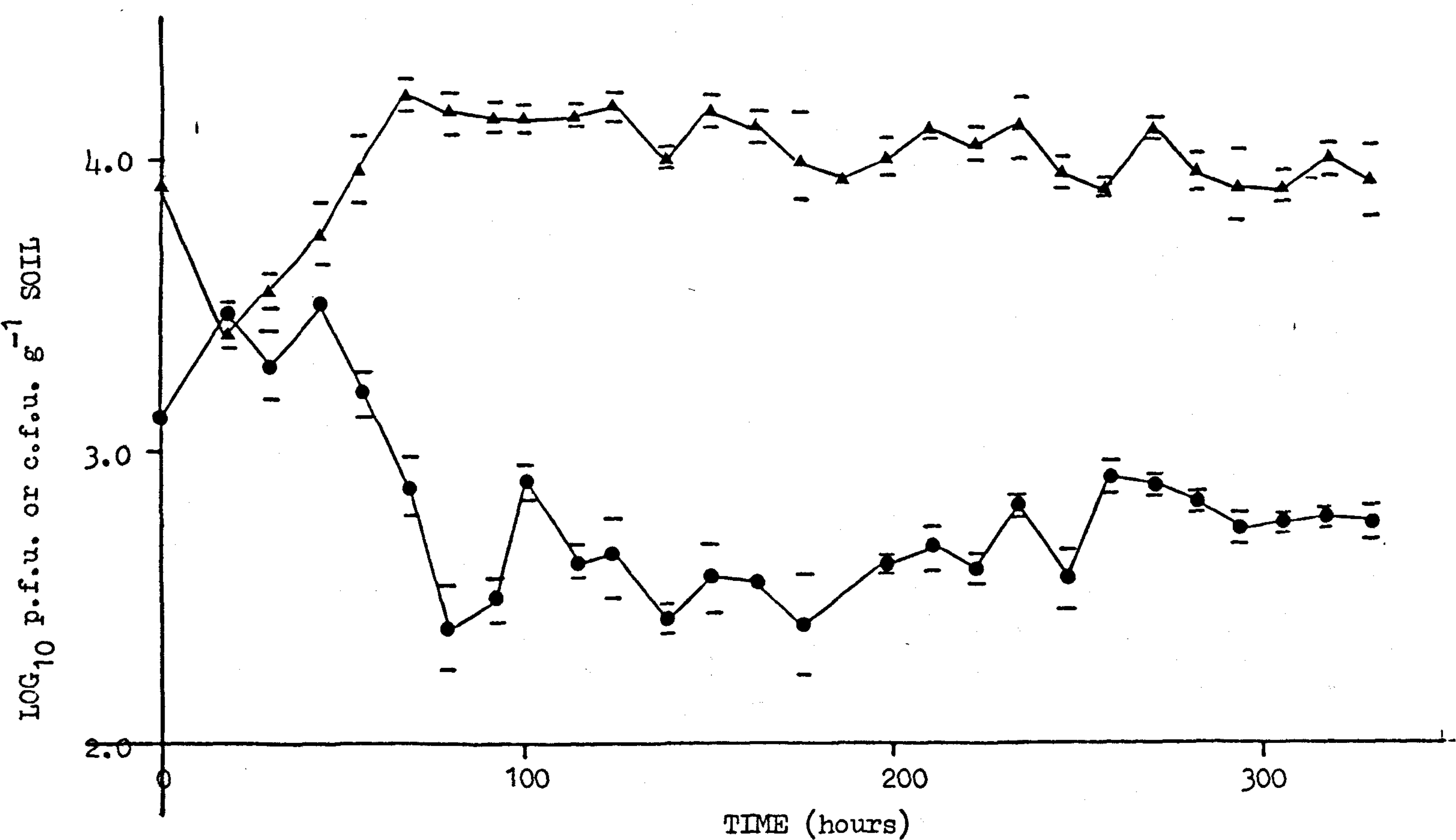


Fig. 20:- Numbers of phage (p.f.u.) and strepomycetes (c.f.u.) recovered from a Freshfield nature sand-dune soil following the addition of MX8 + Ømx8

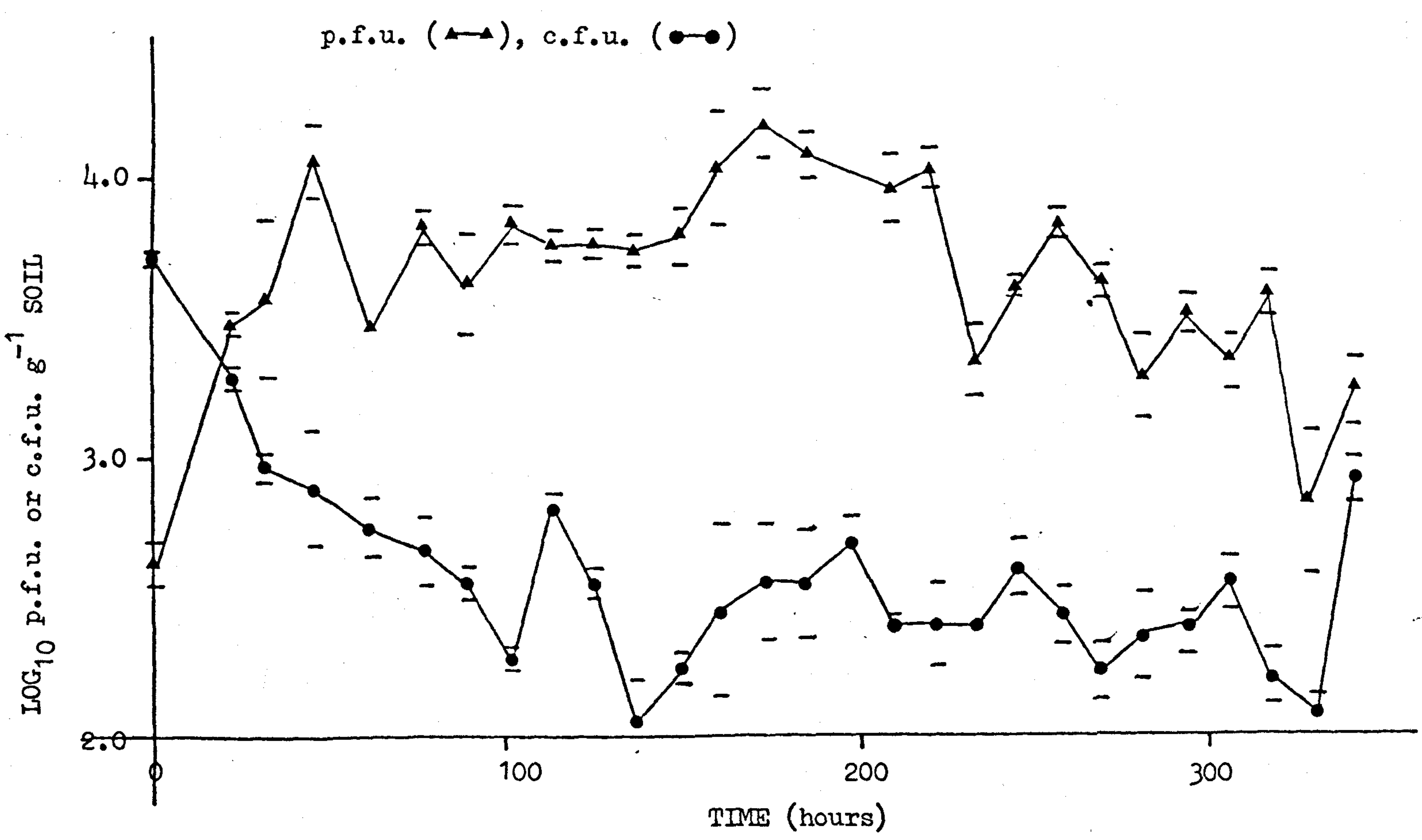
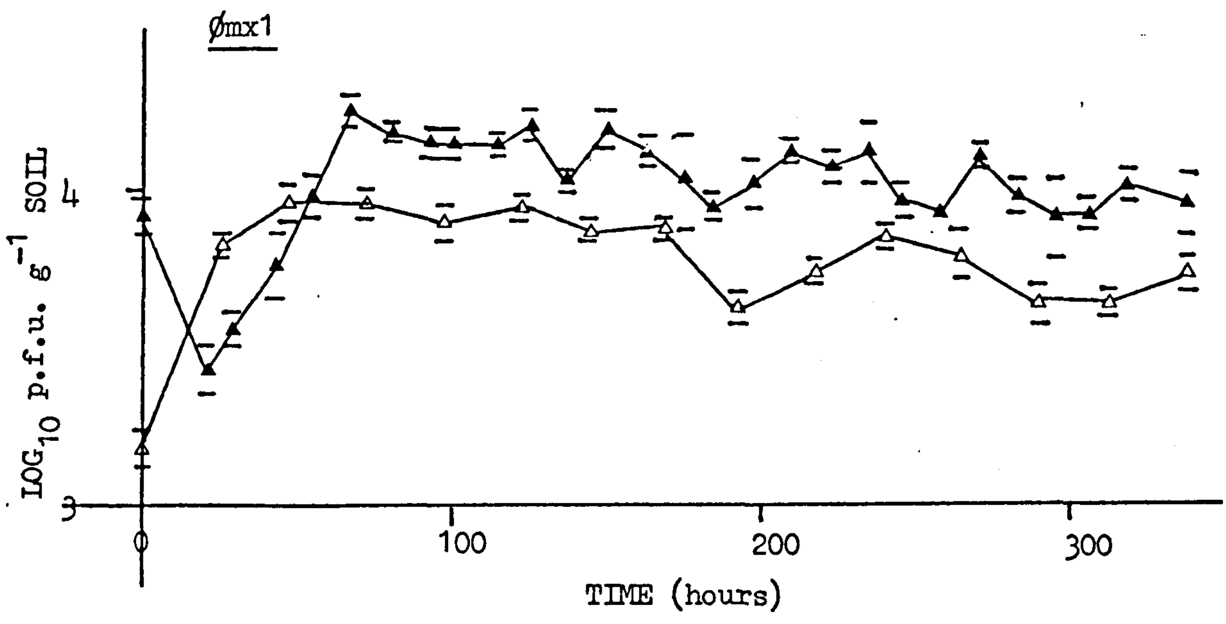


Fig. 21:- Comparison of the observed alteration of c.f.u. and p.f.u. recovered from the control soils together with the corresponding plot from the streptomycete-phage interaction soils

p.f.u. - control soil (△—△), interaction soil (▲—▲)

c.f.u. - control soil (○—○), interaction soil (●—●)



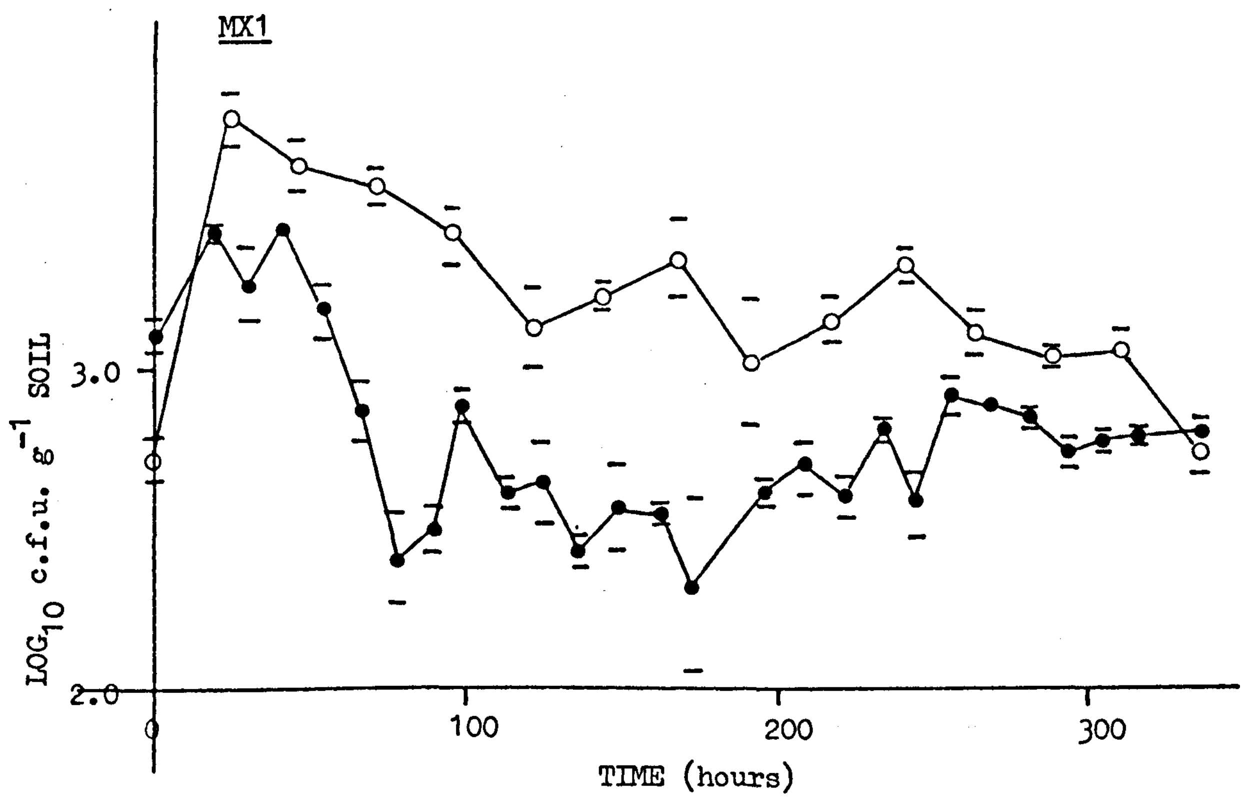
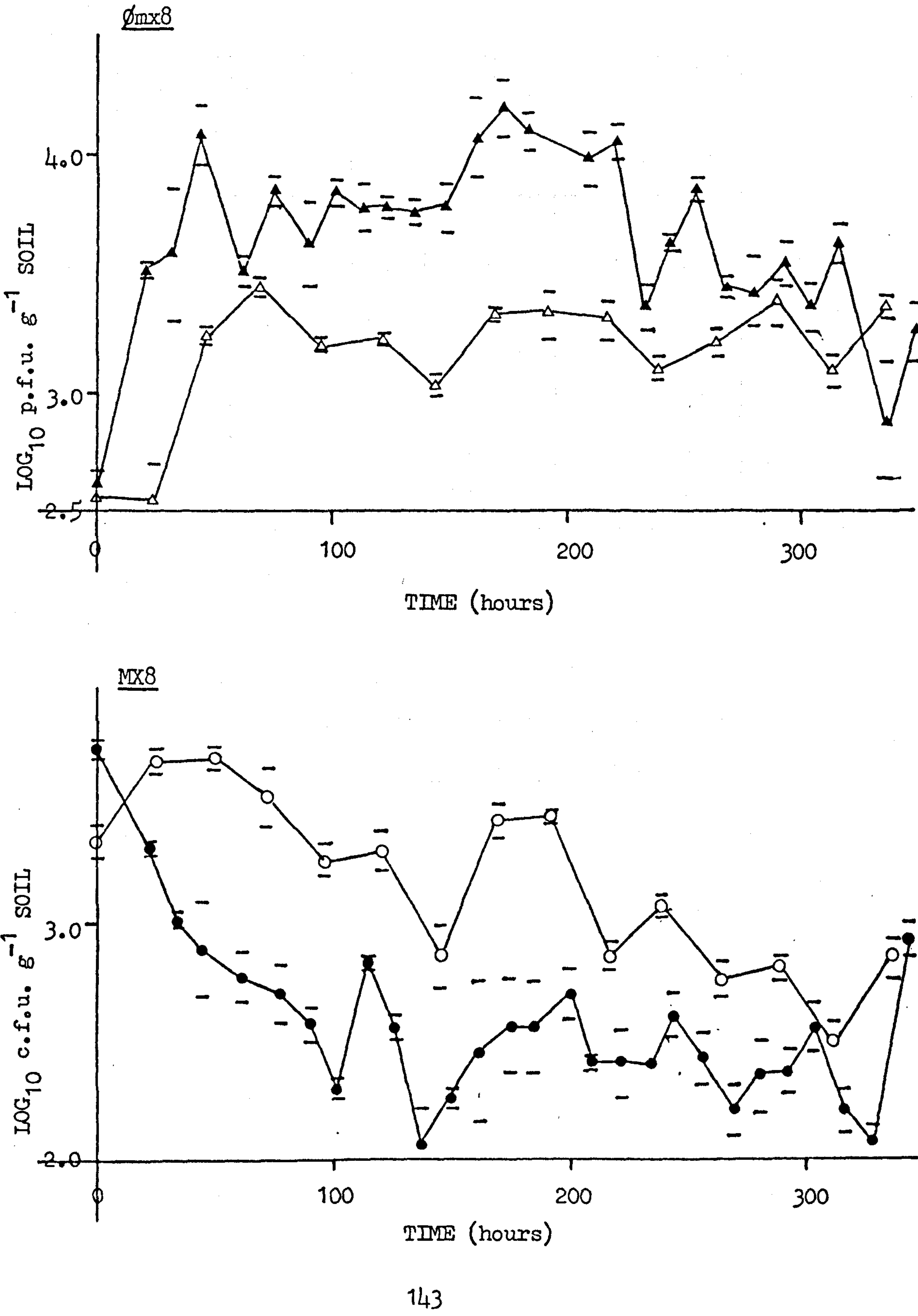


Fig. 21:- cont.



SOURCE OF DATA		HOST + PHAGE	
		MX1 + Ømx1	MX8 + Ømx8
PHAGE GAINS	Upper burst size	128.99	106.46
	Lower burst size	654.87	560.313
HOST LOSS		2093.62	1362.41

Table 21:- Calculated host infections from observed phage gains and host losses for both MX1 + Ømx1 and MX8 + Ømx8

ORIGIN OF INFECTION EFFICIENCY	BURST SIZE (p.f.u./cell)	HOST + PHAGE	
		MX1 + Ømx1	MX8 + Ømx8
SOIL EXPERIMENT	100 19	$3.5 \times 10^{-6} \pm 1.23 \times 10^{-6}$ $2.1 \times 10^{-5} \pm 8.34 \times 10^{-6}$	
SOIL EXPERIMENT	66 13		$1.85 \times 10^{-5} \pm 6.5 \times 10^{-6}$ $9.37 \times 10^{-5} \pm 3.3 \times 10^{-5}$
BROTH EXPERIMENT (Chapter 2 Section VII)		4.53×10^{-9}	4.144×10^{-8}

Table 22:- Infection efficiencies for streptomycete-phage systems MX1 + Ømx1 and MX8 + Ømx8 derived from both soil and broth experiments ($\pm$ standard error)

IV. RESULTS AND DISCUSSION

Figs. 19 and 20 present the observed behaviour of the two interacting populations in the two streptomycete-phage and spore systems, MX1 + Ømx1 and MX8 + Ømx8, following their addition to a Freshfield mature sand-dune soil (pH 7.0), for a protracted period of time. The phage populations are initially composed of those phage which were added to the soil and possibly some members of the indigenous phage population which exhibit lytic cycles for the host added. Later the population also includes those phage which are produced from successful infections. The number of phage particles assumed to be present in the soil can be affected by the recovery method used and natural losses due to inactivation by adverse environmental conditions. The recovery method used here, designed by Lanning & Williams (1982), was shown to facilitate the best recovery of both Ømx1 and Ømx8 from four soils. Natural losses have been shown to occur for the two phage in earlier work, (Chapter 2, section IV). The number of phage recovered is, therefore, likely to be an underestimate of those actually present in the soil. Hence the attribution of streptomycete loss to phage infection will be an overestimate. The apparent losses will include not only those removed by successful infections and those naturally lost, but it was also a reflection of any clumping of streptomycete spores or colonies and any inefficiencies in the recovery of the streptomycete from the soil.

In both systems, (MX1 + Ømx1 and MX8 + Ømx8), an interaction between host and phage is seen to occur but it is only small in magnitude. The change in phage numbers with time is similar to that expected in monoculture (with a lag phase, an exponential phase and a plateau phase) except for the absence of a lag phase which probably reflects the frequency of sampling. Within the first 72h after inoculation, phage replication has commenced and is completed, and it appears that after

this initial interaction there is either little detectable or no further interaction during the period studied. In fact numbers of Ømx8 decreased. There was a corresponding decrease in host numbers to the phage increase and then limited host loss. Data previously obtained on streptomycete spore germination and subsequent growth in soil (Chapter 2, section IV) indicate that during the time in which the phage are replicating, the host is present either as germ tubes or young mycelial tips and will therefore not have an extensive network within the soil. In order then for the phage to replicate on streptomycetes which are at such an early stage in their developmental cycle they must be within close proximity of the available host. This may be affected by the attachment of both the germinating spore and phage to the same colloidal particle, and/or germ tube extension towards the phage particle. Hence an extensive mycelial network is not required for noticeable phage growth within this soil environment.

The number of hosts estimated to be infected is shown in Table 21. Comparison of the number estimated from phage production to those based on host losses indicate a large discrepancy between the values obtained, which is statistically only significant at the 20% level of probability. The different bases of the two estimates have already been emphasised, phage production being an underestimate and host loss due to infection an overestimate. However in both cases the number of hosts attacked is very small. The extent of the infections is probably restricted by the low germination rates of streptomycete spores observed earlier (Chapter 2, section V) and by other authors (Lloyd, 1969; Mayfield et al., 1972). Alternatively, the contact between the hosts and phage may be insufficiently close to lead to an infection, conditions may be unsuitable for adsorption, or a phage may be physically unable to infect a host because it is adsorbed to a colloidal particle. When the number of hosts infected is

based upon the phage production estimates the values obtained are relatively constant for both MX1 and MX8, however when based upon the streptomycete loss, the number of hosts infected is nearly 2x larger for the MX8 + ∅mx8 system than for the MX1 + ∅mx1 system. This may reflect a difference between the systems and especially the numbers of phage and streptomycetes present initially. In the MX8 + ∅mx8 system less than 1.19×10^3 p.f.u. g^{-1} air-dried soil (the concentration originally thought to be added) were introduced into the soil and consequently numbers of phage were less than in the MX1 + ∅mx1 system. That this occurred reflects an inherent difficulty with the preparation of the phage stocks. Phage stocks were prepared in water so that no additional nutrients were added to the soil. Phage require a suitable electrolyte to remain stable in water, e.g. NaCl (Adams, 1959). Therefore the storage of phage overnight in water during their preparation is likely then to lead to their inactivation and a subsequent decrease in numbers and this is probably the cause of the decreased addition of ∅mx8 to the soil. Also when dealing with the addition of small numbers of phage any slight discrepancy will be compounded.

However this does still indicate that an interaction occurs even with the addition of a small number of phage. More MX8 spores were initially present in the soil and therefore may have provided more infection opportunities, but since streptomycete loss is an overestimate and it is difficult to assess how many streptomycetes have been lost through phage infection, no concrete conclusions about the differences between systems can be drawn.

Table 21 includes the number of hosts calculated to have been infected based upon the burst sizes previously determined from the one step growth experiments performed in broth (Chapter 2, section III). Attributing a burst size derived from a broth experiment to a soil system

has to be carefully considered. It has been shown that different broths can give rise to different burst sizes (Barry & Goebel, 1951; Gold, 1959; Kolstad & Bradley, 1967), and therefore it is likely that the burst sizes will also differ in soil. The measured burst size may also be affected if the added host and phage take part in other replication cycles. MX1 can support at least two other phage and Ømx8 replicates in two additional hosts (S. Lanning, personal communication). Both MX1 and MX8 are Streptomyces albidoflavus strains, a common soil streptomycete; it would then be likely that other compatible phage or hosts for the relevant phage or host would be present within a soil system. The presence of another host in the soil for both phage is indicated by the behaviour of the phage controls, Fig. 21. A similar behaviour is observed in both systems for phage alone added to the soil (control) and for phage and host added (soil experiment) indicating that an indigenous host(s) is available. However the magnitude of the phage populations varies in both systems the number of phage observed in the control is always less than those observed in the soil experiment. Therefore the addition of streptomycete spores and phage to a soil results in an increased phage population, other than those added, part of which can be accounted for by added phage replicating within indigenous hosts. The streptomycete controls, Fig. 21, also show a similar behaviour to that of the streptomycete in the soil experiment, but the loss observed is smaller than that for the soil experiment indicating that some added streptomycetes are lysed by the indigenous phage population and that in the two systems (MX8 + Ømx8 and MX1 + Ømx1) studied, streptomycetes are infected both by the indigenous phage and added phage populations.

The interactions of other bacteria-phage systems in natural environments have been studied, in particular those of Bacillus species.

Tan & Reanney (1976) investigated the B. circulans + ØST1 system and demonstrated at 37°C a growth profile of the phage similar to that from a monoculture experiment, except that the time scale was transposed into hours rather than minutes. Temperature dependence was exhibited, but pH alterations appeared to have little effect on phage growth. They noted that the number of phage present before soil treatment was very small; after enrichment the titre was very high but the source of the phage which led to these large increases was undetermined. Investigation of the host population indicated that the diversity of the microflora changed over a 10h period, the proportion of sensitive cells decreased but were able to persist, and that after 10h new patterns of phage sensitivity were observed. The interaction of the indigenous population of the thermophile Bacillus stearothermophilus and its phage was studied under enriched soil conditions (Reanney & Marsh, 1973). Again a temperature dependent effect was observed, with little interaction observed below 30°C. High titres of free phage were present only under conditions favourable to host growth and they suggested that under normal soil conditions phage were probably physically associated with the host and that when host growth was promoted, the phage entered into lytic cycles. The Arthrobacter globiformis + indigenous phage system was investigated by Casida & Liu (1974). Phage for A. globiformis were rarely detected unless soil was nutritionally amended. Added host cells were attacked by suitable phage but added phage rarely infected suitable host cells. The indigenous phage and host populations did however produce phage, and it was suggested that the indigenous phage was masked in some way other than lysogeny. A. globiformis is a pleiomorphic bacteria with resistant spherical cells which promote survival, and it was suggested that incubation with added nutrients leads to a non-synchronous change into

sensitive stage, so that at any one time only a portion of the cells could interact with the phage.

It has been shown that streptomycete phage do infect their hosts and other indigenous hosts within a soil system, and that other indigenous host-phage systems interact. What then prevents the host from being totally eliminated from within the soil population and lead to only the small interactions observed?

Colloids have been shown by Roper & Marshall (1974) and silt by Barnett et al. (1984) to protect hosts from lysis by phage. Escherichia coli was protected at least in the short term by an envelope of colloidal material around it (Roper & Marshall, 1974). Plectonema boryanum was protected by silt from phage to a certain extent, which dampened oscillations of the host numbers without decreasing the numbers of phage particles (Barnett et al., 1984). However colloids could enhance some infections if a host spore and its relevant phage were adsorbed onto the same colloidal particle, the phage then being in close proximity to a potential host. Physical separation of the host and phage by soil particles can obviously prevent infection, and so can the immobilization of phage particles in locations which are unfavourable to adsorption, growth and lysis.

Streptomycetes have, as already mentioned, been shown to be distributed heterogeneously in soil (Mayfield, 1969), but there is little information on the spatial distribution of actinophage. If the phage do not mirror exactly the distribution of the streptomycete, it is possible for the host to avoid large numbers of interactions with the phage which could be detrimental to the total streptomycete population. One of the most obvious ways in which a host can avoid a phage is to have the phage genome integrated into its own genome, thereby preventing any more of the same phage from infecting it, i.e. undergo a lysogenic

association. Reanney & Akermann (1982) stated that most phage genomes in nature probably exist integrated into the DNA of their host cells and this was supported by the G-C contents of the bacteria and their respective phage. They were very similar and therefore suggested that selection had treated phage DNA as a normal constituent of the bacterial DNA. Rautenstein (1970) also stated that lysogeny in actinomycetes was widespread, but that temperate phage were more specific than virulent phage which were polyvalent. This means that a temperate phage needs to find one particular host but having done so it will be 'safe' until a particular set of favourable conditions occur so that it can enter its lytic cycle. A virulent phage needs a wide range of hosts since they are not likely to be available at any one time and place. Temperate phages have been isolated for streptomycetes by several authors (Lomovskaya et al., 1971; Rangarajan, 1966; Dowding & Hopwood, 1973; Ogata et al., 1981; Stuttard & Dwyer, 1982). Such associations can play an invaluable role in the protection of the streptomycete from a further lytic infection and also afford some protection to the phage itself.

The infection efficiencies, "a" calculated from the two streptomycete-phage systems (MX8 + Ømx8 and MX1 + Ømx1) are presented in Table 22, along with those infection efficiencies obtained from broth experiments. Those values obtained from soil are higher than those from broth experiments, obviously reflecting the differences between the two experimental systems, i.e. soil and broth, and indicating that a phage is more likely to infect a host in a soil than in a broth culture in an equivalent time period. The soil system is much more complicated than the broth system, and the free movement which would be observed in broth would be impeded in soil by the soil particles and colloidal materials. Restricted movement with a smaller volume would make it more likely for contact to be achieved. It is impossible to equate the densities of the

phage and host in the two experimental systems because of their physical differences, e.g. the presence of large amounts of particulate matter in the soil, but it may be that the host and phage are more densely packed in the soil than in the broth experiment. Colloidal materials may provide closer contact between the streptomycete and the phage. A more comprehensive study of a range of soils, and indeed different broth conditions together with variations in the densities of the phage and streptomycetes would provide more information on this phenomenon.

Therefore, the interaction of two streptomycete-phage systems, MX1 + ∅mx1 and MX8 + ∅mx8, added to the Freshfield sand-dune soil have been investigated. Interactions were observed for both systems although its extent was limited. Indigenous hosts for the added phage were shown to be present in the soil for both systems. Some host loss was accounted for by inactivation and inefficient recovery. The phage behaviour observed resembled that obtained in monoculture except for the absence of a lag phase, possibly due to the limited sampling frequency. Host behaviour was compatible to that of the phage. The infection efficiencies in both systems were higher in soil than those obtained from broth systems. They reflected the differences between the systems, the main ones being the increased particle density which could increase adsorption chances by restricting the movement of the phage and streptomycete and by providing solid surfaces for adsorption. Variation within the two systems was observed, and further investigations on a range of soil types were suggested, to ascertain the possible causes of the increased infection efficiency.

CHAPTER 4

MODELLING THE INTERACTION OF STREPTOMYCETES AND THEIR PHAGES

I. INTRODUCTION

The modelling of bacteria-phage systems is not new, having been performed for Escherichia coli with various phages by several authors (e.g. Levin et al., 1977; Gaspar et al., 1979). However the streptomycete-phage system has received little attention either in the natural environment or the laboratory. One contributory factor to this, is the mycelial nature of streptomycetes which renders precise definition of the host population complex.

Models are imperfect abstractions of real systems, but can represent extremely powerful tools for the ecologist because they can provide the means to make predictions. In doing so they also force the clarification of ideas about the system being studied. Initially models are very basic, probably involving only a few parameters, and therefore may contain erroneous assumptions due to their simplicity. However, as more is learnt about the system being studied, models can often be adapted to take account of new information. As stated by Smith (1982)

> 'Nature is lavish in detail but many of these are refinements of more basic behaviour. Models attempt to reach beneath the refinements to the basics.'

The mathematical description of a system has the potential to analyse the data of experimental models or actual empirical field results to explain the results on the basis of objective and precisely defined relationships (expressed as mathematical formulae). It will also have theoretical properties which may lead to hypotheses which can be experimentally tested.

So far, in this work the individual parameters of the streptomycete-phage interaction have been investigated. The next step is to fit them into the previously chosen model, the Nicholson-Bailey model of insect-parasitoid interaction.

As mentioned in Chapter I the interaction of streptomycetes and their phages is analogous to that of a predator-prey system, particularly that of an insect-parasitoid system. There are two interacting orgainisms, one of which, the phage/parasitoid, can be detrimental to the host population (in this case the streptomycete). Phage can reduce the size of the host population and if conditions are 'favourable' may totally eliminate the host population. However, under certain conditions the two interacting organisms may coexist and populations oscillate in size, even if such oscillations are not regular.

The modelling of predator-prey systems has received much attention and has led to the production of many models and adaptations of them. Traditionally models are presented in one of two mathematical forms, either difference or differential equations. Differential equations are appropriate to life cycles where continuous reproduction is occurring because they deal in changes over small time intervals. Difference equations deal with population changes over discrete time units and therefore have the merit of easily including some time delays which are prominent in the real world.

The model described below for the interaction of streptomycetes and their phage in the natural environment is a modification of the Nicholson-Bailey model used for the description of the interaction of insect parasitoids and their hosts. It was first proposed in 1935, and being framed in difference equations it is one of the earliest models of a system with discrete generations. Some authors have commented on the likeness to the Lotka-Valterra model of predator-prey

interactions (Lokta, 1925). May (1973) stated that the stability properties of the two models are essentially indistinguishable from each other when the Lotka-Volterra model is in the appropriate equation form. Royama (1971) stated that Nicholson & Bailey proposed their model as an alternative for parasitism to the Lokta-Volterra model, but failed to notice that the scope of their model was already covered potentially by the Lotka-Volterra equations. The Lotka-Volterra equations therefore give the solution to populations with continuous generations, whilst the Nicholson-Bailey equations provide the solution of the same system in difference equations.

The Nicholson-Bailey model is a useful model for describing the interaction of streptomycetes and their phage because as already stated the interaction is very similar to that of the interaction of insect parasitoids and their hosts. Parasitic insects (parasitoids) develop as larvae on or in a single host individual from eggs laid on or near the host. They usually consume all or most of the host body, killing the host and then pupate. The free living adult parasitoid emerges from the pupa and starts the next generation anew by searching for hosts in which to ovipost. They tend to attack only one host stage and may have one or more generations to one of the host. Already similarities are apparent between the life cycle of insect-parasitoid system and the streptomycete-phage system. The main similarities are shown in Table 23.

The basic Nicholson-Bailey model describes host and phage dynamics as:-

$H_{t+1} = e^{r}(H_t - H_a)$ (1) where H_t & H_{t+1} = hosts at time t and t+1

$P_{t+1} = c\ H_a$ (2) where P_t & P_{t+1} = phage at time t and t+1

INSECT-PARASITOID SYSTEM	STREPTOMYCETE-PHAGE SYSTEM
Insect parasitism delays the death of the host until the larvae are fully developed.	Streptomycetes are not lysed until the new phage particles are fully assembled.
Only the female adult parasitoid searches for prey and therefore only one set of parameters is required to described the outcome of search.	In the streptomycete-phage system all phage are thought to be identical and hence only one set of parameters is needed to describe the outcome of search.
A host parasitized by a given species tends to yield a constant number of parasitoids in the next generation.	In the streptomycete-phage system an average number of phage produced is a property of a specific streptomycete-phage system.

Table 23:- Similarities between the insect-parasitoid systems, and the streptomycete-phage systems.

H_a = number of hosts attacked

r = intrinsic rate of increase

c = phage production

The host numbers, in this case the number of spores, germ tubes and mycelial tips, are on the one hand determined by the intrinsic rate of increase "r", and on the other by the number of hosts which escape infection ($H_t - H_a$). The phage population changes in size, either increasing or decreasing, according to the number of attacks, and on the number of phage produced per attack. As already stated the model is framed in difference equations. Royama (1971) stated that representation of the interaction by this method is logical, because after parasitism (in this case infection) the infected host does not disappear immediately.

Further development of the model is needed to include a measure of "H_a" (the number of hosts attacked) in the form of a combination of parameters "a" (infection efficiency as previously described in Chapter 2, section VII) and "P_t" (phage available to infect the host). As already shown (Chapter 2, section VII) the number of parasitoid attacks can be described by the Poisson distribution, which indicates that the attacks are distributed randomly among the available hosts and results from random 'searches' of the phage. Nicholson & Bailey (1935) stated that by definition random searching is completely unorganized, and that so long as individuals or groups of individuals search independently, the searching within thepopulation will be wholly randomized and completely unorganized. It is unlikely that phage do anything else other than search randomly because of their very nature as inert particles outside the host cell. Therefore the number of hosts attacked is:-

$$H_a = \begin{pmatrix} 1 - \text{the probability of the host} \\ \text{not being infected} \end{pmatrix} H_t$$

The probability of not being infected has been shown to be $\exp(-aP_t)$. Therefore:-

$$\text{Number of hosts attacked} = H_t(1 - \exp(-aP_t)) \qquad (3)$$

Royama (1971) likens the result of this equation to the law of diminishing returns. Thus, if P_t increases but "a" remains constant the phage are more likely to try to infect hosts which have already been infected and therefore there is an upper limit to the number of phage which can form infections; above this limit no more infections will be made. Substitution of (3) into equations (1) and (2) gives:-

$$H_{t+1} = H_t \exp(r-aP_t) \qquad (4)$$

$$P_{t+1} = cH_t(1 - \exp(aP_t)) \qquad (5)$$

At this stage the inherent assumptions within the model are:-

a) Each phage in the population searches randomly with respect to both host and other phages.

b) The infection efficiency is a constant for a streptomycete-phage system and is unaffected by host and phage densities.

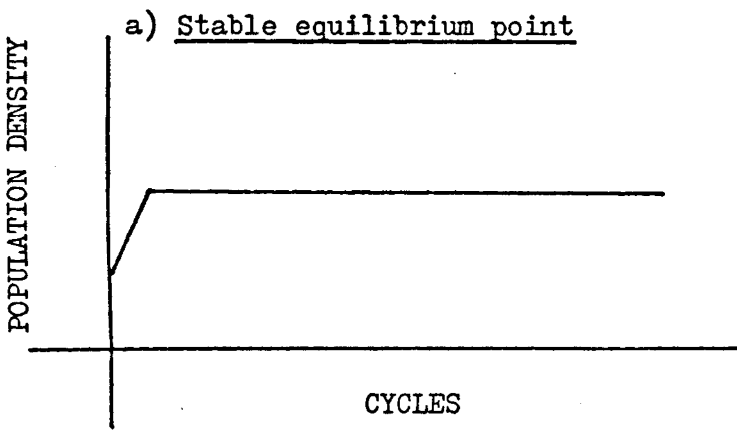
Hassell & May (1973) showed that the model in this form can only describe an unstable equilibrium state, and that any deviation from this condition will lead to host-parasite oscillations of increasing amplitude. Beddington, Free & Lawton (1975) eliminated this unrealistic behaviour by including a density-dependent parameter which leads to the self-regulation of the host. This parameter, "K", represents the carrying capacity of the environment and was included in a manner which was representative of the form found in the logistic equation. The two equations then become:-

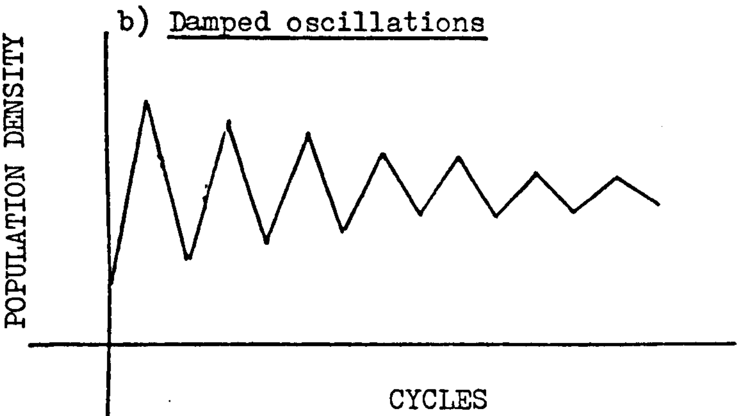
$$H_{t+1} = H_t \exp(r(1-H_t/K)-aP_t)$$

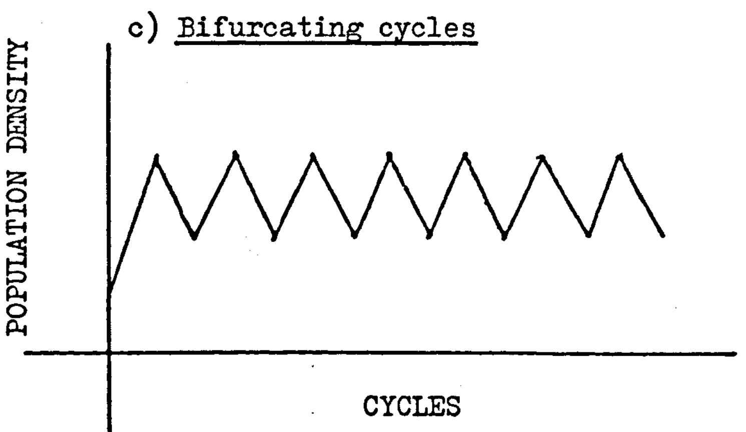
$$P_{t+1} = cH_t(1-\exp(-aP_t))$$

Thus the introduction of a density-dependent function relating to the host population has the potential to cause varying host dynamical behaviour depending on the value of the intrinsic rate of increase. This behaviour can range from oscillatory damping to a stable equilibrium point through to stable cycles, also referred to as 'limit' cycles and bifurcating cycles, through to the extreme case of chaos. (Fig. 22).

In the usage of this model further developments were undertaken to more properly reflect the streptomycete-phage system. In particular it was necessary to calculate the intrinsic rate of increase of the streptomycete host in a way that incorporated the stage structure exhibited by the population. To do this a matrix model was utilized in which three stages were recognized namely spores, germ tubes and mycelial tips. As explained in Chapter 2, section V, the resulting intrinsic rate of increase describes the increase in all of the three previously defined stages of the streptomycete life cycle and thus the equation describes the increase in streptomycetes from spores through the life cycle of germ tubes followed by mycelial tips and the final production of new spores. Therefore "H_t" and "H_{t+1}" describe the numbers of spores, germ tubes and mycelial tips. However spores which do not germinate are inaccessible to phage in terms of availability for infection and thus since the equation deals with infectable units then some parameter must be introduced to deal with those spores which do not germinate either because of insufficient nutrients or due to natural decay of spores. The parameter "s" was used to deal with the proportion of spores which germinate to the successive stages of germ tubes and mycelial tips. Parameter "s" (the proportion of spores which germinate) was determined from work (Chapter 2, section V(A)) which examined spore germination in a







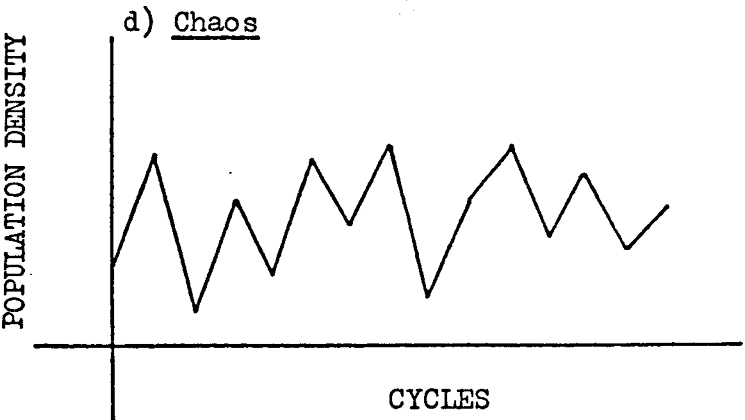


Fig. 22:- Host dynamical behaviour which may be observed on the introduction of a host density dependent function

natural soil. The values for the two systems (MX8 and MX1) are shown in Table 24.

The other modification made was the inclusion of a parameter (z) to account for those phage which are lost naturally in the soil. This has been shown to occur (Chapter 2, section V) and the values used for each of the streptomycete-phage systems are shown in Table 25; these values represent the proportion of phage available to infect host units. Therefore, the final model to be used for the modelling of the interaction of the streptomycete-phage system is:-

$$H_{t+1} = s(H_t \exp(r(1-H_t/K)-aP_t))$$
$$P_{t+1} = zcH_t(1-\exp(-aP_t))$$

As already noted the introduction of "K" into the model leads to the observation of a variety of dynamic behaviour of the host population. The introduction of phage/predator/parasitoid can likewise have a similar effect of varying the dynamic behaviour of both the populations. The possible types of dynamic behaviour observable are:-

a) Stable equilibrium points reached either in a single cycle or by damped oscillations.
b) Stable limit cycles.
c) Chaos resulting from divergent oscillations, or phage increasing without limit and leading to the extinction of the host, or the loss of the phage from within the system.

To examine theoretically the effects of the introduction of a phage/predator/parasitoid into the system there are two types of analysis available:-

a) Local stability analysis - this deals with the effects of small perturbations upon a system and is

HOST	
MX1	MX8
0.13429	0.112761

Table 24:- Values for parameter "s" for MX1 and MX8

PHAGE	
Ømx1	Ømx8
0.656	0.8344

Table 25:- Values for parameter "z" for Ømx1 and Ømx8

examined in a standard local stability analysis.

b) Global stability analysis - this deals with the effects of large perturbations from equilibrium points within a system.

If a point returns to its original point after the perturbations then it can be said to be stable either locally or globally, depending on the size of the perturbations employed. However, local stability does not imply global stability and indeed a locally stable point is only said to be globally stable if on being far away from a stable point the system converges to that point from all other points. The concepts of local and global stability are absolute - either a point is locally or globally stable or it is not.

The properties of the model used here have been examined in two ways:-

1) Phenomological approach Multiple cycles of interaction were carried out by simulation and the results examined.

2) Analytical approach The effects of small displacements to equilibrium points were investigated.

The modelling of two streptomycete-phage systems (MX8 + Ømx8 and MX1 + Ømx1) was undertaken with data obtained from the experiments described earlier (Tables 24, 25 and 26). Parameters were altered to observe the effect of changing values of parameters on the dynamics of the system and to obtain their relative importance on the outcome of the interaction.

PARAMETER		STREPTOMYCETE-PHAGE SYSTEM	
		MX1 + Ømx1	MX8 + Ømx8
INFECTION EFFICIENCY (11 days^{-1})	BROTH	3.198×10^{-8}	3.626×10^{-9}
	SOIL	$3.5 \times 10^{-6}/2.1 \times 10^{-5}$	$1.85 \times 10^{-5}/9.37 \times 10^{-8}$
CARRYING CAPACITY (infectable units g^{-1} soil)		8.54×10^{6}	6.39×10^{6}
BURST SIZE (p.f.u./cell)		100/19	66/13
INTRINSIC RATE OF INCREASE (11 days^{-1})		5.762	5.669

Table 26:- Data for use with the streptomycete-phage model

II. GENERAL METHODS

1) Phenomological approach A computer program was devised for the repeated cycling of the interaction under investigation for use with an Apple II microcomputer.

2) Analytical approach Stability analysis of the Nicholson- Bailey model and its derivatives have been undertaken for various models by several authors, (e.g. Hassell & May, 1973; Beddington, Free & Lawton, 1975; Hassell, 1978). The analysis requires the derivation of a second order difference equation from the model either in terms of H or P. This yields a quadratic solution (Appendix 1) the roots of which show whether returns to the equilibrium state are possible, and if so whether returns are exponentially or oscillatory damped. The approach enables a local stability analysis of the consequences of very small perturbations from an equilibrium population value to be examined. The investigation of the behaviour of populations in the vicinity of the equilibrium point can then expose and demarcate those parameters values that result in damping from those that lead to divergent oscillations. The two regions of parameter space (instability and stability) are divided by a line of neutral stability in which the system continues to oscillate at an amplitude determined by the extent of the initial perturbation. In the case of the Nicholson-Bailey model stability diagrams usually consist of the intrinsic rate of increase "r", plotted against "q", where:-

$q = H/K$ where H = host equilibrium value after predator action.

K = carrying capacity of the environment

"q" therefore measured the extent to which the host is depressed below its equilibrium point (the carrying capacity of the

environment) by the phage. If "q" tends to 1 then the phage population is having little effect on the host population, whereas if "q" tends to 0 the phage is having a large effect on the host population.

III. RESULTS AND DISCUSSION

The approach taken in the modelling of the interaction was to initially examine the result of the interaction as derived from experimentally derived parameters, and then to examine the effect of altering the value of each parameter in a sensible manner to observe their role and importance in the interaction.

a) The interaction observed under values obtained experimentally

A selection of the outcomes of the interactions of streptomycetes and their phages obtained from simulations are shown in Figs 23 & 24 for both of the streptomycete-phage systems investigated. The initial host and phage values were varied within those limits of those observed by:-

a) Kutzner (1981) for the streptomycete values (10^4 - 10^7 c.f.u. g^{-1})

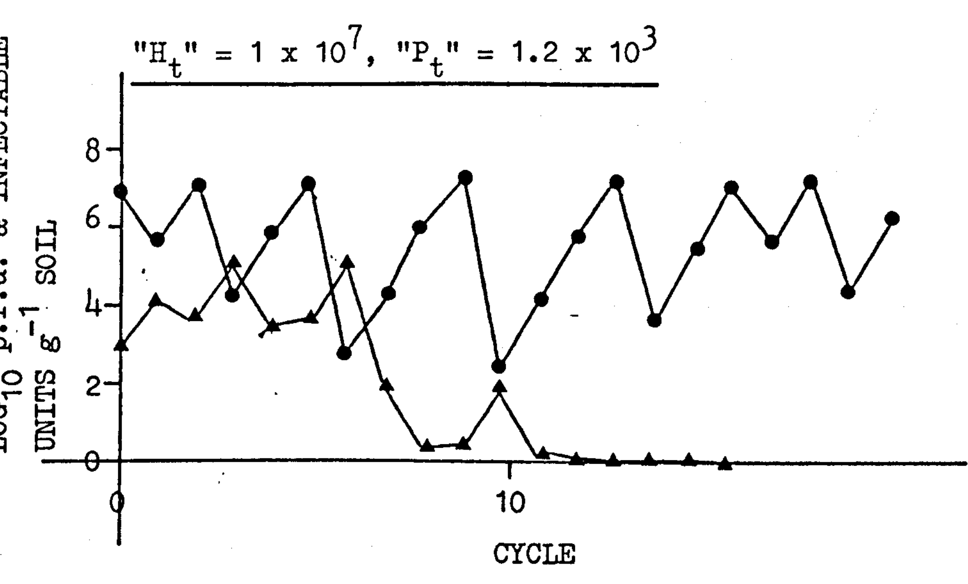
b) Lanning & Williams (1982) for the phage values. The values used in this case were the maximum at which the individual phage were extracted (Ømx1 at 800 p.f.u. g^{-1} and Ømx8 at 1.2×10^3 p.f.u. g^{-1}), and the maximum total number of phage extracted (4.7×10^7 p.f.u. g^{-1}).

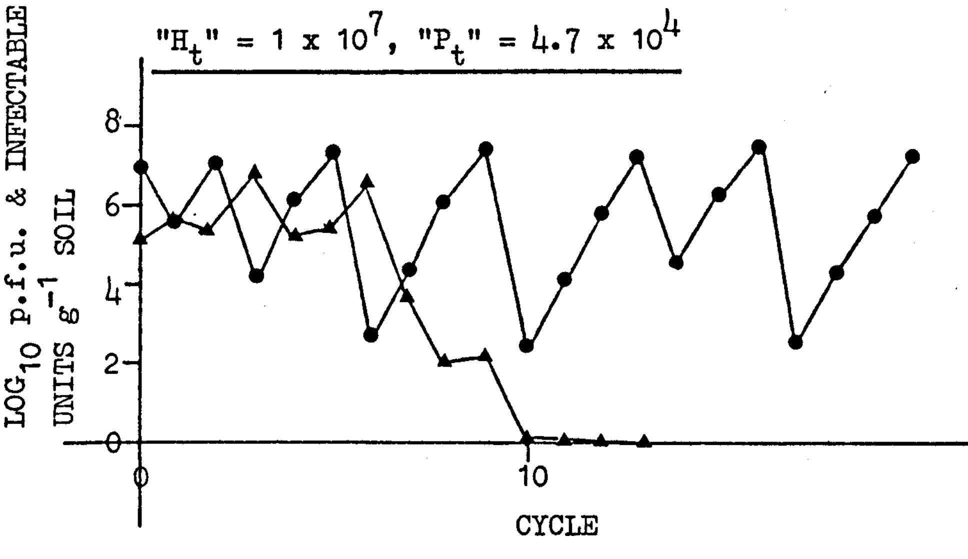
The results indicate that with the sets of parameters used one would not expect to observe the co-existence of the two interacting organisms.

Fig 23 shows the outcomes for the data with the infection efficiency of the order of that obtained from a broth experiment, ($\times 10^{-8}$ for MX1 + Ømx1 & 10^{-9} for MX8 + Ømx8). Only a few selections are shown as the alteration of the initial population sizes of both the streptomycete and the phage had no effect on the nature of the final outcome. However, it could affect the length of the interaction specifically for the MX1 + Ømx1 system (Table 27). In all cases the phage was lost whilst the host continued to cycle for a lease 50 cycles. For the MX1 + Ømx1 system, a sustained but irregular oscillation was observed, although it did

Fig. 23:-

a) MX8 + Ømx8





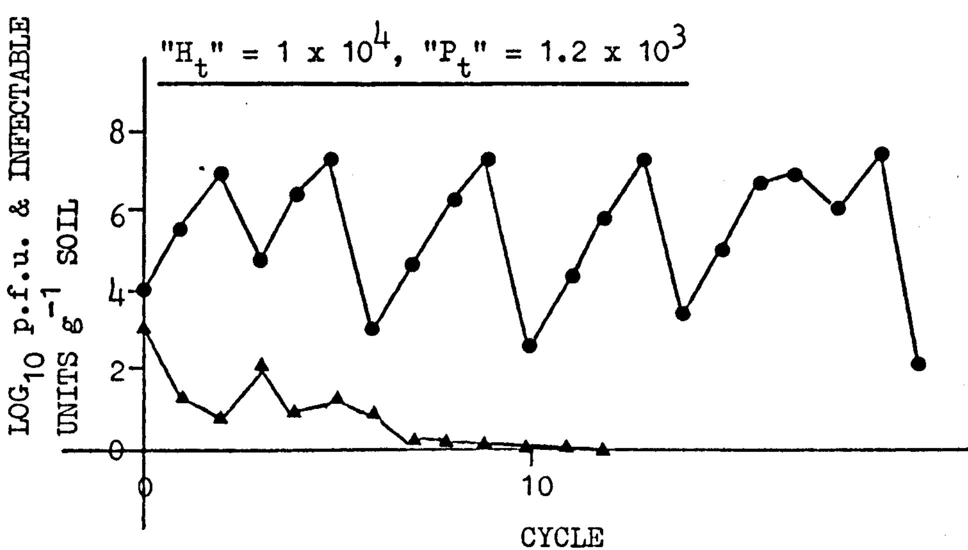
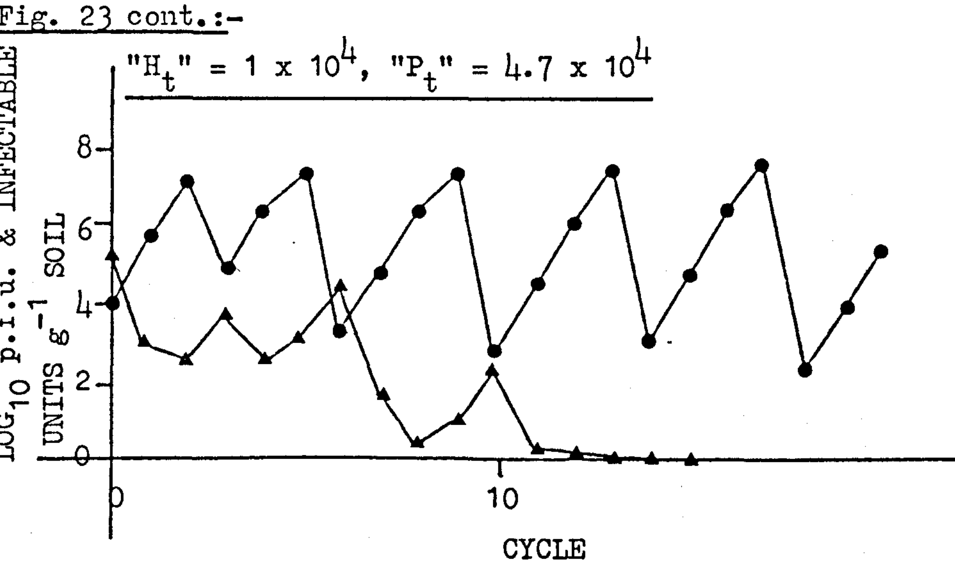


Fig. 23 cont.:-



b) MX1 + Ømx1

"H_t" = 1 x 10^7, "P_t" = 1.2 x 10^3

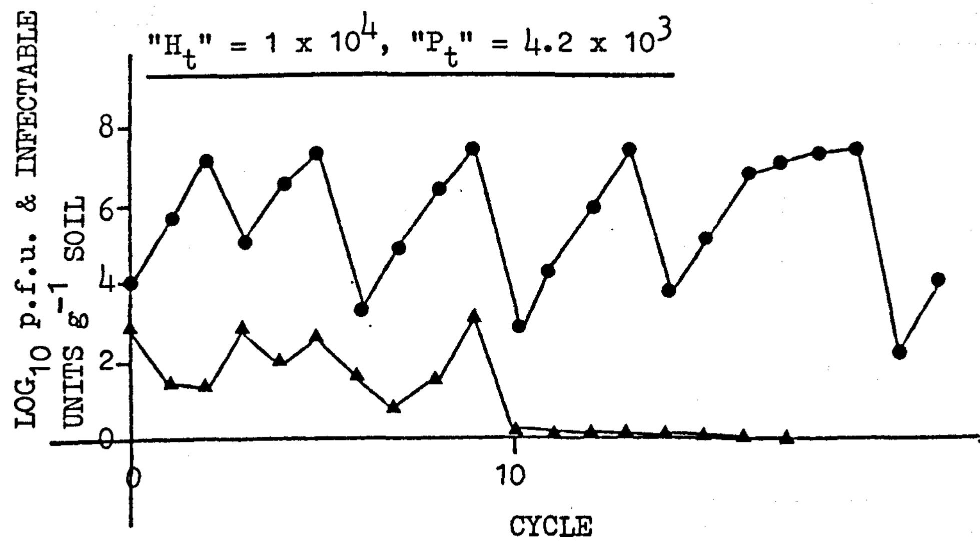
LOG_{10} p.f.u. & INFECTABLE UNITS g^{-1} SOIL

CYCLE

"H_t" = 1 x 10^7, "P_t" = 4.7 x 10^4

LOG_{10} p.f.u. & INFECTABLE UNITS g^{-1} SOIL

CYCLE



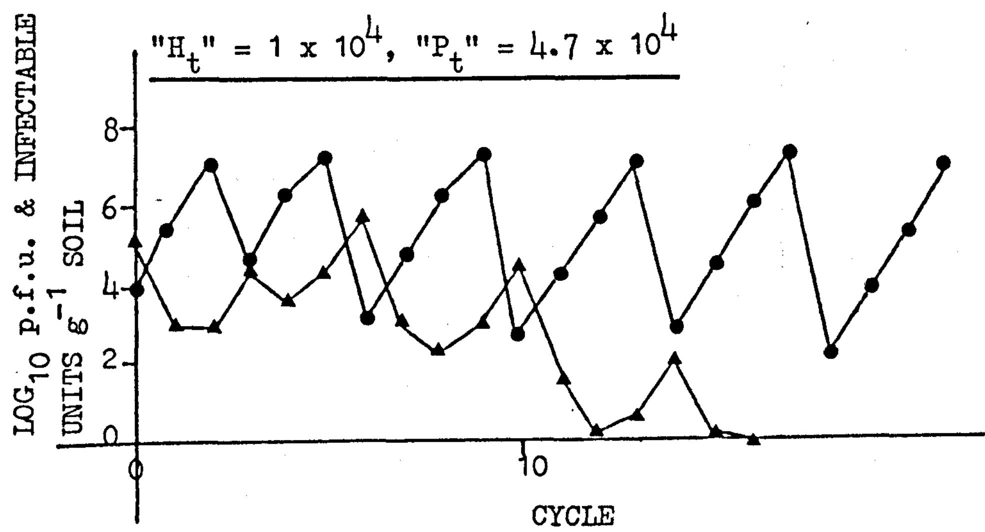


Fig. 23 cont.:- The simulatory behaviour observed for the MX8 + Ømx8 and MX1 + Ømx1 systems with broth derived "a" value and varied initial phage and host population sizes. p.f.u. (▲—▲), infectable units (●—●).

Fig. 24:- Simulatory behaviour observed for the MX1 + Ømx1 and MX8 + Ømx8 systems with soil derived parameter "a"

p.f.u. (▲—▲), infectable units (●—●).

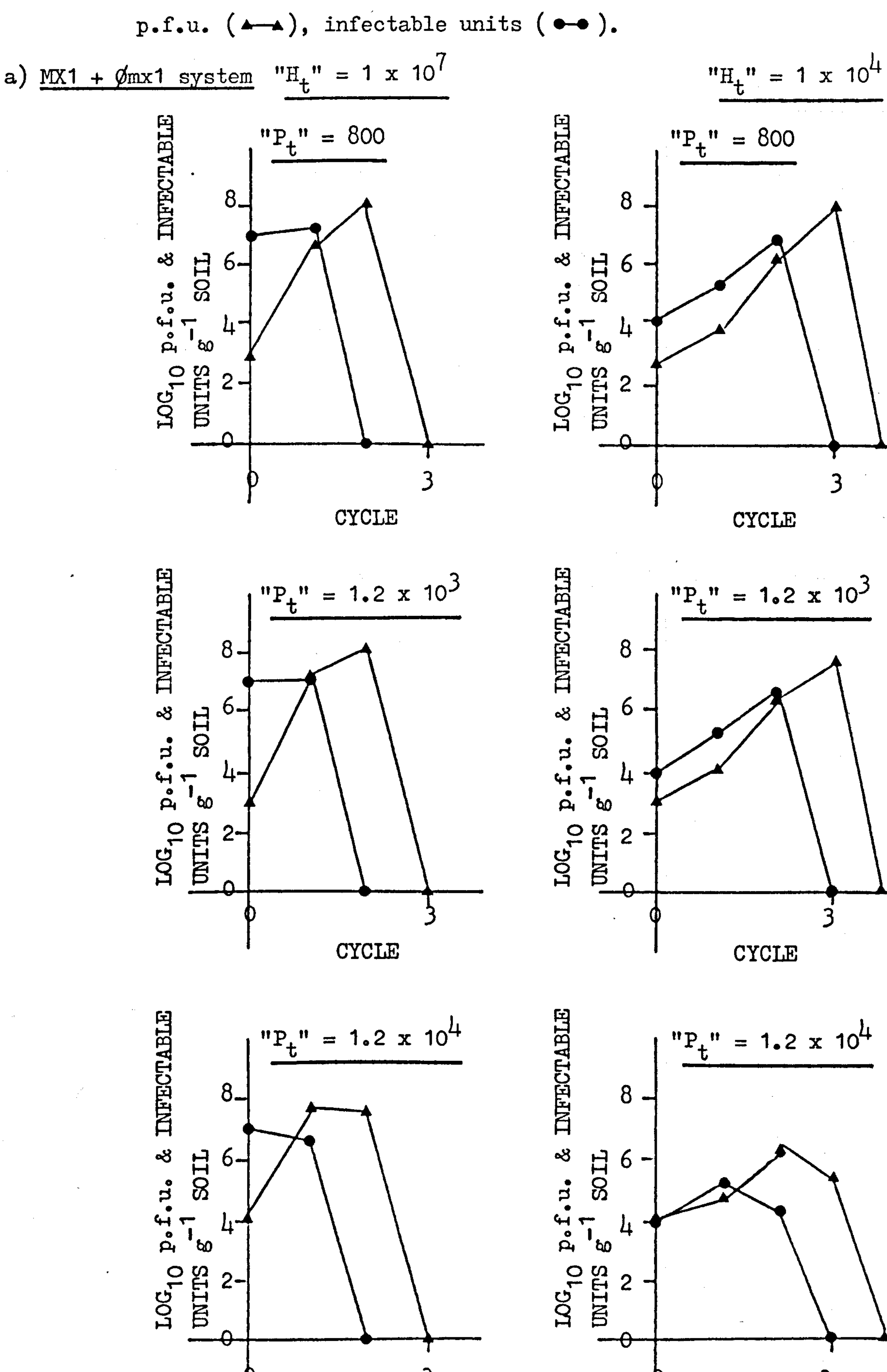


Fig. 24:- cont.

b) MX8 + Ømx8 system

HOST NUMBERS (c.f.u. g^{-1} SOIL)	PHAGE NUMBERS (p.f.u. g^{-1} SOIL)	
	1.2×10^3	4.7×10^4
1×10^4	17	16
1×10^5	28	11
1×10^6	75	44
1×10^7	139	22

Table 27:- The number of cycles of co-existence observed for the MX1 + Ømx1 system

eventually break down. This may indicate that at around "a" = 10^{-8} there may be a value which enables sustained oscillations to occur. The phage although not surviving, does have an effect on the subsequent host cycling. The patterns of cycles are not identical to those observed when the host alone is growing. However, it is difficult to quantify the effect since the host oscillations are irregular anyway, and the phage effect is not dramatic, i.e. it does not alter the nature of the interaction. Therefore, with the value of "a" derived from the broth experimental system the phage is unable to make sufficient infections of the host to sustain an interaction or to totally eliminate the host population, no matter what were the initial sizes of the populations.

Fig 24 presents the outcomes observed when the "a" was that observed under soil conditions. In all cases the streptomycete was eliminated from the system by the phage and was necessarily followed by the phage itself. This means that all streptomycetes in the infectable state were lysed. However it does not mean that all the streptomycete population was lost because there will still be ungerminated spores present within the system.

The stability diagrams for the four individual cases are shown if Fig 25 and indicate that there are regions of stability when the broth-derived "a" values are used. Thus it is possible to obtain stable regions but they are limited, instability being the predominant feature of the diagram. When the soil-derived "a" values are used there are no stable regions, indicating that using only this simple model and incorporating no other features there is little possibility of a stable interaction.

Therefore with the value of "a" set at that obtained from a broth experiment, the phage would be expected to be lost and the host continue

Fig. 25:- The effect of parameter "a" derived from soil and broth experiments on the stability boundaries for the MX8 + Ømx8 and MX1 + Ømx1 systems.

= Stable (X = damped oscillations)

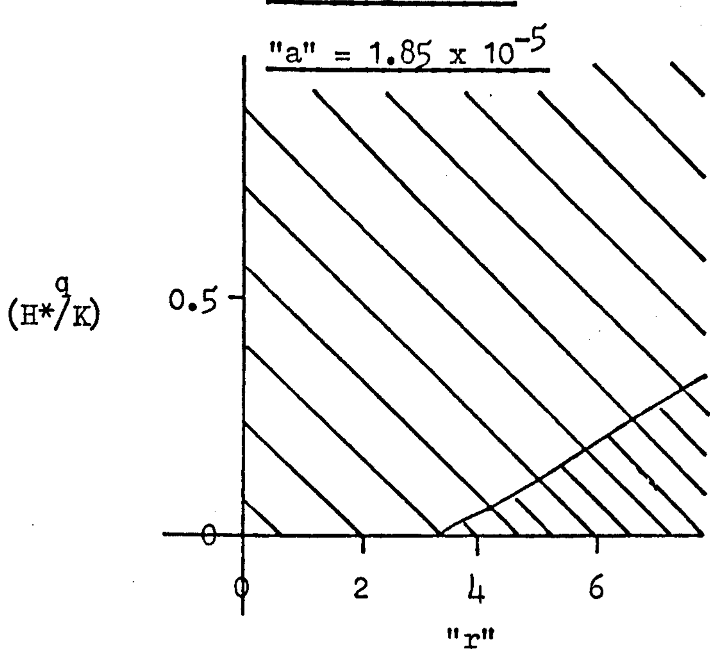
= Unstable fluctuations

= Extinction of phage

a) MX8 + Ømx8 system ("c" = 66)

Soil experiment

"a" = 1.85×10^{-5}



Broth experiment

"a" = 3.626×10^{-9}

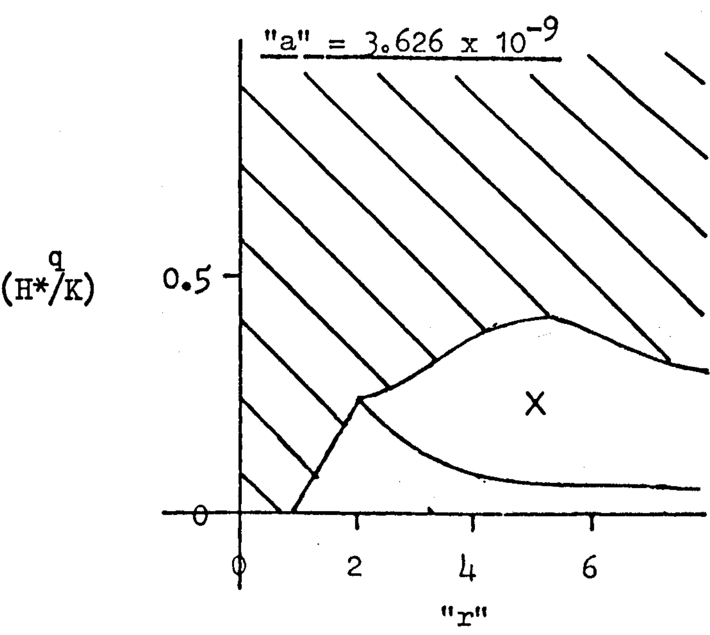
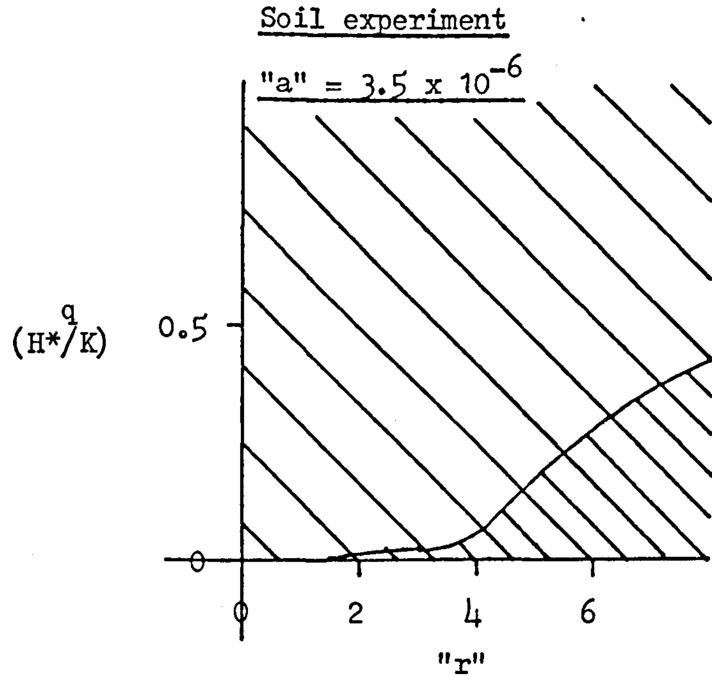
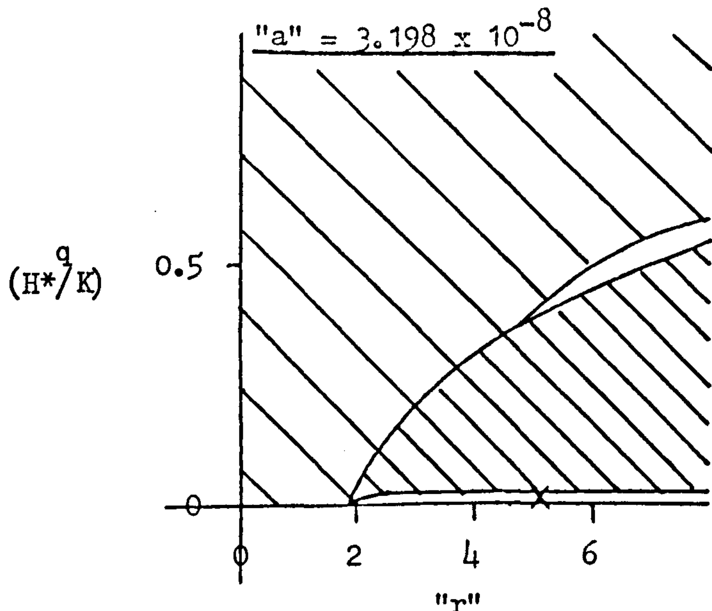


Fig. 25 cont:-

b) MX1 + Ømx1 system ("c" = 100)





to cycle; whereas with the value of "a" from a soil system would be expected that both populations would become extinct, at least so far as the infectable components of the streptomycete are concerned. Since streptomycetes and phage do occur in a soil together, the model cannot be representative of the complete phenomenon in the soil as a whole, and may only in this simple form be applicable to microsites in the soil. Therefore it is necessary to change the individual parameters to mimic the heterogeneous nature of the soil, and ascertain their effects on the interactions which could occur in the natural environment.

b) The effect of altering the infection efficiency, parameter "a"

The infection efficiency is obviously one of the most important parameters in the model as it determines to a large degree the extent of the interaction which occurs. In this case it is the likelihood of a phage encountering a streptomycete within the 11 day cycle period and infecting it. As already shown (Figs 23 & 24) the infection efficiencies derived from broth and soil experiments both result in chaotic interactions for both the MX1 + Ømx1 and MX8 + Ømx8 systems. The former leading to the loss of the phage only and the latter to the loss of both interacting organisms. What then is the effect on the interactions caused by the alteration of the infection efficiency? Table 28 indicates the events which occur following the alterations of the infection efficiency. For the MX1 + Ømx1 system two interactions were observed, changing from the loss of the phage to the loss of both the phage and the host as "a" increased. There was no observed co-existence. The MX8 + Ømx8 pair showed three types of interaction, all of which were chaotic. As "a" increased towards "a" = x 10^{-8}, the host alone cycles with the phage lost; at "a" = x 10^{-8} sustained but irregular oscillations were observed. At "a" = x 10^{-7} the interaction was once again that of phage loss, above this value both of the interacting

INFECTION EFFICIENCY	DYNAMIC BEHAVIOUR OBSERVED
a) MX1 + Ømx1 - 1×10^{-7}	Phage lost, host cycles
2×10^{-7} -	Host & phage lost (total chaos)
b) MX8 + Ømx8 - 2×10^{-8}	Phage lost, host cycles
3×10^{-8} - 4×10^{-8}	Sustained oscillations
5×10^{-8} - 3×10^{-7}	Phage lost, host cycles
4×10^{-7} -	Host & phage lost (total chaos)

Table 28:- The effect of alteration in parameter "a" on the simulatory behaviour observed

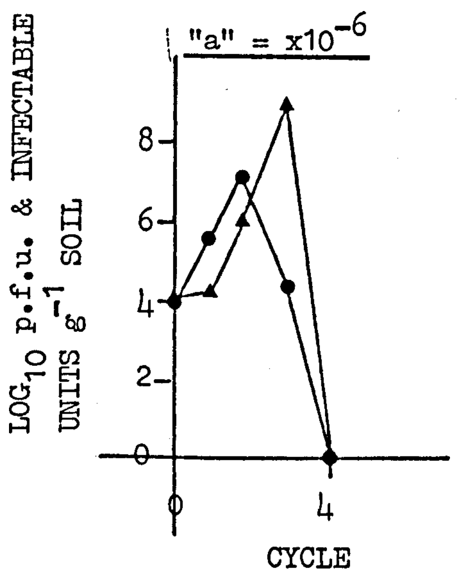
populations were lost from the system. Fig 26 presents a selection of the simulations of the behaviour observed, and in particular the sustained interaction of the MX8 + Ømx8 system. All the observations are chaotic. The oscillations of the host cycling alone when compared to those of the host oscillatory hehaviour at the lower infection efficiencies indicate that the phage has little effect on the host behaviour (Fig 27).

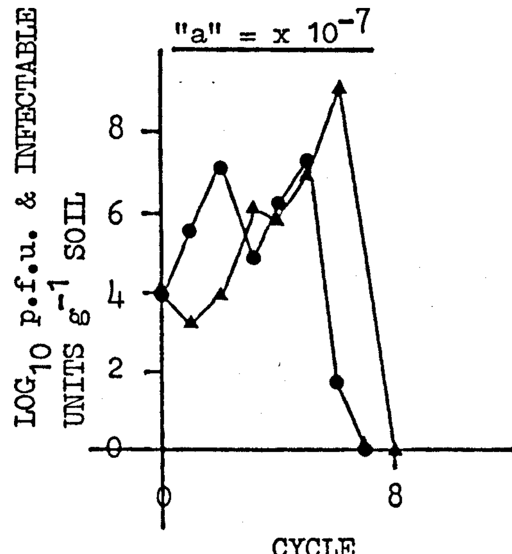
Alteration of the inital host and phage populations at "a" = x 10^{-8} can help to indicate whether these initial populations are important in determining the extent and nature of the interaction in both systems. Therefore, if the initial populations were important one would expect to observe for the MX8 + Ømx8 system a change from the sustained oscillations, and for the MX1 + Ømx1 system a possible change to the sustained oscillations. In both cases no alteration in the nature of the observation was observed. The MX8 + Ømx8 system still shows sustained oscillations, but because of the nature of the host oscillations it is impossible to decide what is the exact effect of the increasing or decreasing phage population, although the host oscillations are different at each host concentration for the varied phage population. For the MX1 + Ømx1 system, altering initial population size does not lead to any alteration in the interaction overall but can have a considerable effect on the extent of the interaction, (Table 27). Therefore it appears that although initial host and phage population sizes are not the most important factor in determining the nature of the interaction, they can play a considerable role in the length of the interaction. If the "a" value is near to that which would produce co-existence, the size of these populations may be the deciding factor as to whether or not sustained oscillations are observed for an extended period.

Fig. 26:- A selection of the simulatory behaviour observed with varied "a" value for both the MX1 + Ømx1 and MX8 + Ømx8 systems.

p.f.u. (▲—▲), infectable units (●—●).

MX1 + Ømx1 system





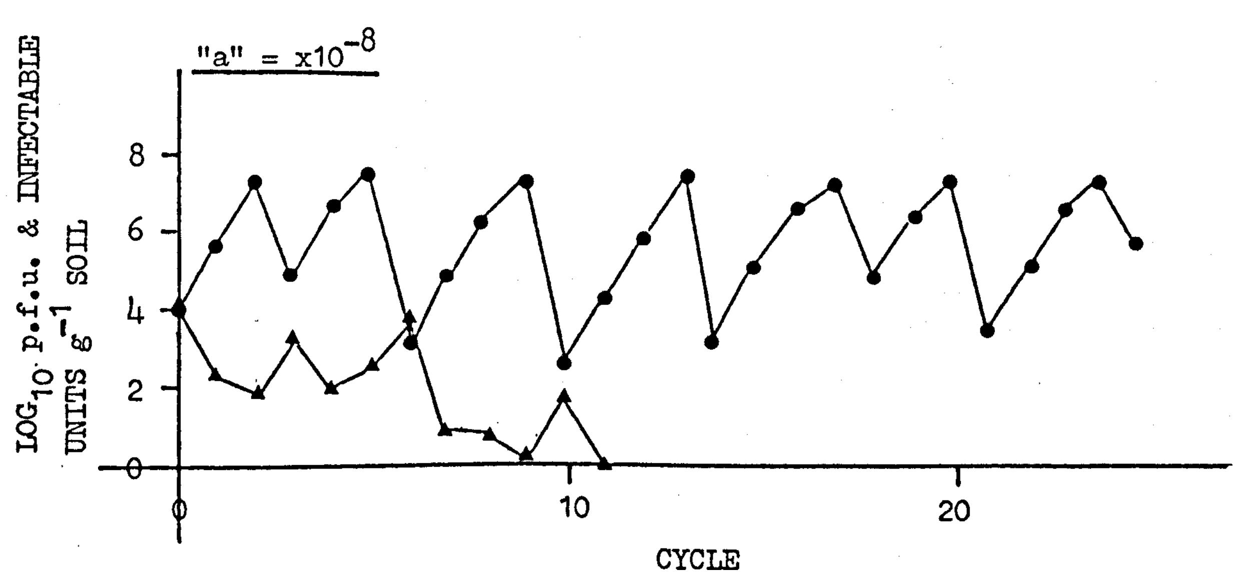


Fig. 26 cont.:- MX8 + Ømx8 system

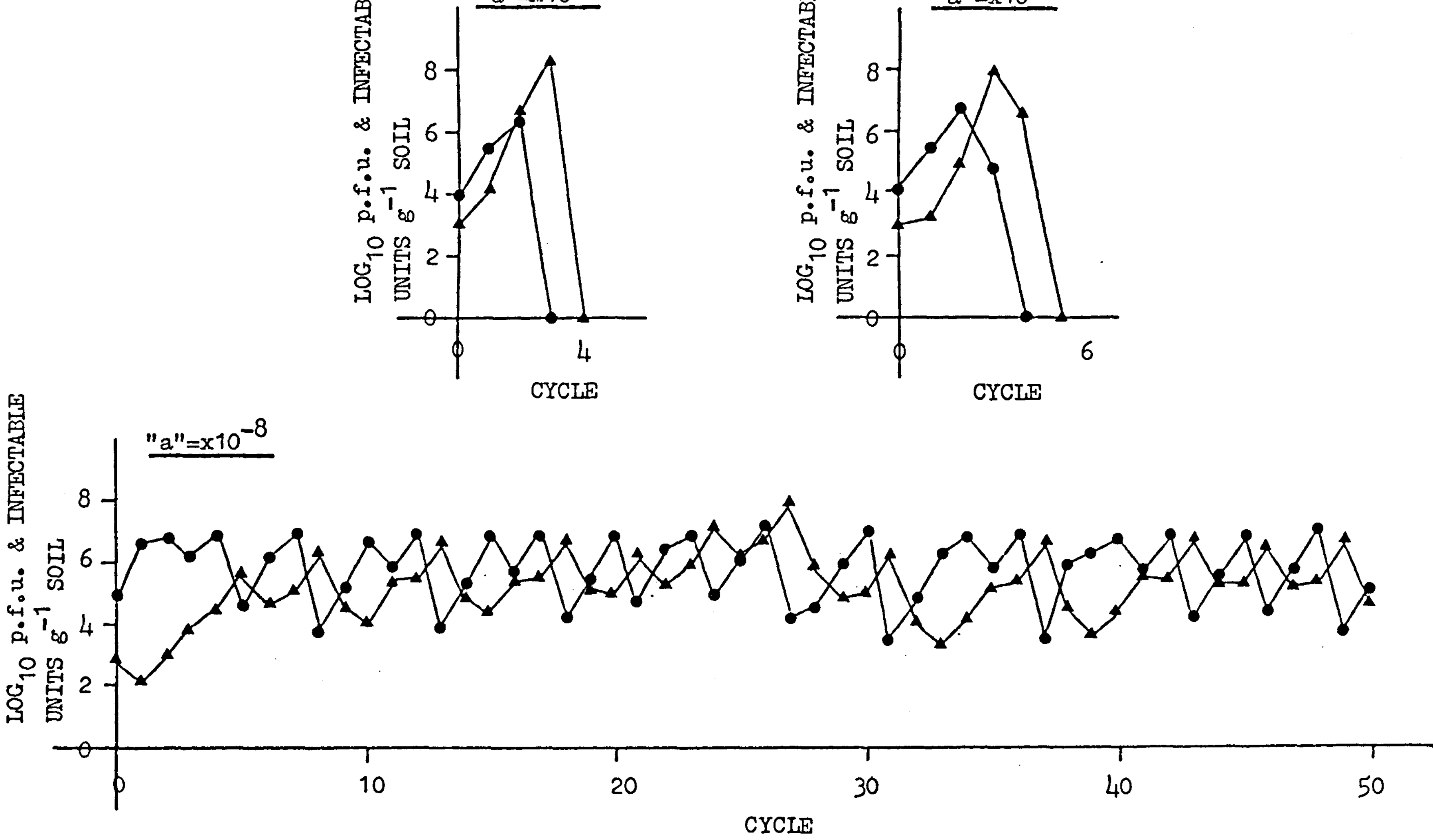
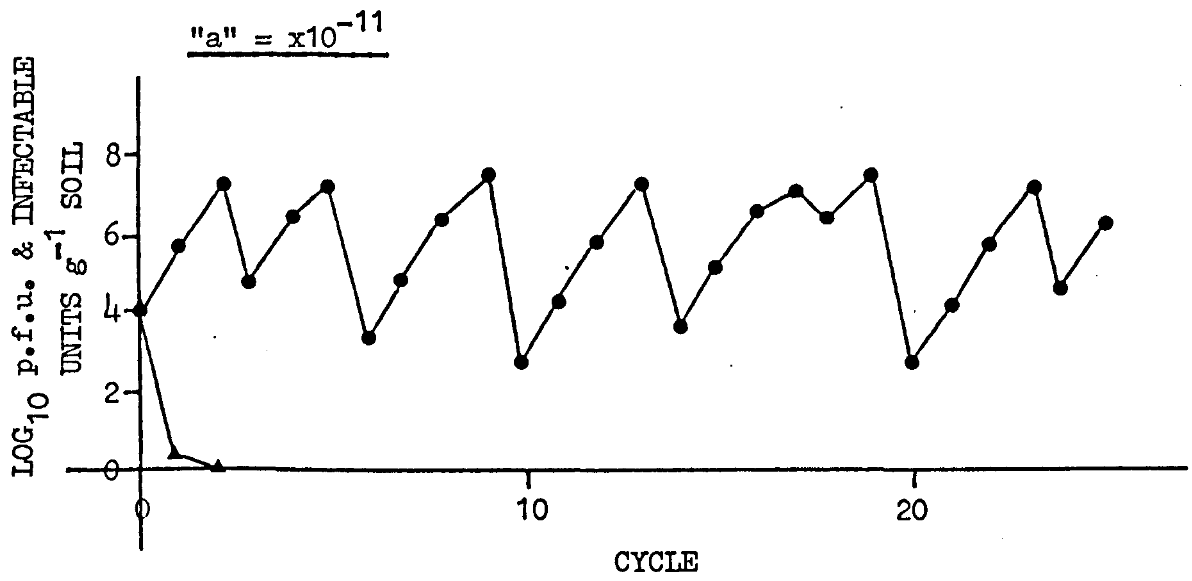
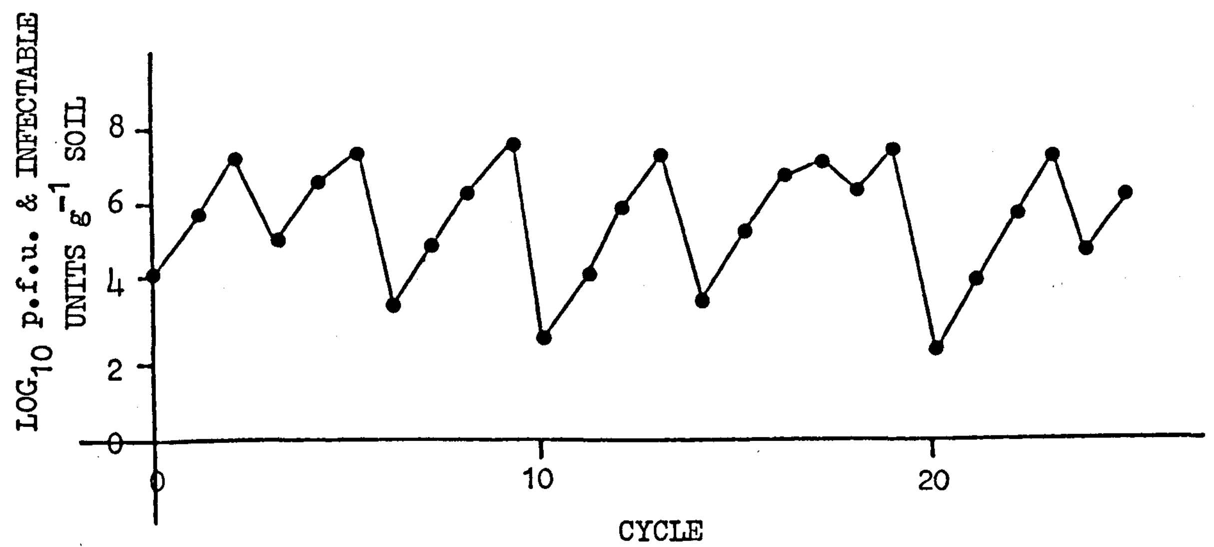


Fig. 27:- A comparison of the host behaviour observed alone with that in the presence of phage at a low infection efficiency - "a" = $x10^{-11}$

p.f.u. (▲—▲), infectable units (●—●)





It can be shown with the initial model that only a small change in the infection efficiency is required for a change in the stability of the interaction. Table 29 presents the range of values of "a" for the adapted Nicholson-Bailey model which coincide with the zones of stability for the intrinsic rates of increase of 2 and 3. The introduction of the two parameters "s" and "z" means that the derivation of a stability diagram is not as simple as that for the original model. More than one stability diagram can be produced depending on the values of parameters being investigated. The stability diagrams for a range of "a" are shown in Figs 28 & 29. The infection efficiency can have a substantial effect upon the areas of stability within the diagrams. For both of the systems (MX1 + Ømx1 and MX8 + Ømx8) varied stability domains were observed at each value of "a", however at some values, the diagrams are identical. Above "a" = x 10^{-9}, the main region of stability disappeared and at "a" = x 10^{-7} there are no stable regions of interaction at all. At low infection efficiencies below x 10^{-10} (MX1 + Ømx1) and x10^{-9} (MX8 + Ømx8) there are large regions of stability which decrease at "a" = x10^{-12} indicating the fine balance maintained between too many and too few interactions to achieve a stable outcome. The points within the observed large zones of stability at various "a"s are not globally stable only locally stable. This is demonstrated by the absence of stable interactions in simulations by initial streptomycete and phage starting populations not equal to those of the equilibrium densities and thus indicates that the initial phage and host densities can play a role in the outcome of the interaction (as has been previously indicated). The diagrams also indicate that there is a dependency between the value used for "a" and that used for the burst size, "c" on the outcome of the interaction. Similar results occur in the stability diagrams when "a" =x10^{-10} and "c" = 10 and when

INFECTION EFFICIENCY RANGE	STABILITY TYPE OBSERVED
a) "r" = 2	
$- 1 \times 10^{-8}$	Increasing oscillations (Unstable)
$9 \times 10^{-9} - 5 \times 10^{-9}$	Damped oscillations
4×10^{-9} -	Exponential oscillations
b) "r" = 3	
$- 1 \times 10^{-8}$	Increasing oscillations (Unstable)
$9 \times 10^{-9} - 7 \times 10^{-9}$	Damped oscillations
$6 \times 10^{-9} - 4 \times 10^{-9}$	Exponential oscillations
3×10^{-9}	Oscillations increase (Unstable)

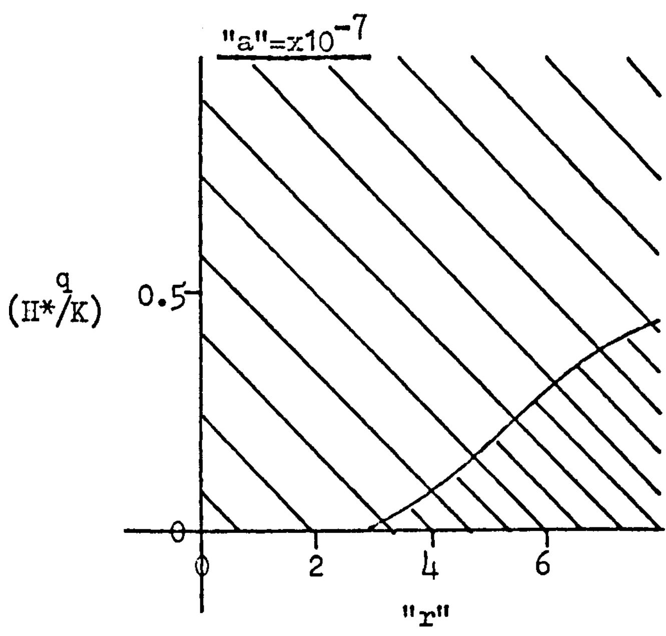
Table 29:- The behaviour observed over specific parameter "a" ranges for the MX8 + Ømx8 system ("c" = 66)

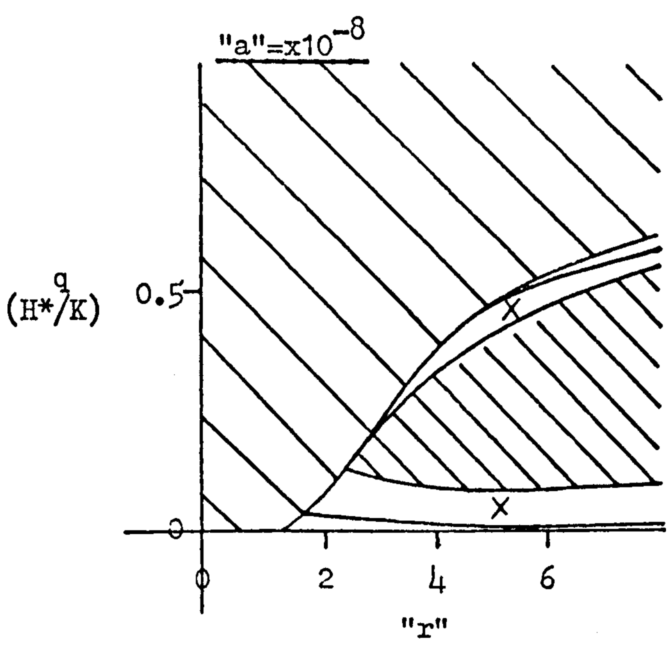
Fig. 28:- The effect of alteration in parameter "a" on the stability boundaries for the MX1 + Ømx1 system ("c" = 100)

= Stable (X = damped oscillations)

= Unstable fluctuations

= Extinction of phage





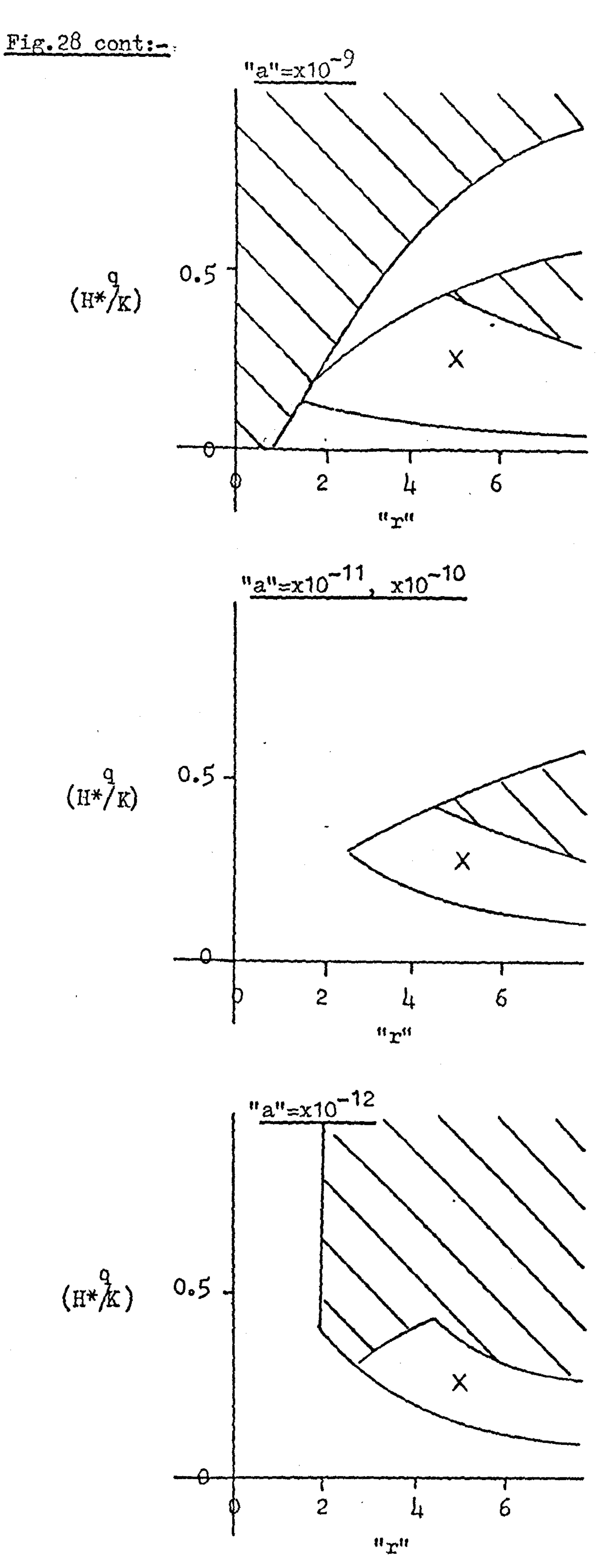


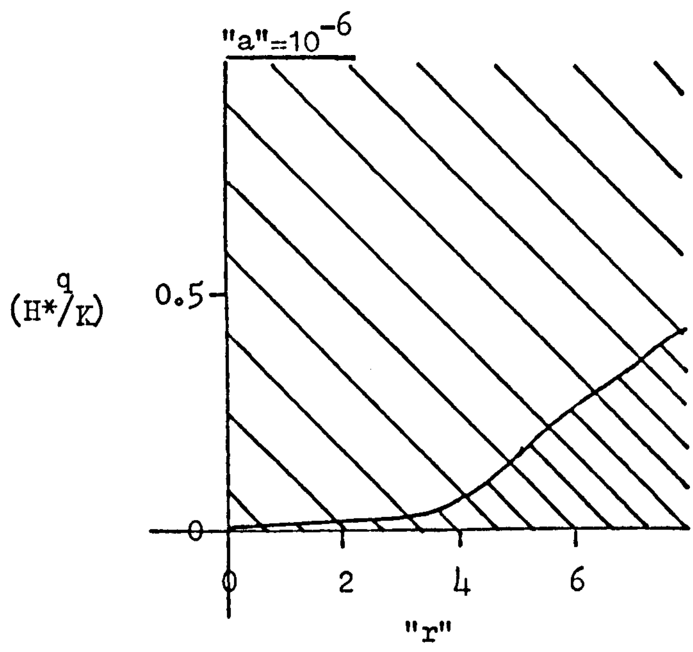
Fig.28 cont:-
"a"=x10^{-9}
0.5
(H*^{q}/K)
0
0
2
4
6
"r"
X
"a"=x10^{-11}, x10^{-10}
0.5
(H*^{q}/K)
0
0
2
4
6
"r"
X
"a"=x10^{-12}
0.5
(H*^{q}/K)
0
0
2
4
6
"r"
X

Fig. 29:- The effect of alteration in parameter "a" on the stability boundaries for the MX8 + Ømx8 system ("c" = 66)

= Stable (X = damped oscillations)

= Unstable fluctuations

= Extinction of phage



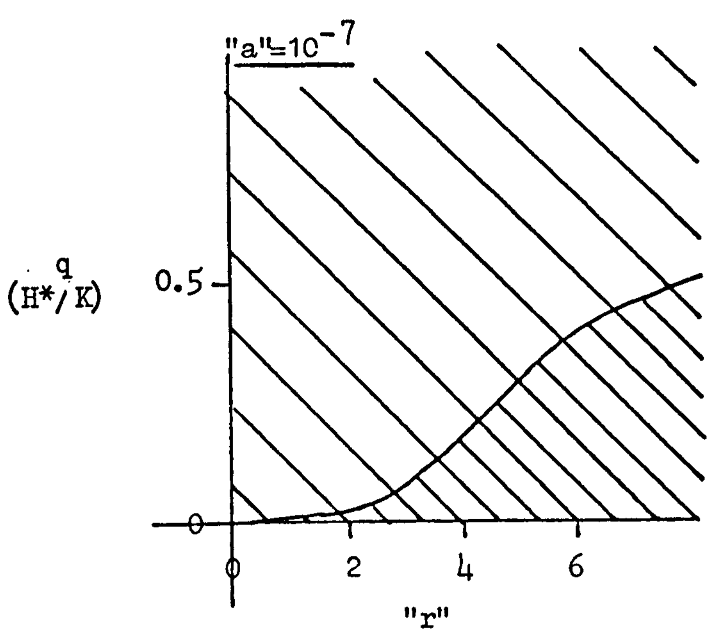
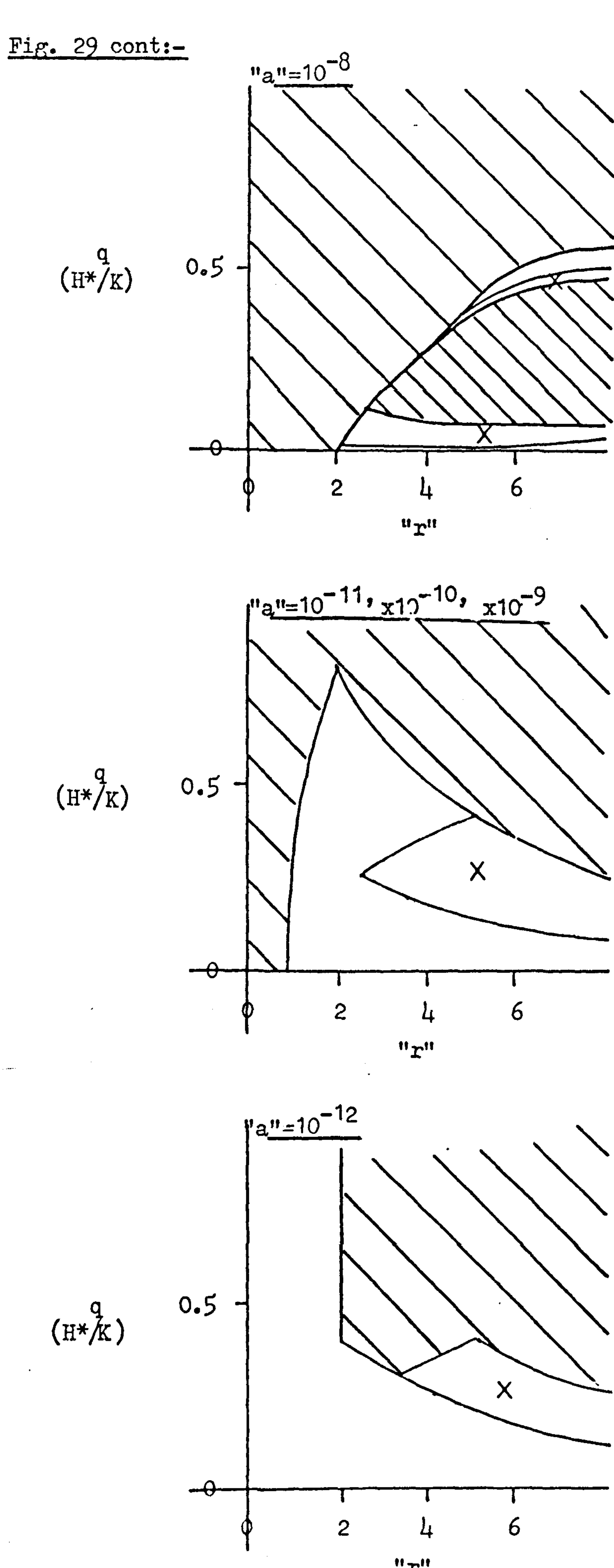


Fig. 29 cont:-



"a" = x 10^{-9} and "c" = 1.

Therefore the value assigned to "a" is a very significant factor in determining the nature of the interaction observed.

The nature of the infection efficiency in this model is one of the major criticisms levelled at the Nicholson-Bailey model (Royama, 1971; Hassell & May, 1973). It was stated that a single constant value for 'a' for a particular set of environmental conditions, is unrepresentative of the situation which occurs in nature and that therefore it was too simplistic a representation of "a" because it is likely to be affected by the densities of either one or both of the interacting populations. Holling (1959) stated that

> 'the only functional responses that have been demonstrated with real animals however are the S-shaped responses of small mammals and the responses of parasites which have continually decreasing slopes. Neither response is linear.'

It is difficult to imagine a situation where a phage's rate of attack could increase indefinitely with increased host or phage density without there being some interference in the populations. As already shown, a constant value for "a" was not observed with changes in phage and host density and therefore the criticism also applies to this modelling of the streptomycete-phage interaction.

Royama (1971) examined the Nicholson-Bailey model critically and in particular the parameter "a" with its associated assumptions. He stated that random encounters could only be found if the number of predators is independently high, the time for searching is unlimited and the density of prey is low. The problem of producing random search does not apply to this system because, as already stated, the interaction of hosts and phage is based upon random encounters. However, even if the phage did not individually encounter hosts randomly, Rogers (1972) stated that for parasites at least, a single parasite will not search randomly over a short period but a population as a

whole throughout a large area might do so.

Hassell & May (1973) reviewed models which have been presented by several authors which allow for the effect of either host or parasite (=phage) densities on "a", but still with the inclusion of random search (e.g. Holling, 1959; Hassell & Varley, 1969; Royama, 1971; Hassell & Rogers, 1972; Rogers, 1972). Therefore there is available literature to provide a basis for adapting the model to include the effects of phage and streptomycetes densities in the interaction. The inclusion of the effect of varying host and phage densities would be one of the next steps to take in making the model more realistic and representative of the streptomycete-phage interaction in soil, or any other environment.

Having looked at the consequences of the infection efficiency is there any way in which it can vary in the natural environment? "a" reflects the ability of the phage to infect hosts during its searching lifetime, which in this case was 11 days, and therefore any alteration in the environment could lead to an alteration in "a". Obviously any factor which prevents or limits, access of phage to a susceptible host without affecting the viability of either the host or the phage will modify the extent of the interaction and thus alter the infection efficiency. One of the most obvious factors is the presence of colloids and perhaps the size of other particles. Introduction of particulate material into a system may restrict the movement of either host or phage, and therefore the soil matrix offers a large active surface area which enables sorptive interactions between the phage and/or host and soil particles to occur (Ruddick & Williams, 1972; Marshall, 1974; Sykes & Williams, 1978; Sobsey et al., 1980). Streptomycetes are usually found in association with organic particles and 'attached' in some way, possibly by the mycelial network, to them. The main movement would then be observed in the spore stage. Spore movement has been examined by

Ruddick & Williams (1972), who intimated that it can be achieved by adsorption to arthropods and by water percolation. The amount of movement is related to their morphology and surface properties, hydrophobic spores being less well suited to dispersal by water. The movement of streptomycetes in the infectable stage is restricted, as it is unlikely that a mycelial network will be easily moved. Nevertheless outgrowth caused by the development of the mycelium may result in the increased chance of meeting phage by being able to bridge gaps which were unbridgeable to the phage and therefore make the streptomycete more accessible to the phage.

The adsorption of phage particles to colloidal materials and sediments has been observed by several authors (Roper & Marshall, 1974; Bystricky et al., 1975; Sykes & Williams, 1978; Schiffenbauer & Stotzky, 1983) and thus will restrict their movement. Marshall (1974), in a review of adsorption of viruses onto surfaces in soil and water, states that the adsorption of viruses to soil particles is significant and readily influences the movement of viruses through soil columns and presents data collected which indicate virus adsorption form 78-100% in a range of soils. Sobsey et al. (1980) stated that virus retention in soil (and therefore adsorption) is influenced by soil type. Therefore the effect of colloidal materials can work in both ways; by adsorbing the phage to particles it restricts their free movement and therefore can reduce the infection efficiency observed, if however they are restricted in the same area or onto the same particle as the streptomycete it can increase the infection efficiency. Roper & Marshall (1978) investigating the Escherichia coli + a bacteriophage system found that colloidal material can protect the bacteria from the phage if the particles were small enough to form a protective coat, otherwise no protective effect was observed.

Since the infection efficiency is a measurement of adsorption ability then any environmental factor which affects the adsorption of phage to the host whilst leaving both still viable will affect "a". These environmental factors have already been discussed when "a" was examined (Chapter 2, section VII), e.g. ionic composition & temperature.

It is more likely therefore that "a" will vary not only between different soil types, but also within a single soil due to the heterogeneous nature of the environment. Since then "a" plays a significant role in determining the outcome of the interaction, it is likely that there will be several different interactions occurring within one soil alone, due to the effect of host densities and environmental conditions. Further investigations must be performed to discover what can alter the nature and extent of the interaction within a soil system, and to determine the exact response of the infection efficiency to the changes in host density which result from the interaction and from the heterogeneous distribution of the streptomycetes.

c) The effect of the introduction and alteration of parameter "s".

The inclusion of parameter "s" has been previously explained, its role being to represent the proportion of spores produced which will germinate and produce new potentially infectable units. The inclusion of this parameter not only more properly represents the growth and availability of the streptomycete within the soil, it also alters the stability of the host alone and that observed in stability diagrams of the interaction under consideration. May (1974) examined the oscillatory behaviour of biological populations with non-overlapping generations. In particular he examined the first of the Nicholson-Bailey equations:-

$$H_{t+1} = H_t \exp(r(1-H_t/K)) \quad (1)$$

and therefore the behaviour of the host in the absence of the parasitoid. He stated that some of the very simplest non-linear difference equations even for single species exhibit a spectrum of dynamical behaviour. Table 30 presents the dynamical behaviour observed at different intrinsic rates of increase, and shows that the value assigned to parameter "r" can have a profound effect on the behaviour of the host. Examination of Table 30 indicates that at the intrinsic rates of increase obtained for the two systems under investigation, (MX8-"r" = 5.669, MX1-"r" = 5.762), the resultant host behaviour would never be stable but always that of chaos. He defined chaotic behaviour as any 'r' value for which there are cycles of period 2, 3, 4, 5, ..., n,...., where n is any positive integer, along with an uncountable number of initial points for which the system does not settle into any finite cycle. A similar result of oscillatory behaviour was also derived by Cook (1965).

Table 31 presents the change in dynamical behaviour of the host under varied "s" values. It shows that for intrinsic rates of increase of 1 and 2 there must be a certain proportion of the host continuing to the next cycle or the host will not be able to sustain itself and losses will be incurred. It is possible to observe stable equilibrium points up to an intrinsic rate of increase up to 4 and bifurcating cycles up to "r" = 5. At "r" = 6 and above, only chaotic behaviour was observed. Fig 30 presents the behaviour observed at the two intrinsic rates of increase utilized for the two systems investigated along with that observed in the absence of "s". The inclusion of parameter "s" affects the proposed growth of the streptomycete by decreasing the depth of the oscillations. Table 32 indicates that at the two intrinsic rates of increase observed it is possible, with the inclusion of "s" to observe

DYNAMICAL BEHAVIOUR	VALUE OF THE INTRINSIC RATE OF INCREASE
Stable equilibrium point	2 > r > 0
Stable cycles of period 2^n	
2-point cycle	2.526 > r > 2.000
4-point cycle	2.656 > r > 2.526
8-point cycle	2.685 > r > 2.656
16, 32, 64	2.692 > r > 2.685
Chaos	r > 2.692

Table 30:- Dynamics of a population described by the difference equation 1.

(From May, 1974)

INTRINSIC RATE OF INCREASE	DYNAMICAL BEHAVIOUR OF THE HOST			
	HOST LOSS	STABLE EQUILIBRIUM	BIFURCATING CYCLES	CHAOS
1	- 0.35	0.375 - 1.000		
2	- 0.10	0.150 - 0.975	1.000	
3		- 0.375	0.400 - 0.775	0.800 -
4		- 0.100	0.150 - 0.250	0.275 -
5			- 0.150	0.175 -
6				0.050 -

Table 31:- The range of "s" values required to observe the stated dynamical behaviour of the host at the listed intrinsic rates of increase

Fig. 30:- The oscillatory behaviour of MX8 and MX1 in the presence and absence of parameter "s"

a) MX8

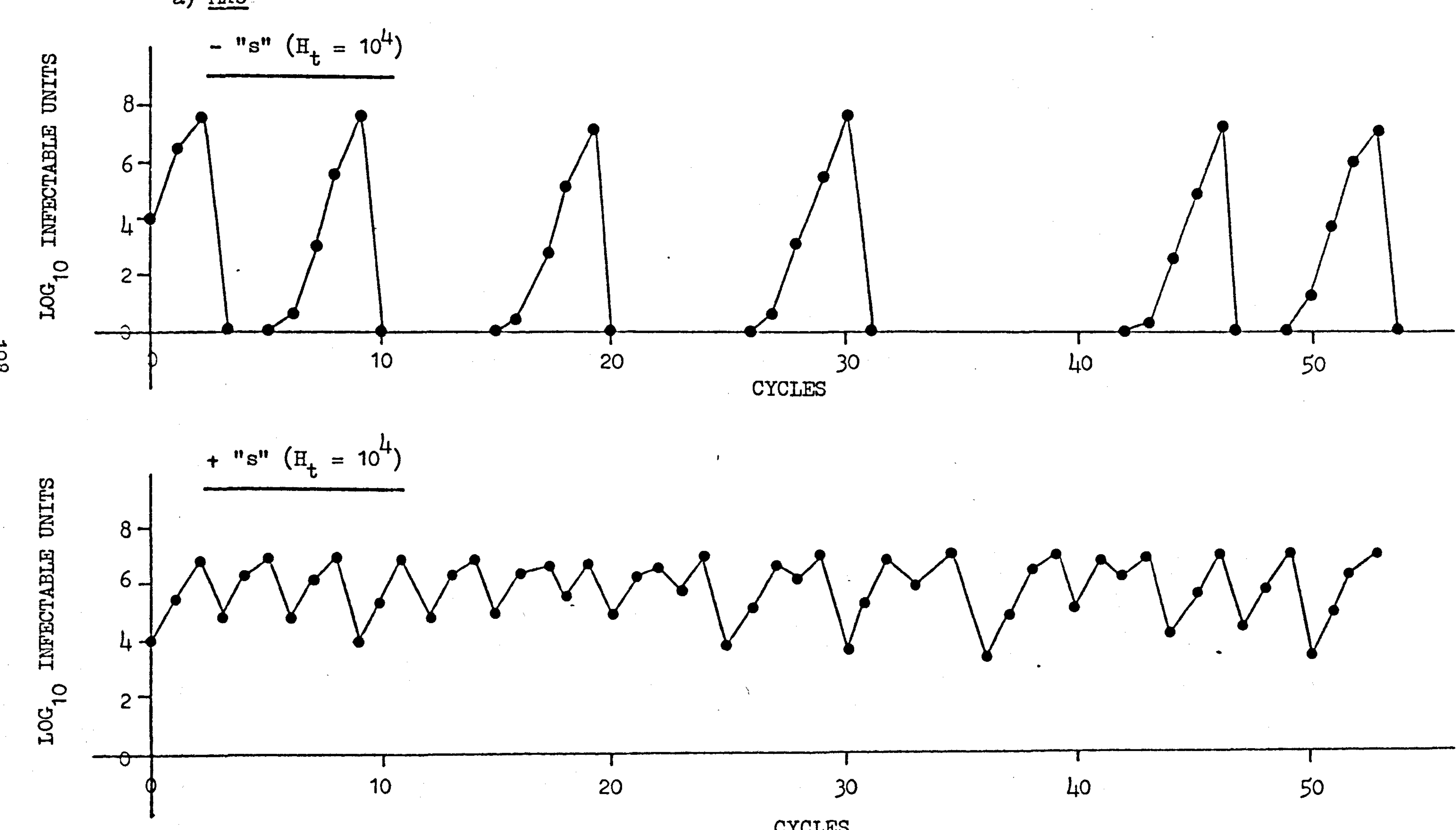
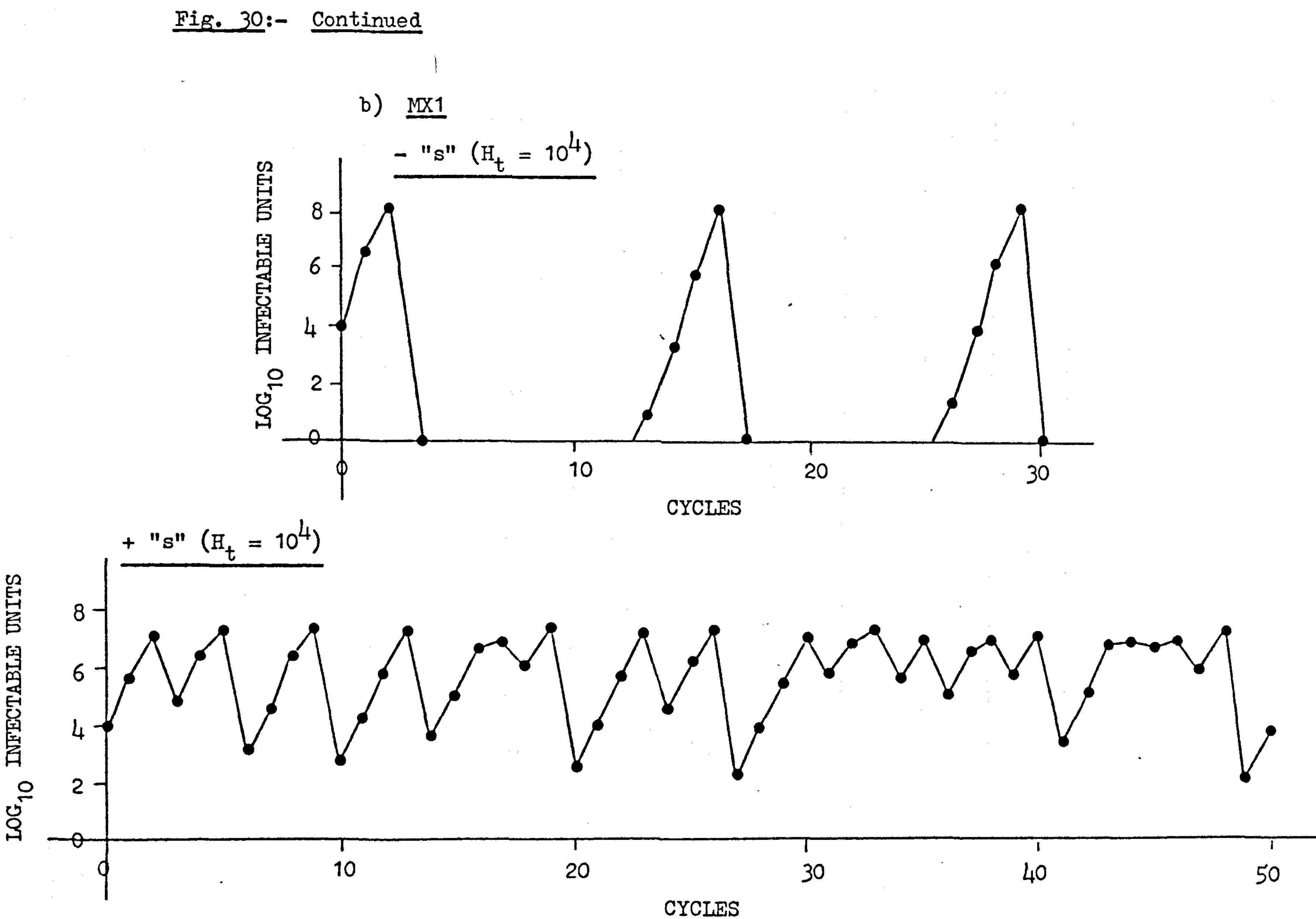


Fig. 30:- Continued

b) MX1



	INTRINSIC RATE OF INCREASE ("r")	
DYNAMICAL BEHAVIOUR OBSERVED	5.672 (MX1)	5.669 (MX8)
STABLE EQUILIBRIUM POINT	- 0.02	- 0.02
BIFURCATING CYCLES	0.03 - 0.04	0.03 - 0.05
CHAOS	0.05 -	0.06 -

Table 32:- "s" values at which the stated behaviour can be observed

both stable equilibrium point behaviour and bifurcating cycles. However these are only observed at very low values of "s", = 0.04 for MX1 & = 0.05 for MX8. Table 33 demonstrates the effect seen when "s" is equivalent to those used for the two streptomycetes under investigation. It indicates that at the values used for "r" and "s" in this work, the host behaviour still falls into that region of behaviour defined as chaotic, the host never falling into any defined cycle. The representation of streptomycete growth with respect to infectable units as chaotic would appear to be justified if the nature of the increase is examined. A single spore germinates to produce a large mass of mycelium, and therefore a sudden surge in the available infectable points on the streptomycete is observed. The subsequent breakdown of the mycelium to produce a large quantity of spores leads to a decrease in the infectable units. This together with the spasmodic growth of the streptomycete which is thought to occur in soil would not appear to have the attributes for a stable growth pattern.

If then "s" directly affects the host behaviour pattern what is the effect on the stability diagrams for the model in which it is involved? The introduction of "s" and "z" into the model leads to the alteration of the roots of the quadratic equation which describes the behaviour of local stability points in such a way that the stability diagrams are now dependent on a number of parameters (Table 34). The effect of these parameter on the interaction can only be investigated by altering their value and observing their effect on the stability diagram produced. If they are very important in the interaction then the diagrams will alter substantially for small changes in the parameter value. Fig 31 presents the effect of increasing "s" value on the nature of the stability diagrams. As can be seen the effect of altering "s" is very limited, even though its introduction does alter the nature of

	PARAMETER "s"	
DYNAMICAL BEHAVIOUR OBSERVED	0.13429 (MX1)	0.112761 (MX8)
HOST DECLINE	0 - 2	0 - 2.125
STABLE EQUILIBRIUM POINT	2.125 - 4	2.5 - 4.125
BIFURCATING CYCLES	4.125 - 4.50	4.25 - 4.50
CHAOS	4.75	4.75

Table 33:- Range of intrinsic rates of increase at which the stated host behaviour can be observed

COMPONENT OF BEHAVIOUR DETERMINANT EQUATION	
b	$1 - rq + azcH \exp[[\ln[1/s]] - r[1 - q]]$
c	$[aHzc][1 - [rq \exp]]\ln[1/s]] - r[1 - q]]]]$

Where conditions of stability are dependent on the roots of the equation:-

$$\lambda^2 + b\lambda + c = 0$$ (see Appendix 1 for explanation of use)

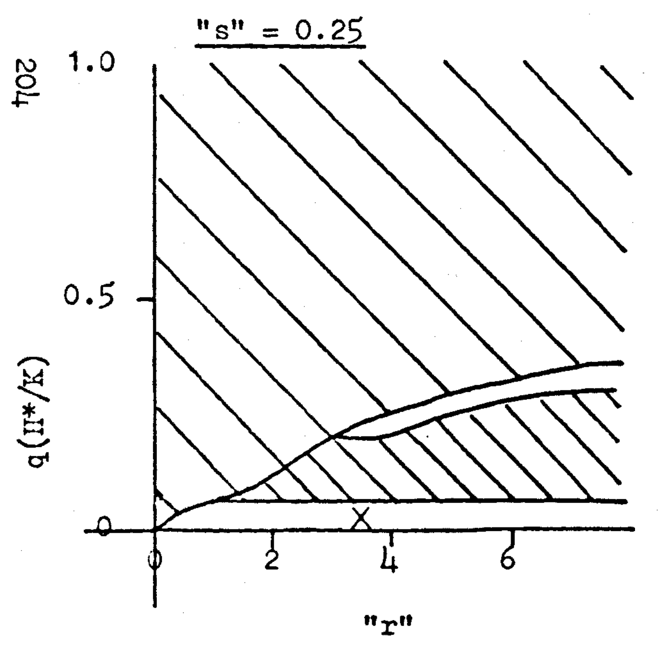
Table 34:- Parameters upon which stability boundaries may be dependent

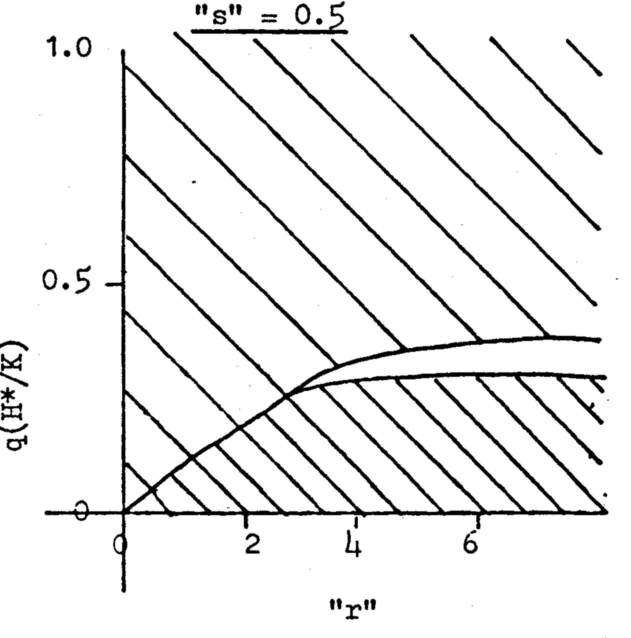
Fig. 31:- The effect of alteration in parameter "s" on the stability boundaries for the MX8 + Ømx8 system ("a" = x 10^{-8}, "c" = 100)

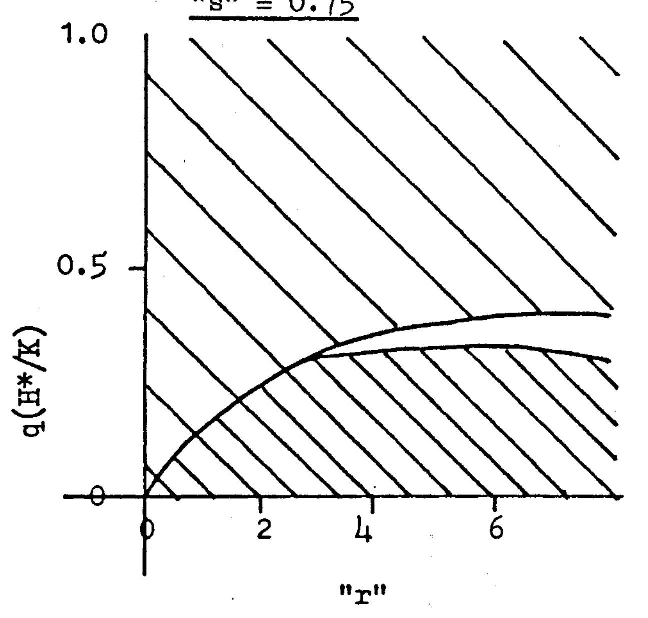
= Stability (X = damped oscillations)

= Unstable fluctuations

= Extinction of phage







the stability diagrams. It does not alter the shape of the diagram, but it does extend the regions of the zones observed. It has little effect on the local stability because a stability diagram investigates the depression of the host caused by the phage and not by any other parameter, and "s" is not a directly effective in the interaction; its effect is seen on the availablity of the host. This effect of "s" would then be expected as similar initial host and phage populations might be expected to show different outcomes of interaction at differing "s" values.

How then could "s" be altered in the natural soil environment? As 's' is a measure of the proportion of spores germinating in a soil system, then any alteration in this proportion would lead to an alteration in "s". An increase in the nutrient status of the environment could lead to increased "s", or any other increase which makes conditions more favourable for spore germination could lead to increased "s". If the carrying capacity of the environment is constant, then production of smaller colonies could allow for the germination of more spores than if the colonies were generally large in terms of the mycelial tips produced. Therefore it would be interesting to investigate the proportion of spores which germinate in different soil types and in different horizons within a single soil.

d) The effect of the alteration of the carrying capacity of the environment, parameter "K".

The introduction of a density-dependent factor to the Nicholson-Bailey model was made by Beddington et al. (1975) to produce a model which resulted in outcomes which were of a generally more realistic nature. In the absence of this density-dependent regulation oscillations of increasing amplitude are always the result of any interaction. Such unstable interactions have only been observed under

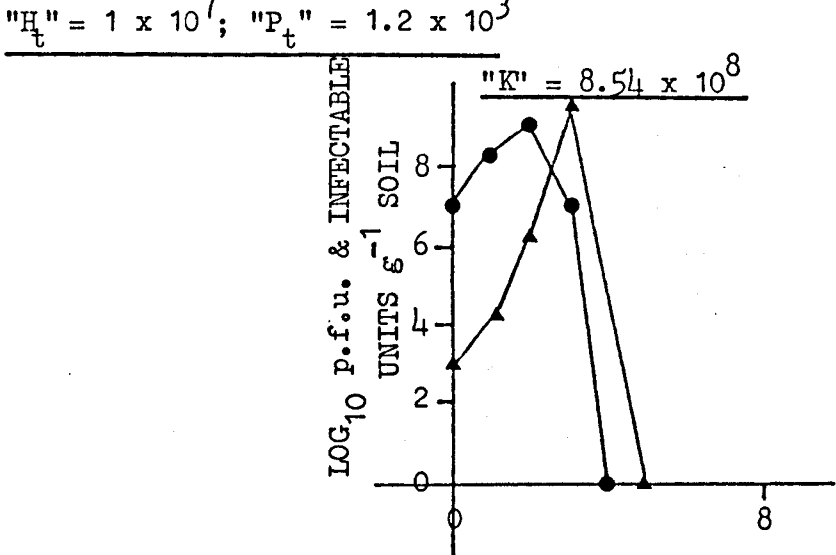
laboratory conditions for insect-parasite interactions, whereas insect-parasite interactions in nature are generally stable (Hassell & May, 1973). Beddington et al. (1975) also stated that the inclusion of a density-dependent factor always leads to some sort of stability in a model.

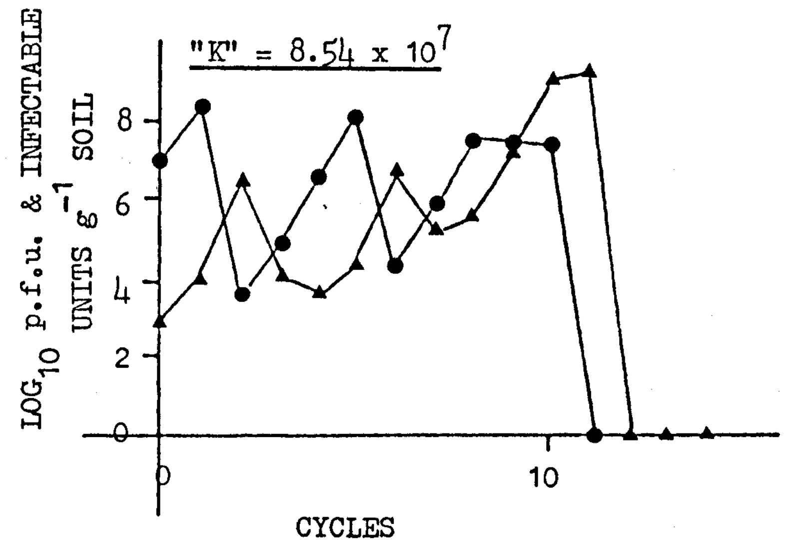
It has already been stated that the experimental value obtained for "K" is thought to be an underestimate of that actually occurring in the soil and therefore the effect of the alteration of "K" is of great interest. Fig 32 presents a selection of the results obtained when the carrying capacity ("K") is altered between 10^6 - 10^8 infectable units g^{-1} soil for the MX1 + Ømx1 system. The host reaction was explored over an initial host density range of 10^4 - 10^7 spores g^{-1} soil, and a phage density of 1.2×10^3 and 4.7×10^4 p.f.u. g^{-1} soil. The infection efficiency was equivalent to 10^{-8} because it is thought that at this value or around it, it would be possible to obtain sustained but irregular oscillations of both the host and the phage. Thus it was possible that any alteration in the carrying capacity could lead to the production of these oscillations. The results obtained indicate that the alteration of the carrying capacity, at least at this value of 'a' will not lead to oscillations which were sustained, either irregular or regular. It can however determine whether the chaotic interaction is to take the form of the phage alone being lost or the greater chaotic event of both the host and phage being lost from the system. As "K" increases, in all combinations of host and phage used, there is an increasing likelihood that both will be lost. Above "K" = x 10^9 infectable units g^{-1}, whatever the original starting concentrations, both organisms were lost. At higher carrying capacities the host will be able to reach a higher concentration and therefore provide more sites for the phage to infect and at this particular "c" value (burst size = 100) the phage will eventually be present in such

Fig. 32:- The simulatory behaviour observed under conditions of varied carrying capacity and initial host population size for MX1 + Ømx1 ("a" = x10^{-8})

p.f.u. (▲—▲), infectable units (●—●)

"H_t" = 1 x 10^7; "P_t" = 1.2 x 10^3





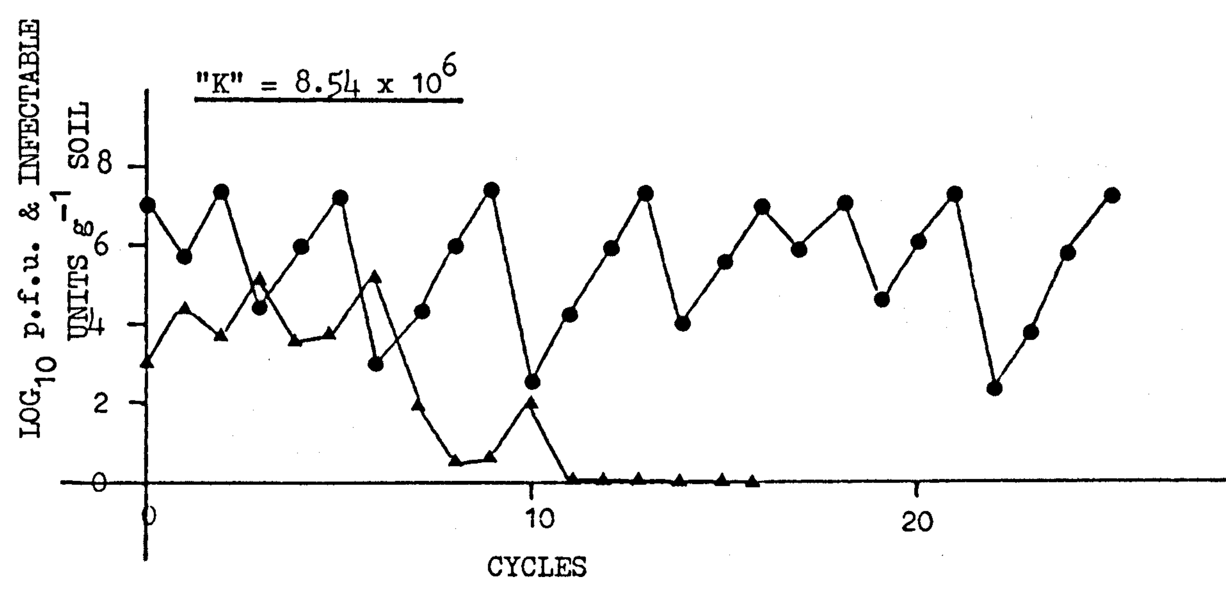
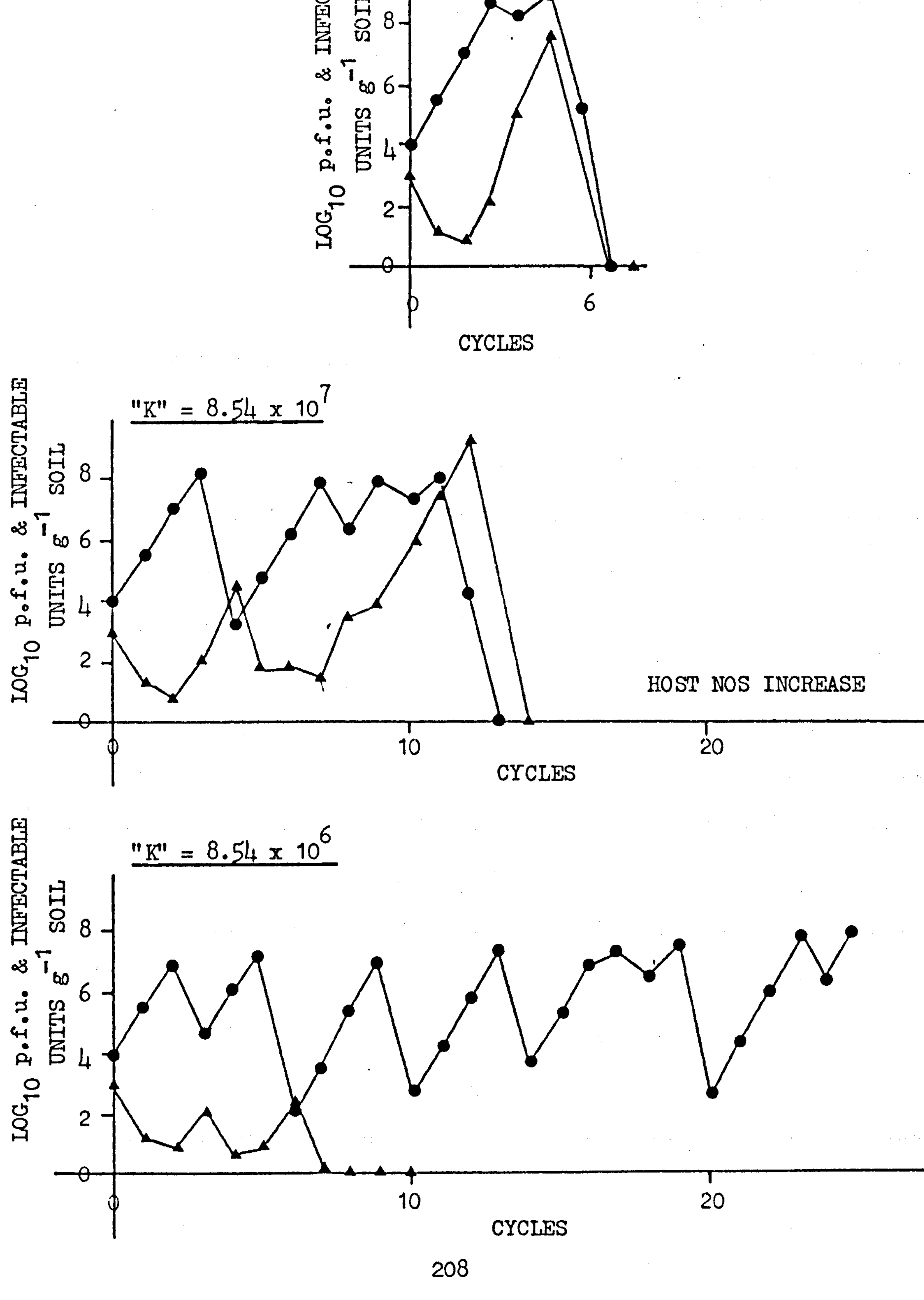


Fig. 32 cont.:-

"H_t" = 1 x 10^4; "P_t" = 1.2 x 10^3



quantities as to be able to eliminate the available host. It is not possible to determine which of the interactions would occur between the streptomycete and the phage. It appears that if the simulated initial host concentration is near to that of the carrying capacity, either above or below it, the host is more likely to survive; possibly this is because there will not be an initial increase in the phage sufficient to eliminate the host from the system.

Therefore the carrying capacity of the environment can play an important role in determining the outcome of at least a chaotic interaction, but this outcome is related to the initial number of hosts units available to be infected and provide further infectable units, and may also be related to the value adopted for the intrinsic rate of increase. Beddington _et al_. (1975) indicated that the lower the growth rate, the further the predator has to depress the prey below its carrying capacity before a chaotic interaction occurs. Also if the value adopted for the infection efficiency is at either extreme _i.e_. it predicts loss of host or phage alone, any alteration in the carrying capacity would not be expected to lead to any alteration in the nature of the interaction observed.

How then can the carrying capacity of a soil for a streptomycete be altered? It could be altered by a change in the nutritional status of the soil, or any environmental changes which make the soil more or less amenable to streptomycete growth. Lowering of the carrying capacity could be achieved by a decrease in the availability of nutrients either through physical unavailability or any environmental condition which lowers the ability of the streptomycete to grow will also lead to a decrease in the carrying capacity. Increased values of "K" would be achieved by an increased availability of nutrients due to the natural decomposition of organic materials within a soil and the release of

nutrients to lower horizons by the downward percolation of rainwater.

Further work is required to examine the way in which "K" is effected by nutritional conditions and changes in environmental conditions, as well as to more accurately determine the value(s) of "K" within a soil.

e) <u>The effect of the introduction and alteration of parameter "z"</u>

The parameter "z" allows for the natural decay of the phage which has been previously shown to occur in an 11 day period. It does not however, allow for this decrease to occur during the 11 day period but removes a proportion of the phage at the start of each cycle. The values calculated for "z" have already been shown in Table 25. Fig 33 presents a selection of the results observed of simulations of the two interacting systems of MX1 + Ømx1 and MX8 + Ømx8 under conditions of various "z", where again "a" was of the order x 10^{-8} for the reasons previously explained. The overall effect of altering the proportion of phage which are available to continue into the next cycle is shown in Table 35. This indicates that the proportion can have a profound effect upon the interaction which occurs at this particular "a" value. For the MX1 + Ømx1 system the "z" value cannot alter the nature of the interaction, only the length of it; as the "z" value increases the length of the interaction increases. For the MX8 + Ømx8 system the proportion of phage continuing into the next cycle has to be greater than 0.723 in order for there to be sustained oscillations, although the pattern of oscillations is not identical at each altered "z". Below this value the phage are lost, the number of cycles that they are able to take part in decreasing as the value of "z" decreases.

The effect of "z" on the stability diagrams is shown in Fig 34, for the MX8 + Ømx8 system. As "z" increases, the stability regions in the diagrams are decreased until when "z" = 1 (all the phage go on to the

Fig. 33:- The effect of alteration in "z" in the interaction observed for the MX8 + Ømx8 and MX1 + Ømx1 systems

p.f.u. (▲—▲), infectable units (●—●)

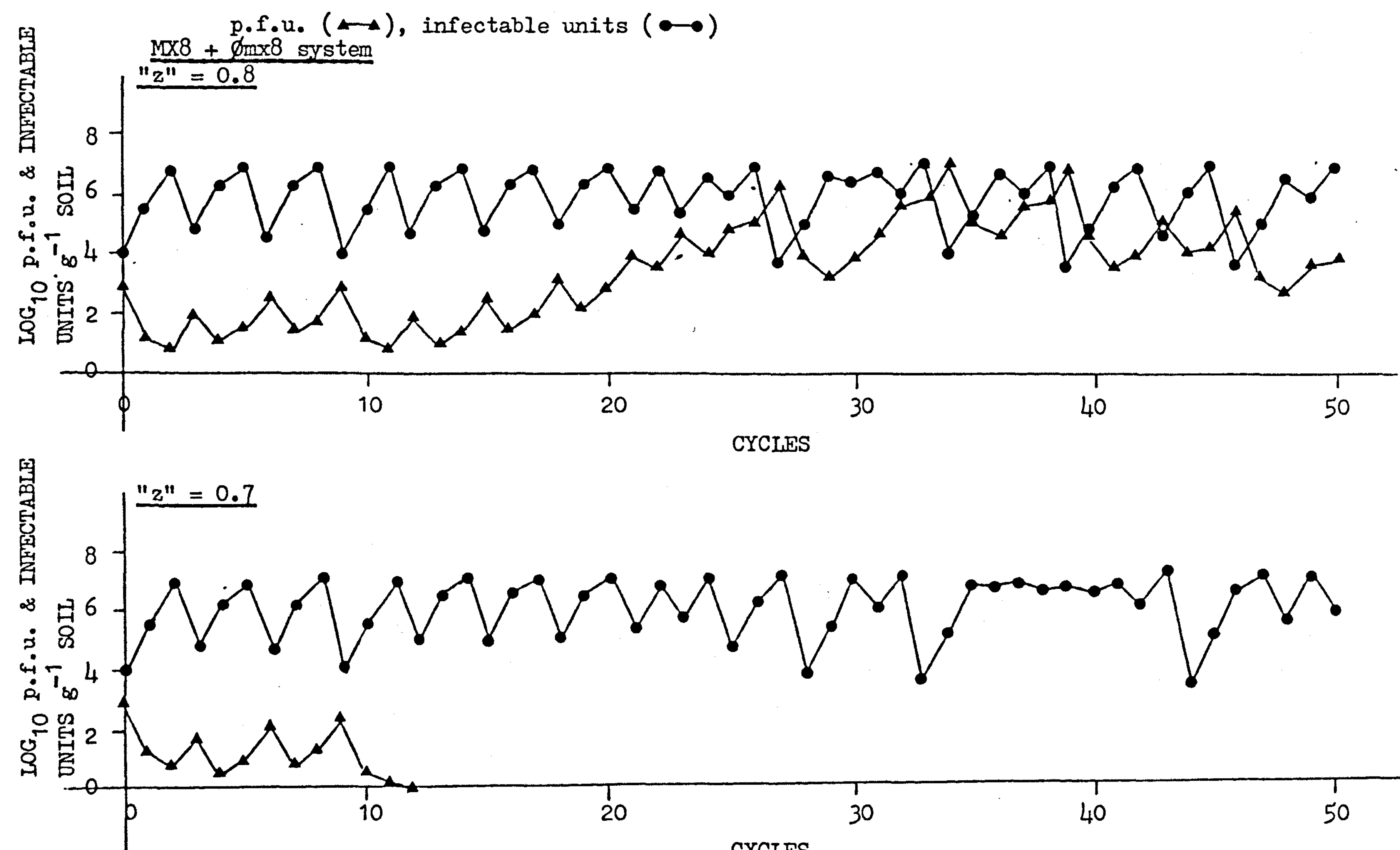


Fig. 33 cont.:- MX8 + Ømx8 system

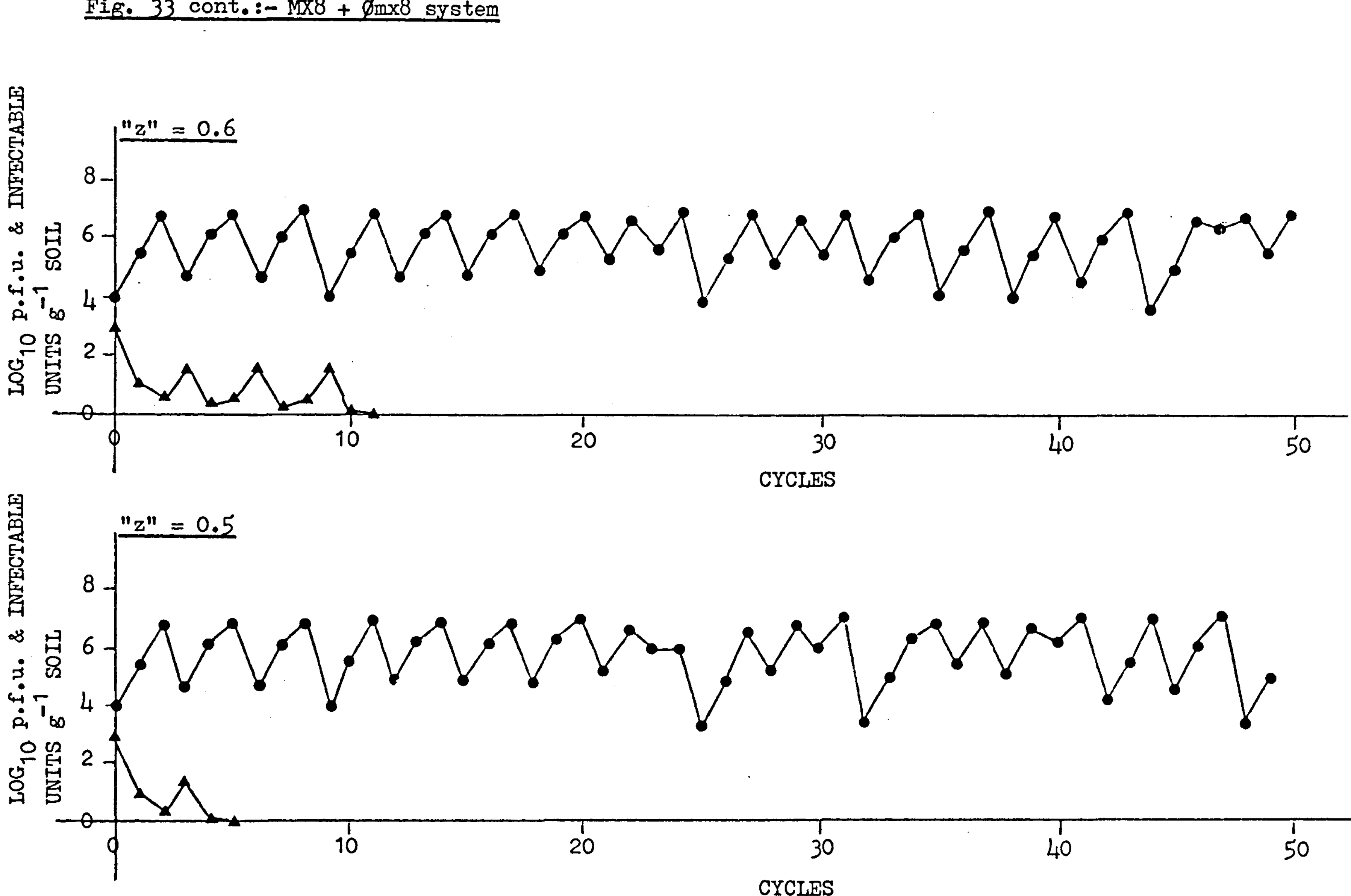
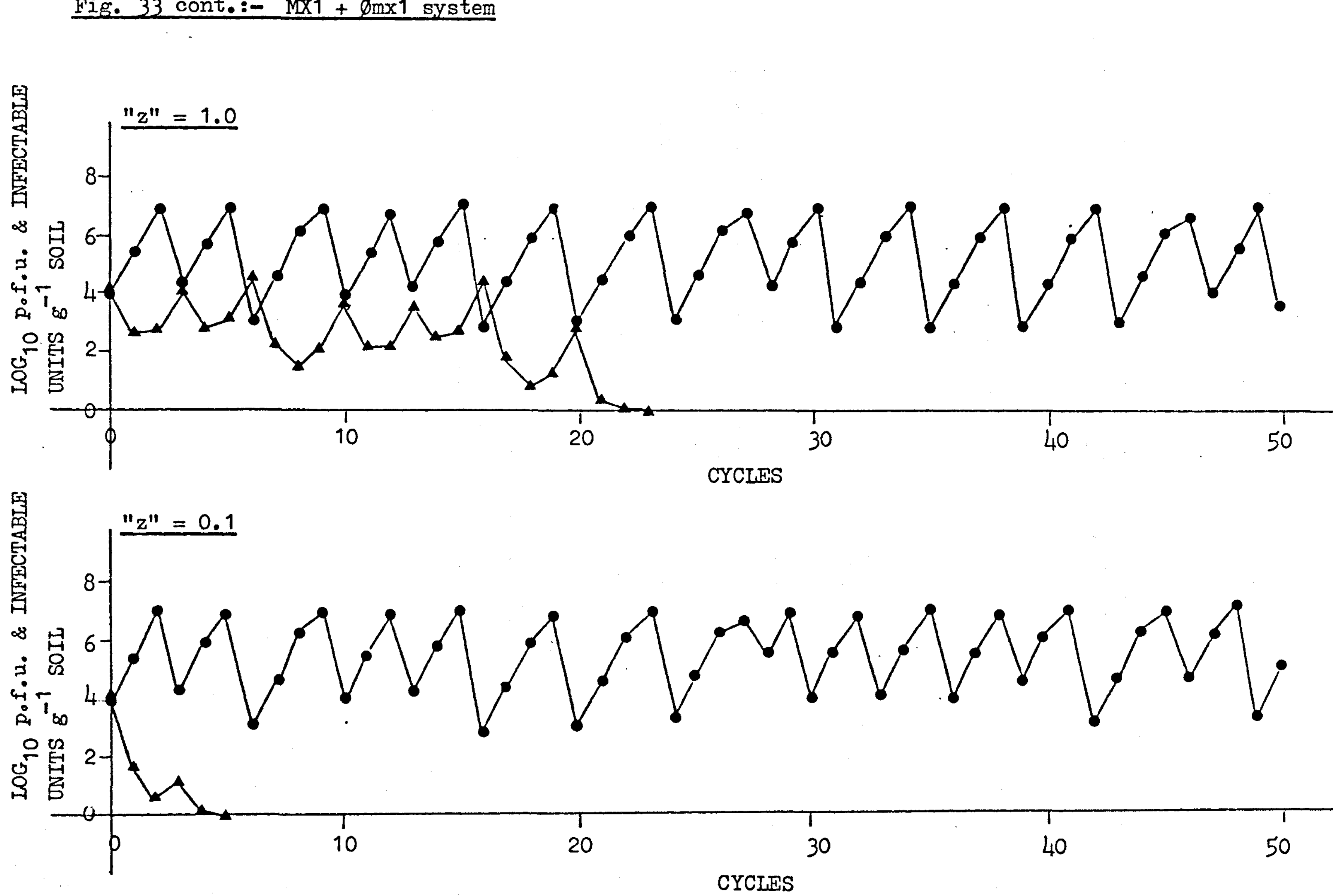


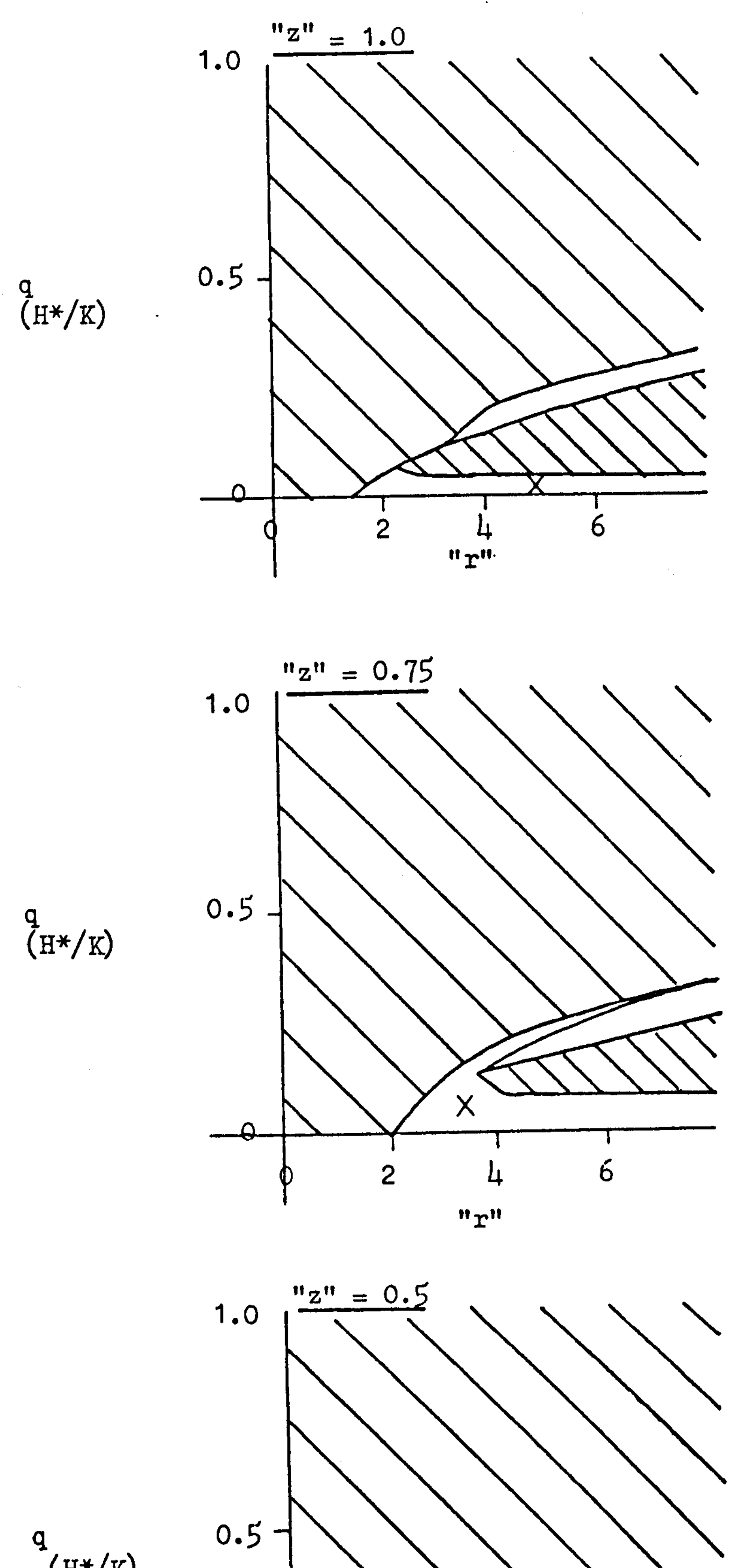
Fig. 33 cont.:- MX1 + Ømx1 system

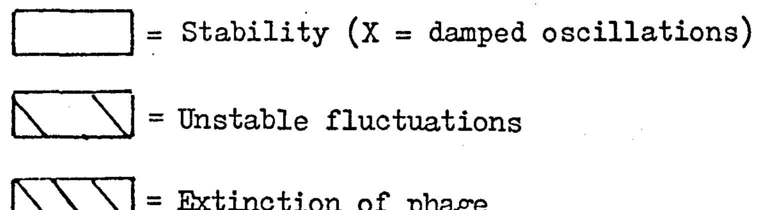


"z"	RESULTING BEHAVIOUR
a) MX8 + Ømx8	
0 - 0.722	Chaos - Phage loss
0.723 - 1.000	Sustained oscillations but not of a regular pattern
b) MX1 + Ømx1	
0 - 1.000	Chaos - Phage loss

Table 35:- The effect of the alteration in "z" on the outcome of the interaction

Fig. 34





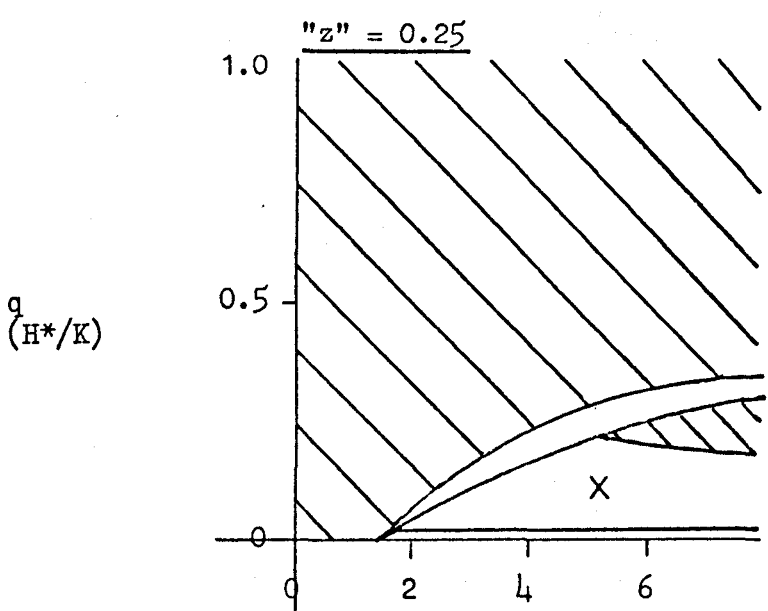


Fig. 34 cont:- The effect of alteration in parameter "z" on the stability boundaries for the MX8 + Ømx8 system.

("a" = x10^{-8}, "c" = 66)

next cycle), there are only two limited regions of stability. An increase in "z" will lead to an increase in the proportion of unstable interactions because it allows for more phage to continue to the next cycle and therefore produce more interactions which will alter the resultant nature of the interaction.

Alteration in the value of the parameter "z" can be a result of the nature of the environmental conditions in which the interaction is undertaken. It is unlikely to be a constant for any phage type either within one soil type or between soil types because of the heterogeneous nature of soil, both chemically and physically. Previous work (Chapter 2, section IV) has dealt with the effects of varied environmental factors, e.g. pH, temperature, on the survival of phage within a soil.

Parameter "z" appears to have an important role in the outcome of the interaction between streptomycetes and their phage. It reduces the number of phage which can be available to 'perform' new infections, and hence when it is increased to its maximum ($z = 1$) an increase in the unstable regions in the local stability diagrams is observed.

f) The effect of changes in the burst size, parameter "c"

The burst size of a particular streptomycete-phage system can change with alterations in the environmental and physiological conditions of the host (Chapter 2, section III). Even changes in the host and phage densities have been shown to affect phage production (Barry & Goebel, 1951). For most of the insect-parasitoid systems for which this model was originally devised, the value of "c" is 1. However as shown earlier for the systems investigated here, and indeed for most host-bacteria systems, the "c" will be in excess of 1. The burst sizes produced in a single experiment have been shown to follow a normal distribution

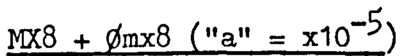
(Delbrück, 1945), and therefore to utilize a single value of "c" even though it is the average measurement, is a limitation of the representation of "c" in this model. It may be possible if the range and frequency of individual burst sizes were obtained from the examination of individual burst size experiments to include a random distribution of the burst sizes based on the frequency of their observation.

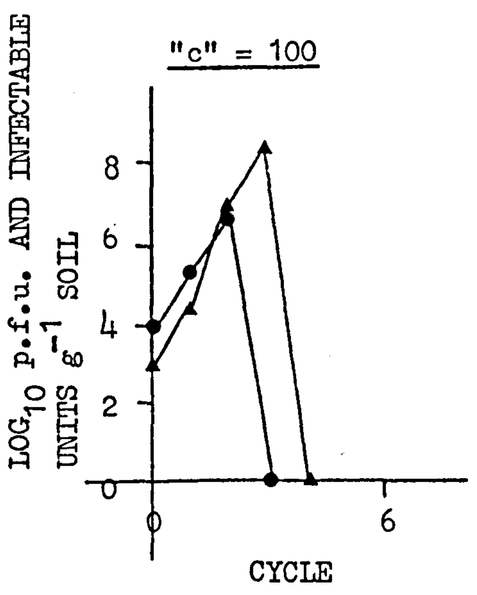
Fig 35 presents the results of the originally derived "a" values together with a variation of "c". The alteration of burst size has little effect on the final outcome of the interaction it always being chaotic. However, in some cases, MX8 + Ømx8 & "a" = 10^{-5} and MX1 + Ømx1 & "a" = 10^{-6}, at the lower burst sizes the host is not always lost from the system.

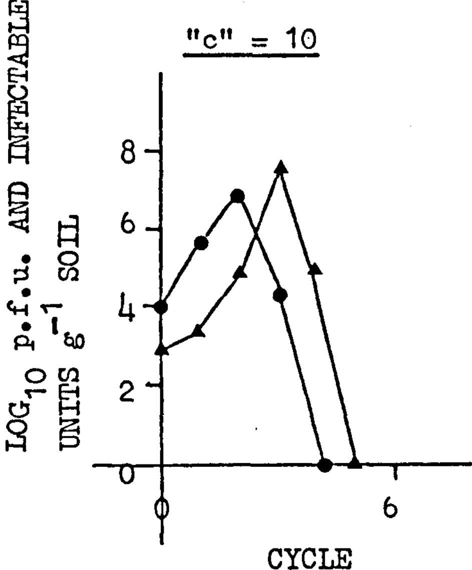
"c" does not have any direct effect on the production of the original stability diagrams (Fig 36), but it can be seen to effect the movement across the zones of stability (Table 36). For the value of "a" originally used, no movement is observed as their reaction falls well into their stability areas. However to observe the same interactions at different ten-fold increases in "c", a ten-fold decrease is required in the value adopted for "a". These observations were seen at "r" = 2 & 3, and "a" falling into the range of $\times 10^{-7}$ - $\times 10^{-9}$. When the effect of "c" on the stability diagram is considered for the model used in this work (that containing parameters "s" and "z"), it is seen that it does have an effect on the stability diagram produced. Why does "c" now become important in the derivation of the stability diagrams? The introduction of the parameters "s" and "z" into the model leads to more complicated roots of the quadratic equation which enables the stability diagrams to be produced. The parameter "c" now appears within these roots and thus its alteration leads to the production of variable stability diagrams, although the changes

Fig. 35:- The effect of altered burst size on the interaction observed for the MX8 + Ømx8 and MX1 + Ømx1 system at "a" derived from soil and broth experiments

Infectable units (●—●), p.f.u. (▲—▲)







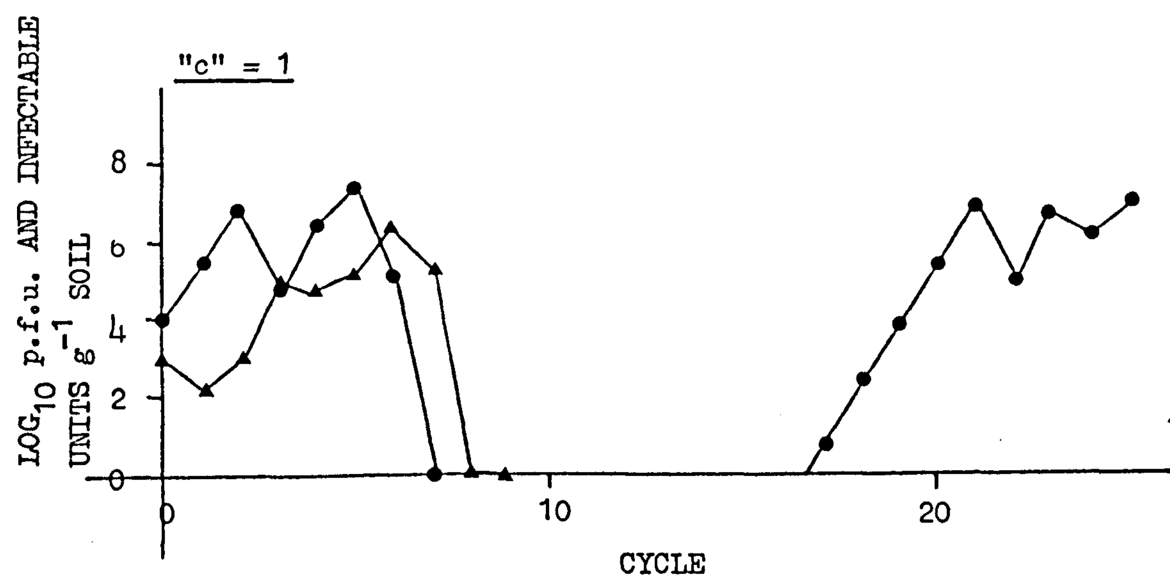


Fig. 35 cont.:- MX8 + Ømx8 ("a" = x 10^{-9})

Fig.35 cont:- MX1 + Ømx1 ("a" = x10^{-6})

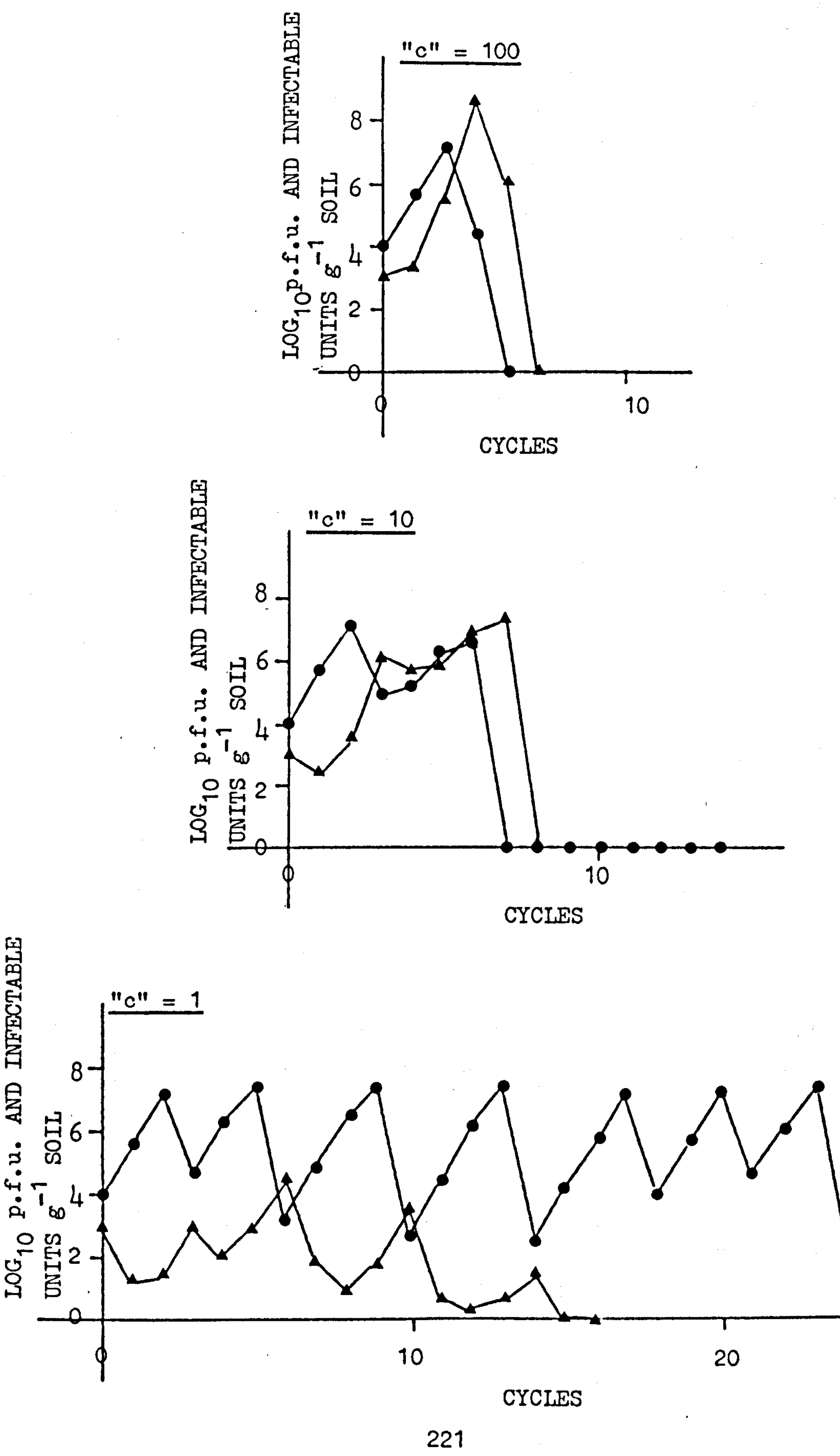
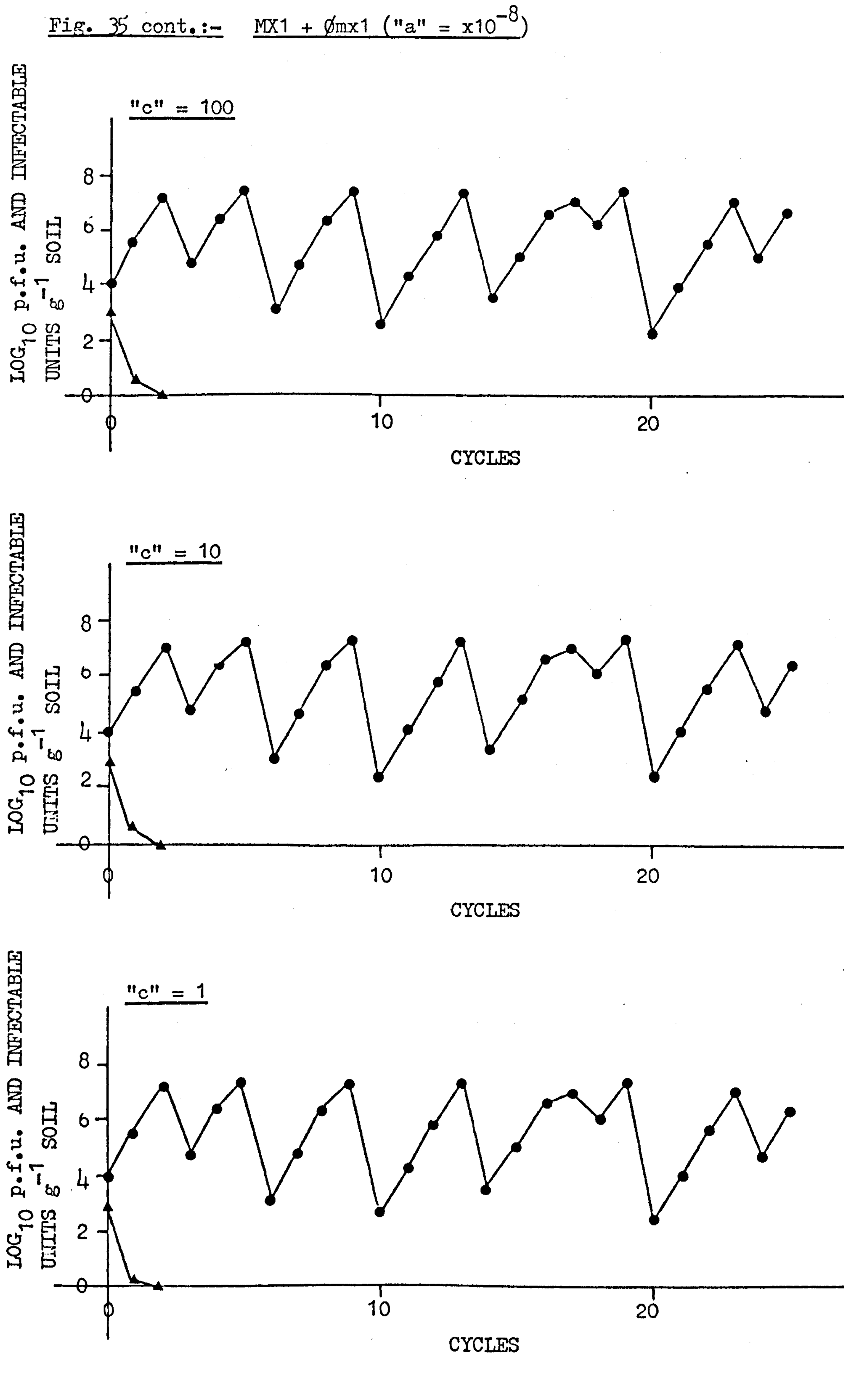
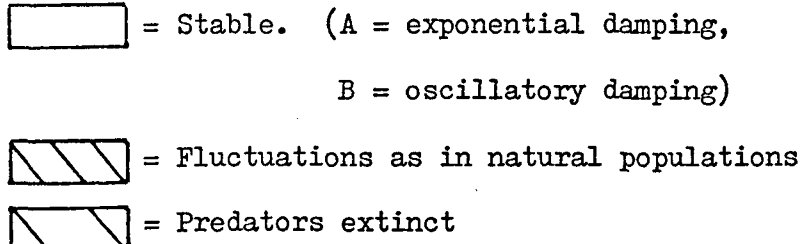


Fig. 35 cont.:- MX1 + Ømx1 ("a" = $x10^{-8}$)





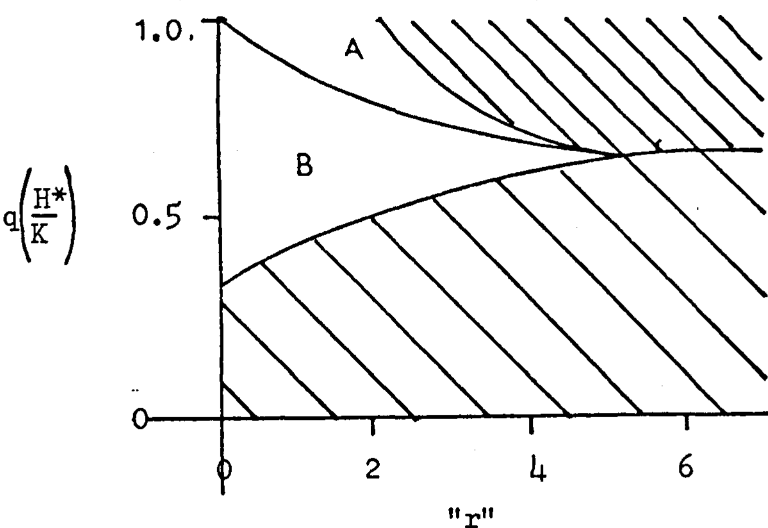


Fig. 36:- Stability boundaries for the original Nicholson-Bailey model

(After Beddington, Free and Lawton, 1975)

INFECTION EFFICIENCY RANGE	STABILITY TYPE OBSERVED
"c" = 100 -7×10^{-9}	Increasing oscillations
$6 \times 10^{-9} - 5 \times 10^{-9}$	Oscillatory damping
4×10^{-9}	Exponential damping
3×10^{-9} -	Oscillations increase without limit
"c" = 10 $- 7 \times 10^{-8}$	Increasing oscillations
$6 \times 10^{-8} - 5 \times 10^{-8}$	Oscillatory damping
4×10^{-8}	Exponential damping
3×10^{-8} -	Oscillations increase without limit
"c" = 1 $- 7 \times 10^{-7}$	Increasing oscillations
$6 \times 10^{-7} - 5 \times 10^{-7}$	Oscillatory damping
4×10^{-7}	Exponential damping
3×10^{-7} -	Oscillations increase without limit

Table 36:- The effect of the alteration in "c" on the behaviour observed in relation to parameter "a" for the MX8 + Ømx8 system. ("r" = 3)

observed may be slight. Fig 37 presents the stability diagrams obtained for varying "c" and "a". These indicate that there are regions of stability (local) in some cases, but that generally as "a" increases these regions decrease with a corresponding increase in "c". For any one particular "a" value it can been seen that as "c" increases, usually th size of the stable region of the interaction decreases. Biologically this is an obvious result, since as the number of phage produced per infection increases the total number of phage in the population will also increase, leading to an increase in the detrimental effect of the phage on the host, thus there is more likely to be an unstable interaction. The points within the regions of stability are at least locally stable due to their derivation, but it is unlikely that they are globally stable since simulations performed (although limited) which would have fallen into a region of stability, do not. The extent of the local stability in this model in unknown because of the lack of investigation into this aspect of the modelling. May (1978) stated that for his model of insect-parasitoid interaction the local stability properties were identical to those of global stability.

The alteration of the burst size of the phage can have an effect on the outcome of the interaction observed, but only at particular infection efficiencies. If "a" is very small or very large the infection efficiency is the only factor determining the outcome of the interaction, but at intermediate values of "a" the burst size can play a major role in the outcome of the interaction.

g) The introduction of a refuge and its effect on the interaction between streptomycetes and their phage.

Until now the model has assumed that all hosts are equally available in the natural environment (soil) both in time and space. However in the natural environment it is more than likely that not all the hosts

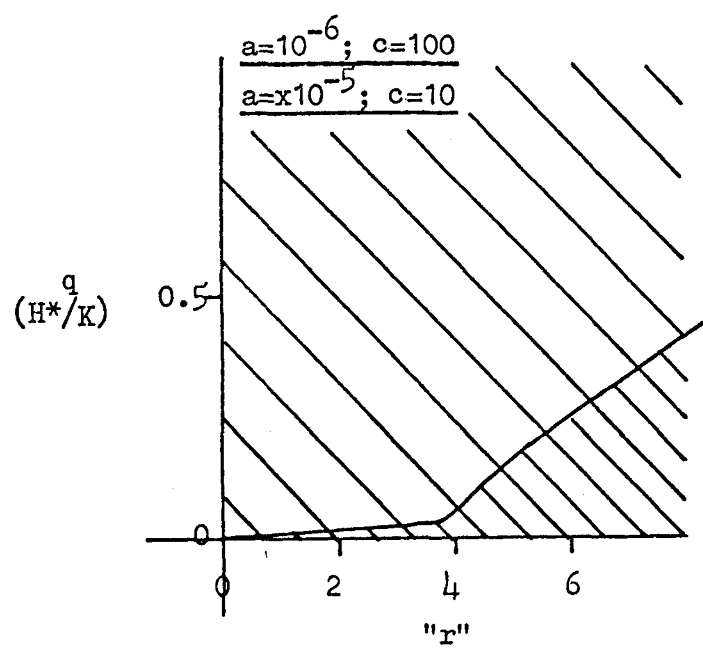
Fig. 37 :- The effect of the alteration in parameters "a" and "c" on the stability boundaries for the MX1 + Ømx1 and MX8 + Ømx8 systems.

= Stable (X= damped oscillations)

= Unstable fluctuations

= Extinction of phage

a) MX8 + Ømx8 system



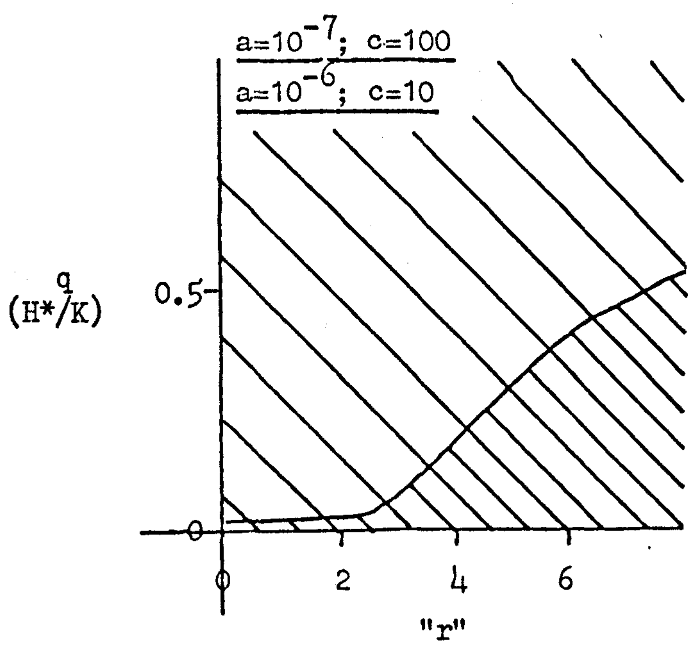


Fig. 37 cont:-

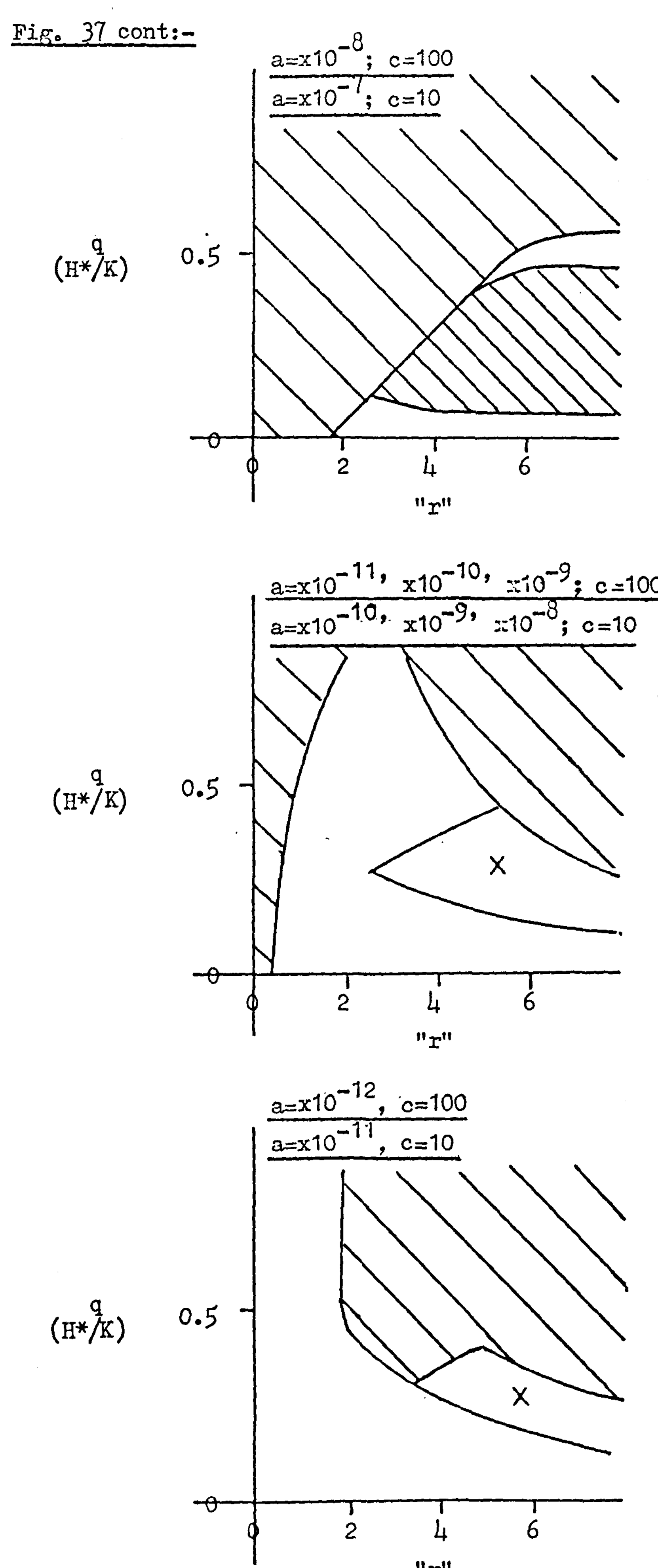


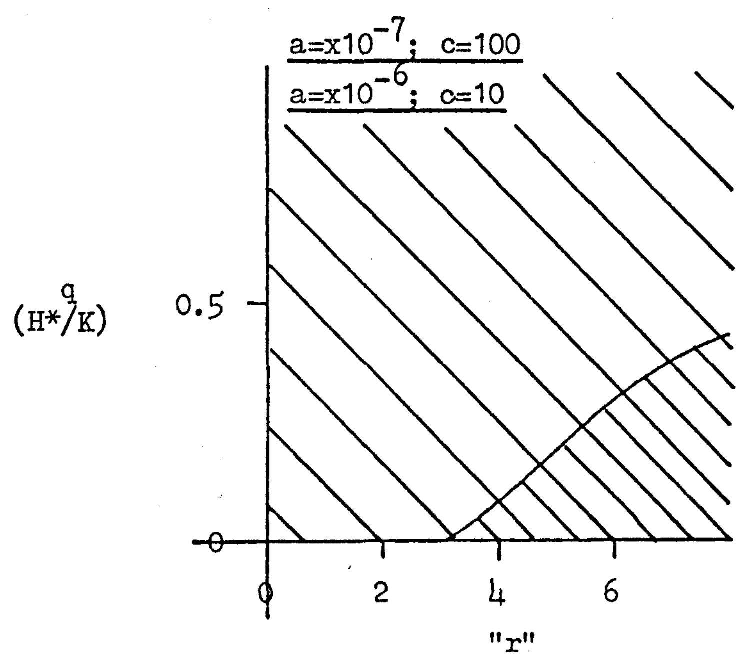
Fig. 37 cont:-

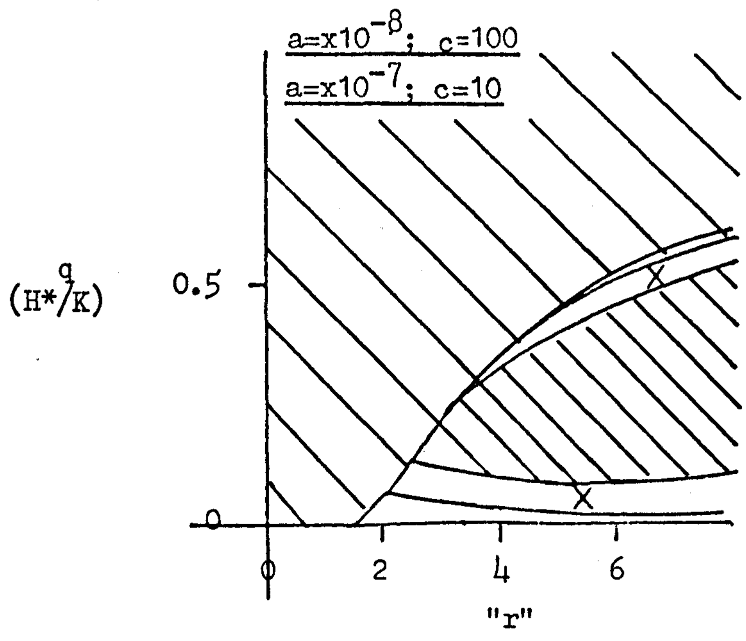
= Stable (X = damped oscillations)

= Unstable fluctuations

= Extinction of phage

b) MX1 + Ømx1 system





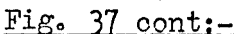
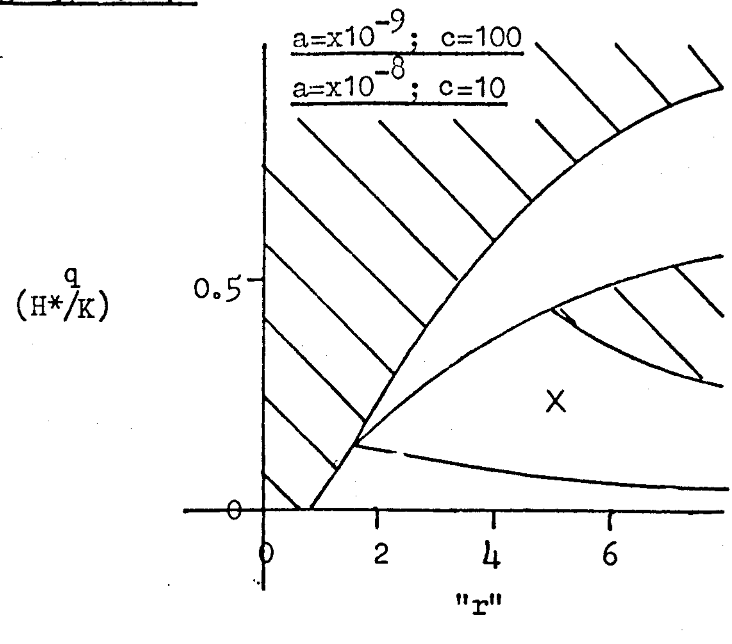
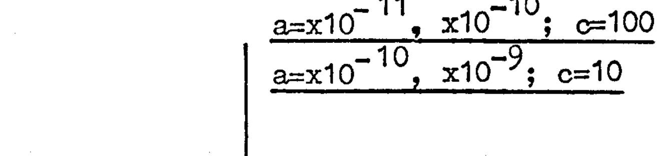
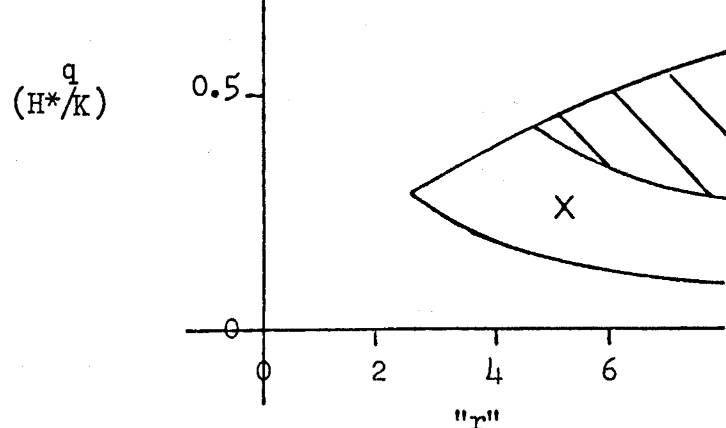
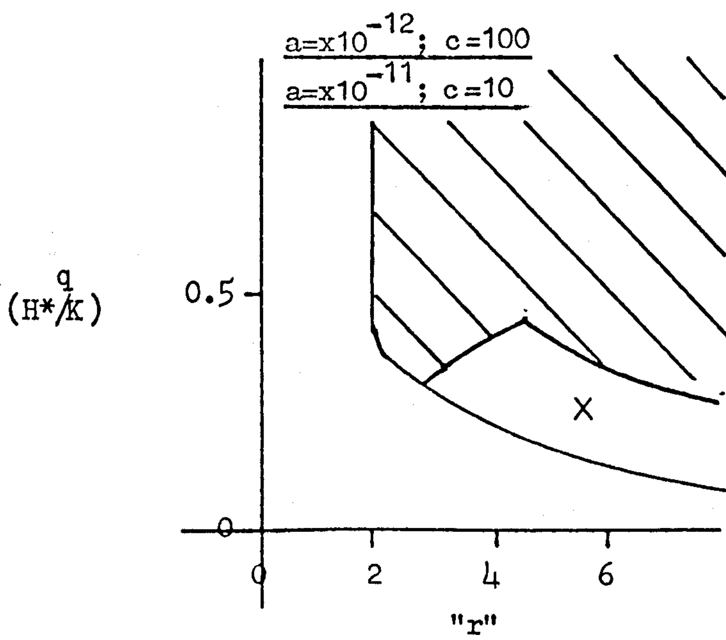


Fig. 37 cont:-


a=x10-9; c=100
a=x10-8; c=10
q
(H*/K)
0.5
0
0
2
4
6
"r"
X


a=x10-11, x10-10; c=100
a=x10-10, x10-9; c=10


q
(H*/K)
0.5
0
0
2
4
6
"r"
X


a=x10-12; c=100
a=x10-11; c=10
q
(H*/K)
0.5
0
0
2
4
6
"r"
X

are equally easy to infect either because they have some sort of defensive mechanism, e.g. lysogeny or because they occupy situations which are inaccessible to phage. This then means that some hosts will have a refuge from the phage. A state of temporal refuge will exist if the host is in its infectable state at a time when the phage is not. This event will not occur with phage as they are always capable of infecting hosts. Streptomycetes are uninfectable in the spore stage of their life cycle. However the parameter "s" deals with the proportion of spores which do not germinate and if they remain as spores they are not in a refuge as the nature of a refuge is such that it implies that a host will be able to grow and propagate without the danger of infection from the phage involved. The application of refuges in this system are when the streptomycete is either unavailable to the phage by way of its position, or it is in a lysogenic association which makes it resistant to infection by a similar phage to that with which it is associated. Distribution patterns have been studied for streptomycetes (Mayfield, 1969) and shown to be heterogeneous, but there is little information on the distribution of phage. However it is not improbable that a heterogeneous distribution of phage also occurs. Refuges may also take the form of environments which although allowing the streptomycete growth are unfavourable to adsorption, infection or to the phage themselves.

Refuges can take a variety of forms, e.g. aggregation indices, but are initially of two very basic forms:-

1) Partial refuge - these are a result of the tendency of ill effects to be aggregated, and are usually used for the presentation of spatial heterogeneity effects. Parasites which can control their own movement tend to remain in an area of densely packed prey, and

therefore those in more sparsely packed areas can more easily escape attack. These are usually presented in the form of aggregation indices and were first proposed by May (1978), who used the negative binomial tó present aggregation.

11) Total refuge - in these refuges all the hosts in the refuge are protected from infection.

In this work only the total refuge has been investigated, althcugh partial refuges may prove to be important when further and supportive work at different host and phage densities has been completed. However Hassell (1978) stated that

> 'a special case of non-randon search, akin to predator aggregation, is where some prey are free from predation within a spatial or temporal refuge, and that aggregation provides a partial refuge not dissimilar to that of a total refuge.'

Therefore this will give some indication as to whether spatial differences can lead to stability.

Two types of total refuge have been used in this work:-

1) Constant proportion refuge

$$H_{t+1} = s((1-\gamma)H_t \exp(r(1-(H_t/K))) + H_t\gamma\exp(r(1-(H_t/K))-aP_t))$$

$$P_{t+1} = zc\gamma H_t(1- \exp(-aP_t)))$$

where γ = the proportion of host available to the phage

11) Constant number refuge

$$H_{t+1} = s(H_o \exp(r(1-H_t/K)) + (H_t-H_o) \exp (r(1-H_t/K)-aP_t))$$

$$P_{t+1} = zc((H_t-H_o)(1-\exp(-aP_t)))$$

where H_o = the number of hosts protected in the refuge

Murdoch & Oaten (1975) stated that it is difficult to find systems which approximate to the assumption of a fixed fraction in a refuge. If one class were invulnerable and that class formed a constant

proportion, then this would be mathematically the same as a constant proportion refuge. However Hassell (1978) stated that a constant proportion refuge appears to be the most common, at least in the insect-parasite world. As no prior information was available on streptomycete-phage systems it was decided to investigate both types of refuge to observe their effect on the interaction.

Fig 38 shows a selection of the nature of the interactions found by simulation with the operation of a constant proportion refuge at two infection efficiencies (one relating to that observed in the soil system and "a" = $x10^{-8}$, a significant value for reasons previously explained). Table 37 presents the nature of the interactions over the whole range of refuge sizes investigated. When "a" = $x10^{-8}$ (that at which a previously sustained but irregular oscillation was observed for the MX8 + Ømx8 system) no alteration in the outcome was observed for the MX1 + Ømx1 system, only an extension of the interaction. However the MX8 + Ømx8 system did show some alteration in the interaction observed. Below γ = 0.8 the phage population is lost although the length of the interaction is extended. When 90% of the host infectable units are available for infection, sustained irregular oscillations are again observed, indicating that the number of hosts and phage taking part in the interaction plays a role in the final interaction. Thus, at this particular "a" the inclusion of a constant proportion refuge cannot turn an unstable interaction into a stable one. When the infection efficiencies obtained from the soil are used in each system ("a" = $x10^{-5}$), then a much more varied behaviour is seen. Table 38 presents the interactions observed and Fig 39 a selection of the simulations. For the MX1 + Ømx1 system, irregular but sustained oscillations were observed until 60% of the host population was available for infection; when between 70% & 82.5% of the population were

Fig. 38:- The effect of the introduction of a constant proportion refuge on the MX8 + Ømx8 and MX1 + Ømx1 systems with "a" = x10^{-8}

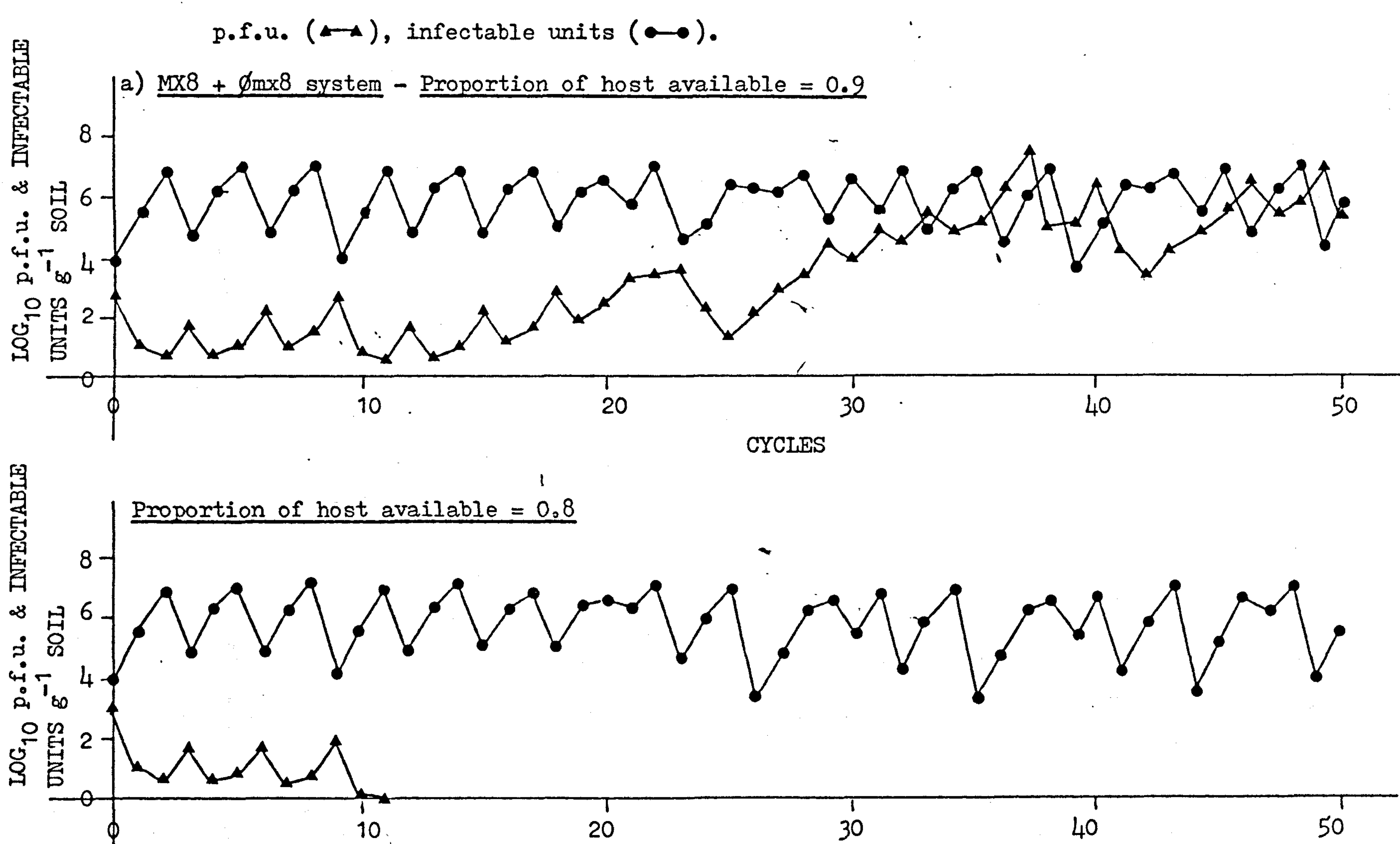
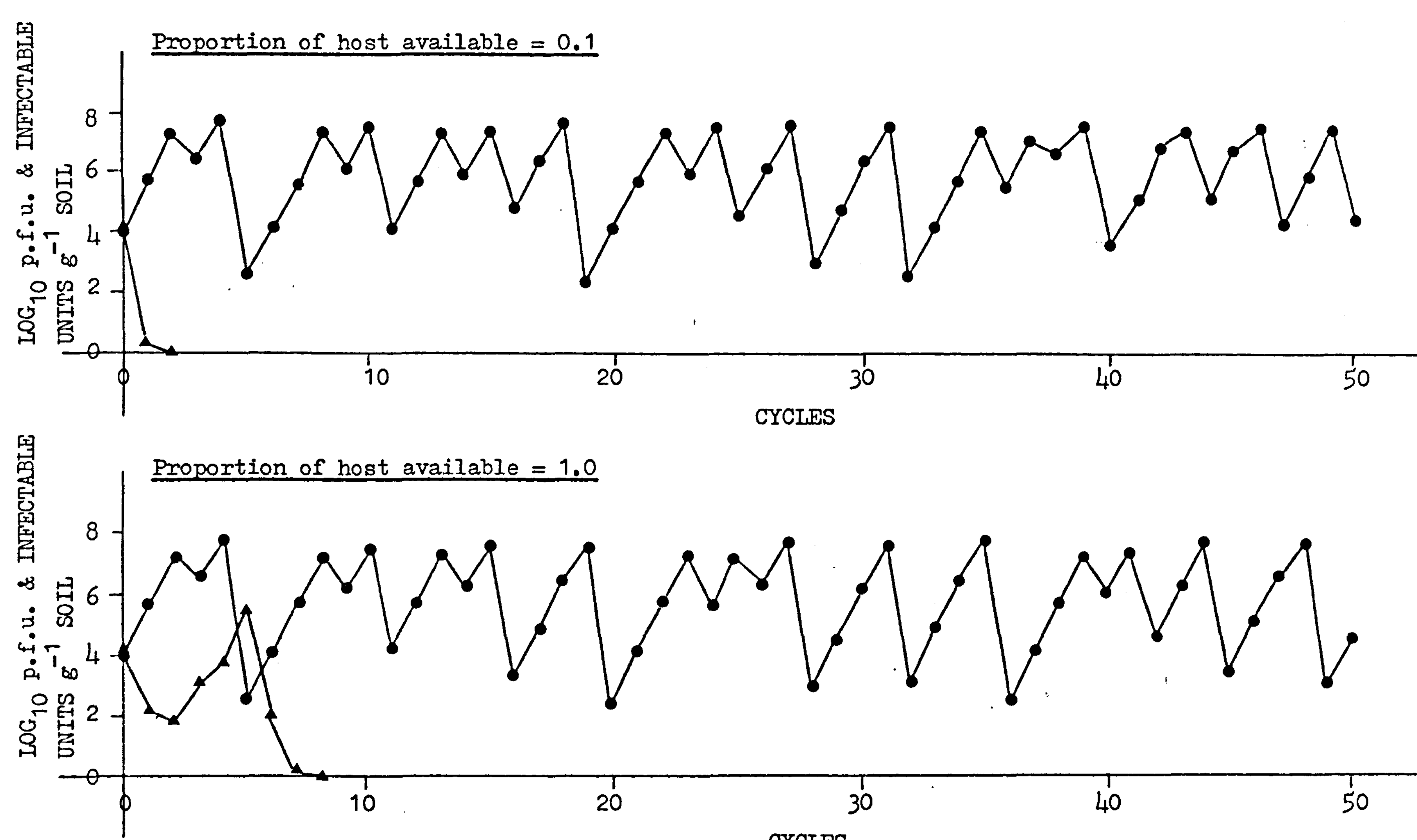


Fig. 38 cont.:-

b) MX1 + Ømx1 system



PROPORTION OF HOST TO REFUGE	DYNAMIC BEHAVIOUR OBSERVED
a) MX8 + Ømx8	
0.1 - 0.8	Phage lost, host continues to cycle
0.9 - 1.0	Sustained oscillations
b) MX1 + Ømx1	
0.1 - 1.0	Phage lost, host continues to cycle

Table 37:- The behaviour observed with the introduction of a constant number refuge at "a" = $x10^{-8}$

PROPORTION OF HOST TO REFUGE	DYNAMICAL BEHAVIOUR OBSERVED
a) MX8 + ∅mx8	
0 - 0.60	Irregular oscillations
0.60 - 0.75	Bifurcating cycles
0.75 - 0.97	Stable equilibrium point
0.98 - 0.99	Bifurcating cycles
1.00	Chaos
b) MX1 + ∅mx1	
0 - 0.7	Irregular oscillations
0.725 - 0.80	Bifurcating cycles
0.825 - 0.975	Stable equilibrium point
0.98 - 0.99	Bifurcating cycles
1.00	Chaos

Table 38:- The behaviour observed with the introduction of a constant proportion refuge at "a" = $x10^{-5}$

Fig. 39:- The effect of the introduction of a constant proportion refuge on the MX8 + Ømx8 and MX1 + Ømx1 systems with "a" = x10^{-5}

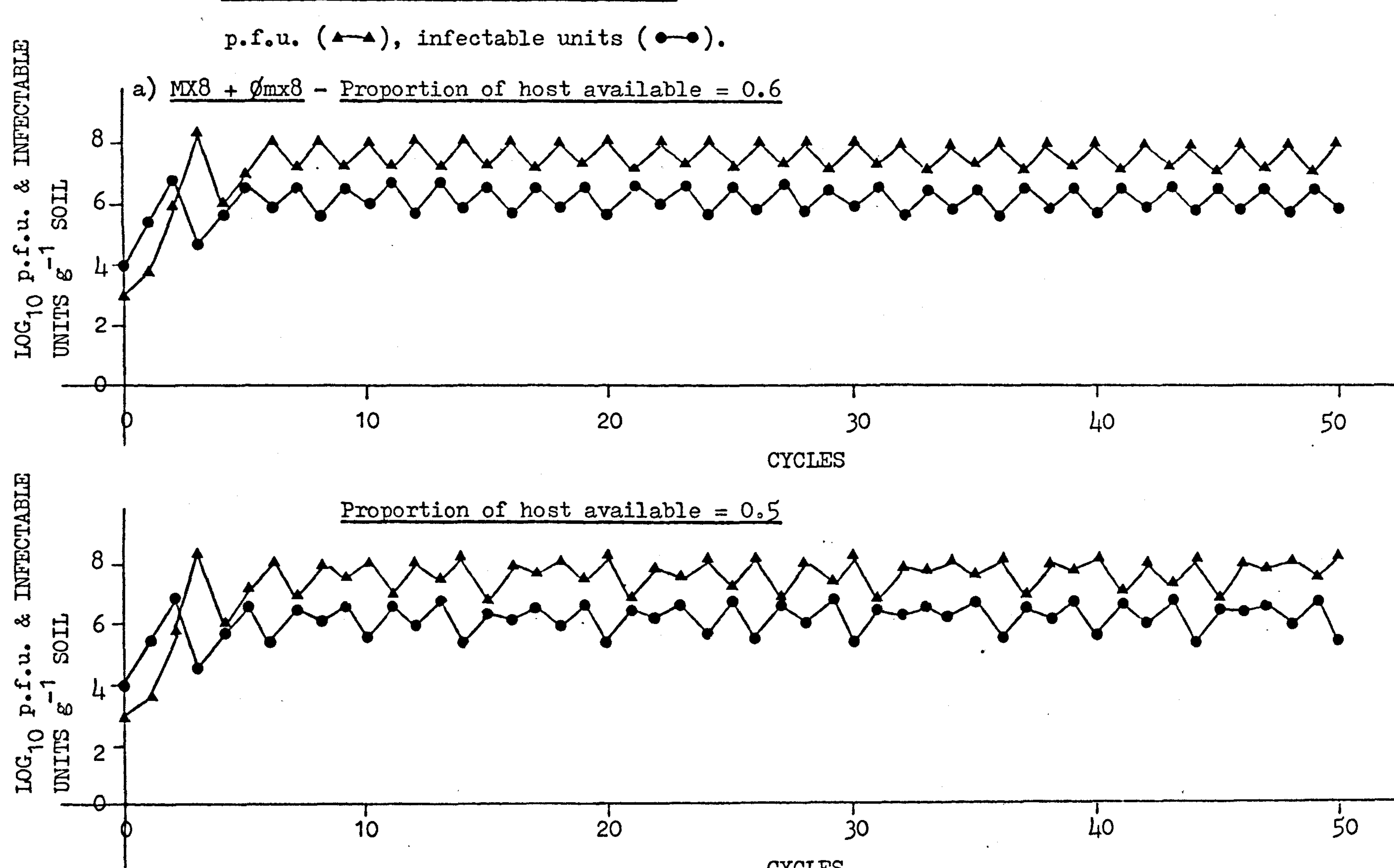


Fig. 39 cont.:-

Proportion of host available = 0.8

LOG$_{10}$ p.f.u. & INFECTABLE UNITS g^{-1} SOIL

0 2 4 6 8

0 10 20 30 40 50

CYCLES

Proportion of host available = 0.7

LOG$_{10}$ p.f.u. & INFECTABLE UNITS g^{-1} SOIL

0 2 4 6 8

0 10 20 30 40 50

CYCLES

Fig 39 cont.:-

b) MX1 + Ømx1 system

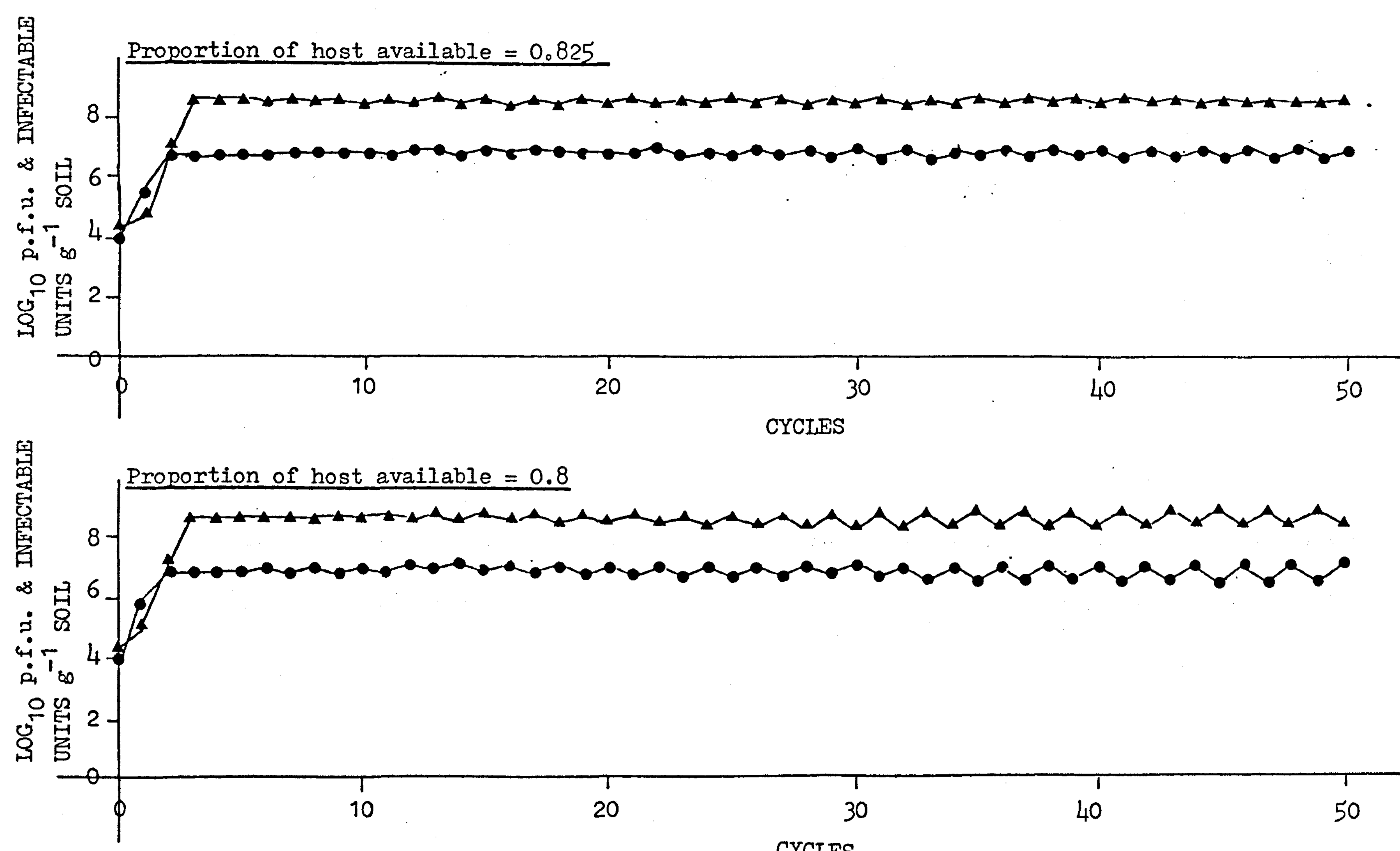


Fig. 39 cont.:-

Proportion of host available = 0.99

LOG$_{10}$ p.f.u. & INFECTABLE UNITS g^{-1} SOIL

0 2 4 6 8

0 10 20 30 40 50

CYCLES

Proportion of host available = 0.97

LOG$_{10}$ p.f.u. & INFECTABLE UNITS g^{-1} SOIL

0 2 4 6 8

0 10 20 30 40 50

CYCLES

available, oscillations of the host and phage were apparent with more phage than infectable hosts being available. When the proportion of hosts available i.e. equal to 98/99% of the host population, damped oscillations were observed. When all of the host population is available, the normal chaotic situation was found.

Changes of behaviour were also observed for the MX8 + Ømx8 system. For $\gamma = 0.7$ a slight variance in the oscillations was observed (Fig 39) however when $0.7 > \gamma < 0.6$ they were regular bifurcating oscillations. Between 0.75 & 0.97 a stable equilibrium point behaviour was observed. Above $\gamma = 0.97$ the cycles were still regular but of longer period, and at $\gamma = 1$ chaos resulted. The phage numbers were always greater than those of the host.

It was therefore possible to observe sustained and stable interaction in both of the streptomycete-phage systems investigated. If a constant proportion refuge was operating within a soil system, sustained co-existence of the two interacting organisms could occur. The refuge would be able to offset the naturally detrimental effects of the phage by replacing the host units which were lost through infection with units which had been produced in the refuge.

The constant number refuge was investigated, again at two different infection efficiencies. At "a" = $x10^{-8}$ the refuge did little to stabilize the interaction observed and also proved difficult to operate, i.e. there was an upper limit to the number of hosts which could be in the refuge due to the density-dependent parameter governing the host growth and production of infectable units. For the MX1 + Ømx1 system, a chaotic interaction was always observed, and for the MX8 + Ømx8 system sustained but irregular oscillations occurred regardless of the ratio of host/refuge. At values of "a" obtained from the soil experiment it was impossible to obtain 'sensible' simulations, as in several cycles

the number of hosts assigned to the refuge was greater than the number of hosts in the whole system. There were then two factors involved in the instability of the constant number refuge:-

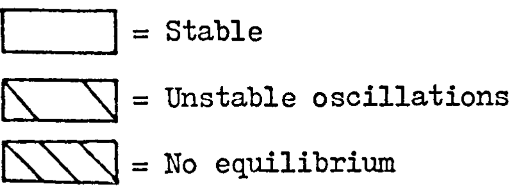
1) The size of the host population assigned to the refuge.

2) The value of the infection efficiency.

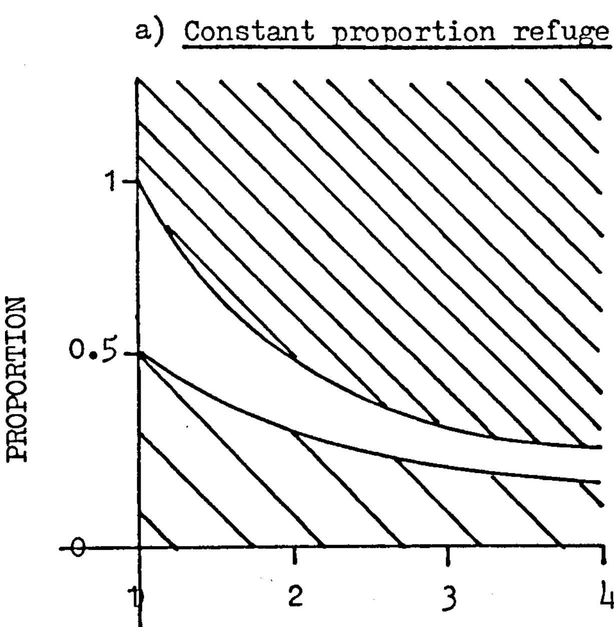
Therefore, the introduction of a refuge of a constant proportion nature leads to a stabilizing of the interaction, but the constant number refuge does not. However, in work which was based on the introduction of these refuges to the simplest version of the Nicholson-Bailey model, when there is no density-dependent parameter, the fixed proportion refuge was shown to be less potent than the constant number refuge. The stability diagrams for these two refuges are shown in Fig 40, and were observed by Hassell & May (1973). They indicate that the introduction of any refuge can confer a large degree of stability to a system. The constant number refuge shows a greater stability area because in the fixed number refuge, the proportion of hosts protected increases as the density of host decreases, whereas in the fixed proportion refuge it remains constant. Hassell (1978), however, stated that

> 'the quantitative effects of refuges can depend sensitively on the model used.'

Bailey et al. (1962) investigated the effects caused when some individuals were more difficult to find than others by altering the value assigned to "a". When "a" = 0 it was the equivalent of a refuge. They showed that this could lead to stability but only if the magnitude of the inaccessible fraction lay within narrow limits, related to the power of increase of the host, otherwise the system was unstable. A refuge is directly related to the ability of a host to escape phage attack and would have no affect upon the imposition of a maximum limit to which a host population can increase, although this limited may vary



a) Constant proportion refuge



b) Constant number refuge

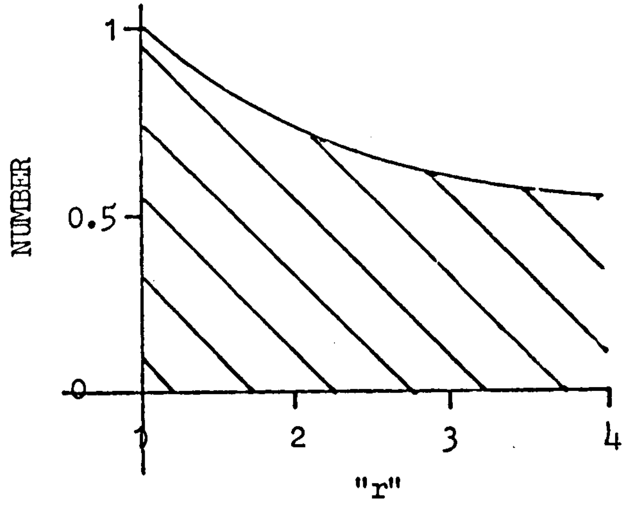


Fig. 40:- The effect of the introduction of a) constant proportion refuge and b) constant number refuge on the stability boundaries (After Hassell & May, 1973)

if a temporal refuge is studied. In this work the inclusion of this density-dependent parameter seems to make the constant proportion refuge more stable than the constant number refuge (the reversal to when "K" is involved), at least at the points examined here. The inclusion of parameter "K" and the values adopted for "r" mean that the host is exhibiting oscillatory behaviour and therefore it is likely that unless a strict limit is kept on the proportion of hosts to the refuge, phage will be lost because no hosts will be available for infection since they will all be in the refuge.

Therefore the effect of the introduction of a total refuge for the host infectable units and spores from the phage has been investigated. The refuge may take many forms. It may be that of spatial differences in the distribution of the host and phage, or conditions which are unamenable to phage adsorption to the strepto-mycete and subsequent infection. Alternatively it may be that of lysogeny, where a host is still in close proximity to the phage but it is unavailable if the phage are of a similar type to that with which it has made lysogenic association. It has been shown that the inclusion of a density-dependent parameter operating on streptomycete growth can render a constant number refuge less stable than that of a constant proportion refuge. It was possible to obtain sustained and stable interactions at infection efficiencies which were previously those of maximal chaos (loss of both the phage and the host). The extent to which either a total number or constant proportion refuge may operate in the soil is unknown, and therefore this section was a hypothetical exploration into this area of modelling. More background information which would help to indicate whether indeed either of these refuges operate in a natural environment is required.

IV) CONCLUSION

The Nicholson-Bailey model has been used to investigate the streptomycete-phage interaction. The model was adapted to include two additional parameters, "s" and "z" which dealt specifically with particular aspects of streptomycete and phage behaviour in soil, i.e. the germination of only a proportion of the streptomycete spores produced from the previous propagation cycle and phage survival. The role of each parameter in the model together with the introduction of refuges, both that of a constant proportion and a constant number refuges, on the outcome of the interaction was examined.

CHAPTER 5

GENERAL DISCUSSION

The interaction of two streptomycetes and their phage has been examined with the use of an adapted Nicholson-Bailey model for insect-parasitoid interactions. With data obtained from both broth and soil experiments the predicted outcome of the interaction in the soil was unstable. This could be termed "chaotic", a term coined by J. A. Yorke (May, 1974) which describes a situation where no matter how long the time of interaction is prolonged there is never a repeated oscillatory pattern. This outcome is obviously unreasonable. If the model applied to a soil predicts a total loss of phage and host, this would suggest that whenever phage and streptomycetes are together the extinction of both would be the result, and therefore one would never expect to find both in the same soil. It is obvious that the interaction of streptomycetes and their phages may have a profound effect on the population numbers of both. However the total elimination would not be the overall expected conclusion, although local interactions leading to the elimination of growing streptomycetes may be expected.

This exemplifies one of the main problems associated with this model. It assumes that all parameters excepting host and phage numbers are constant, and so assumes a constant and homogeneous environment. The soil as an environment is definitely not homogeneous. It is by its very nature a dynamic environment in which conditions are in a state of continual change, promoted by either microbial activities or physical and chemical reactions. Even if it were constant for long periods the nature of the soil would not be homogeneous. McClaren & Skujins (1968) described the soil as a matrix of colloidal systems, with the inert solid particles being complexes of minerals and organic polymeric substances (humus) within which are dispersed organic molecules, inorganic salts and ions, water and gases.

Thus the soil is a heterogeneous mixture of solids, liquids and gaseous components. It is therefore unrealistic to apply a model, and in particular such a simple one as this to a whole environment. It should be more appropriately applied to microenvironments. Differences between microenvironments are not difficult to envisage, e.g. pH, soil colloid concentration, microbial densities, and therefore it follows that the parameters within a model will not be constant between microenvironments, or even within one microenvironment over a protracted time period. Modelling allows these changes to be made if they are reasonable, and therefore allows for the exploration of hypothetical situations in the soil to be investigated. It may also indicate the important factors within an interaction and the changes which need to be affected to make the model more appropriate to the system being investigated.

This was the basis of the investigation undertaken here. In the case of burst size there was data to indicate that this could change within the range of temperatures found in some soils. The infection efficiency can also be justifiably changed, at least over a limited range as it was found not to be constant for a soil of identical source in different experiments. However the other parameters, "K", "r" & "s" were arbitarily changed as no quantitative information was available with respect to their probable alteration within a soil system.

The role of each individual parameter was more difficult to assess in order of their effectiveness; local stability diagrams can indicate their role in the case of particular host and phage equilibrium values, but their reliability for global stability effects is questionable. Simulations can indicate the importance of parameters if they alter the interaction dramatically by changing the nature of the interaction; small changes in the oscillatory behaviour are difficult to quantify because of the irregular nature of the host oscillations. The extent

of the interaction afforded some measure of the effect of adding phage to a streptomycete system because the longer a phage interacts with the streptomycete the more likely it is to have an affect on its behaviour.

For the two streptomycete-phage systems investigated, it was observed that one of the most important factors in the interaction was the value adopted for the infection efficiency. This was only to be expected as it describes the degree of the interaction occurring. If a phage is unable to make sufficient interactions no matter what the other parameters are the phage will be lost from the system. If it performs its task too well it will still be lost from the system as it will eliminate all the available host and therefore will become 'redundant' (lost from the model system). However the stability diagrams indicate a range of infection efficiencies where the other parameters can alter the nature of the interaction at equilibrium densities. In particular the burst size then appears to be important in determining the outcome of the interaction. The burst size can, therefore play an important role in the outcome of the interaction in both of the streptomycete-phage systems but only over a limited "a" range. The local stability of the two streptomycete-phage systems was limited and therefore indicates that at particular population densities, the starting concentrations can be important.

Therefore initial population densities can play an important role in the outcome in that if they differ significantly from that of the steady state they will result in chaos. Once different from the equilibrium values they cannot alter the nature of the interaction, only the extent of it. The proportion of phage which was available (parameter "z") to produce a sustained but irregular oscillation was important, and this is related to the effect of the alteration of "a" and "c" as all affect the number of phage which will be available to the

next cycle of the interaction. It would take a very significant effect to produce a stable interaction because the host oscillates irregularly due to the value of the intrinsic rate of increase. No alteration in the carrying capacity can alter the nature of the interaction, i.e. from stable to unstable. It can, however, be a factor in deciding whether the two interacting organisms would be lost in relation to the infection efficiency.

One effect which produced the significant alteration in the interaction was that of the introduction of a refuge. It was shown to lead to all types of interactions from chaos to stable equilibrium point behaviour. The inclusion of a density-dependent parameter ("K") for streptomycete growth resulted in the effect of the constant proportion refuge appearing to be more potent than the constant number refuge, at the densities and "a" values utilized. This is the reverse of that observed by other authors when no density dependent parameter was present. As already stated, whether these refuges do occur in the soil is unknown but it is likely that they may do so in the form of lysogenic associations, evolution of the host which results in the alteration of adsorption sites, or as a result of physiological changes in the host, or even the host being in a position which is physically separated from the phage, or that the ionic environment may prevent adsorption and thus prevent infection. Further work could investigate these options in detail to find how these possible refuges may operate numerically. Lysogeny could serve not only as a refuge for the host but also as one for the phage when adverse conditions prevail, and this area would be a prime target for subsequent investigation. Although it is difficult to prove that a phage exists in soil as a prophage, it has been suggested by many authors that this may be the predominant phage state within the soil (e.g. Reanney & Akermann, 1982). Several lysogenic streptomycetes have been isolated from soil, (Ogata et al., 1981; Dowding & Hopwood, 1973). The

introduction of a refuge can widen the application of a model from that of microsites to larger volumes especially if the refuge was representing spatial separation of the phage and host.

As already stated the Nicholson-Bailey model is only simple and will therefore include some inherent assumptions which cannot adequately describe the situation in the soil. Hassell & Commins (1978) stated that the Nicholson-Bailey model was an extreme situation where the population is specific to and synchronized with its prey. The first of these assumptions is that the outcome of search/infection is described by a single parameter which is the result of a type I functional response described by Holling (1959), and is a straight line relationship between host density and the number of hosts infected. This parameter was stated by Rogers (1972) to be the only limiting factor on the parasites/phage. Anderson & May (1978) stated that a constant attack rate is a destabilizing feature in a model as it prevents the phage/predator being differentially effective at high prey/host densities. It is unlikely that "a" is unaffected by changes in the host density, as shown in Chapter 2, section VII, and therefore this is one of the main constraints within this model. Bailey et al. (1962) examined the effects which alterations in "a" can have upon the stability observed. They showed that when some hosts are more difficult to find than others, this leads to stability, and that the likelihood of stability increased when hosts for which the parasites had the lowest "a"s were most numerous, the higher "a" categories becoming progressively and rapidly less numerous. If "a" follows a normal distribution the populations were always unstable. Others dealing with the effects of densities on "a" have been DeBach & Smith (1947) and Hassell (1978). Hassell (1978) presented equations which dealt with the inclusion of both type I and type II functional responses. Type II responses include a handling time which is the time required to process

a prey before it can go on to form another infection. This does not apply here as the phage once having made an infection cannot go on to make another. A type III response can be reflected if the phage switches hosts for which a different "a" is observed. The functional response cannot readily be defined as either a type I, II or III response, due to the presence of insufficient data to positively demonstrate the response type. However the infection efficiencies were not constant within the host:phage ratios studied, and therefore the result is not always a constant "a" as is assumed in the Nicholson-Bailey model. The precise functional response may be determined only if further investigations are performed. The infection efficiency may be limited by the number of phage present as, unlike parasitoids, a phage does not inject is DNA and then continue to perform another infection. This limitation will therefore be seen when there are high host densities in excess of the phage population. The representation of the number of attacks by the Poisson distribution involves the assumption that a host could be attacked more than once, because of the definition of the "a" the host can be superinfected by the phages involved. If the calculation of "a" had been based on the total number of phage adsorped in both states then this would include the phage which adsorb to a host which has already been infected which can occur. However, as the number of phage totally or irreversibly adsorbed were similar in both of the streptomycete-phage systems, this would have little effect on the outcome of the interactions.

Other assumptions inherent in the model are that the intrinsic rate of increase is constant with every cycle of the interaction. This means that once a streptomycete has completed a full cycle in a particular environment, this same environment will immediately return to its former state and be ready to support spore germination. The representation of

streptomycete growth as exponential (e^r) means that the number of mycelial tips, germ tubes and spores will increase exponentially. Allan & Prosser (1983) investigated the early filamentous growth of S. coelicolor. They found that the number of mycelial tips increased exponentially. However, the assumption that all stages of the streptomycete growth cycle increase exponentially requires further investigation in a non-limiting environment. A constant carrying capacity assumes that at the beginning of each cycle, an environment will be identical to that at the start of the previous cycle. Slight changes in the nutrient status of an environment could result in the alteration of the carrying capacity. The model is based on the interaction of the two organisms; it therefore, assumes that if a phage does not make a successful infection it is lost from the system. It does not account for the possibility that even though a phage did not make a successful infection in one cycle, it may still be available to the next cycle. Therefore the model underestimates the phage which are available to the next cycle. Similarly it assumes that when all the hosts which are in an infectable state have been lysed, they are completely lost. No allowance is made for the subsequent germination of those spores which had not previously germinated but where still capable of doing so, or for any pulsed input of hosts to the system.

The adapted Nicholson-Bailey model represents only the simple interaction of streptomycetes and phage. It does not include any of the effects of the environmental factors (apart from phage loss) affecting the interaction. Also it has several inherent assumptions derived from its simplistic nature, which are not truly representative of the streptomycete-phage system, or indeed of the insect-parasitoid system for which the model was originally derived. Recognition of these limitations is the first step towards correcting them. However,

when modelling any system it is essential to present a simple, general model which represents the interaction which occurs in an environment in the simplest conceivable situation. Subsequent adaptations of the model can be made to account for the situations in the environment and the additional influences which can affect the population, thereby increasing its relevance to the interactions modelled. Sometimes many distinctive models are required to encompass and determine the effect of environmental factors. These may be needed to take account of the reactions of different species, microenvironments and a wide range of environmental factors.

As previously mentioned, the result of the interaction based upon data for streptomycetes and their phage from both broth and soil experimental systems is chaos. Alteration of "a" can lead to sustained but irregular oscillations of the host and phage indicating that at the simplest form of interaction both could persist together. But what other factors are there occurring in the natural soil environment which might lead to the stabilization of a seemingly generally unstable interaction within an environment in which the phage is able to eliminate the streptomycete?

A complete analysis of factors influencing the interaction should include the effects of spatial heterogeneity, varied hosts (switching), altered "a" and altered host susceptibility. What then is the effect on the modelled streptomycete-phage interaction of the introduction of these variables that could occur in the natural environment? Will they lead to the stabilization of the interaction or do they have no real effect on the outcome? Murdoch & Oaten (1975) stated that

> 'it is an almost a tautology that the predator-prey systems that are seen are those that are in a sense, stable since they have avoided extinction.'

Therefore the existence of streptomycetes and phage in a single soil

indicates that some sort of stability exists, and that there are some mechanisms which protect the streptomycetes. That the streptomycetes exist in the soil indicates they can 'avoid' the large detrimental effects of the phage.

There is much literature available on applications of the insect-parasitoid model due to the ease of studying these organisms. Some of the changes applied to the model cannot be applied to the streptomycete-phage system as parasitoids actively search and move whereas the former system is based totally on random encounters. Bearing this in mind and considering factors not included in the model there are several opportunities for further development of the model to simulate more realistically the situation in the soil environment.

The spatial distribution of the streptomycetes and phage may have an influence in two ways:-

i) The streptomycete is physically separated from the phage and hence is in a refuge.

ii) The spatial heterogeneity may lead to variation in the densities of both phage and host and therefore to an alteration in "a" within microenvironments.

Spatial heterogeneity can only be achieved by the passive movement of the phage and host and this may depend on several factors. Streptomycetes may be distributed by the movement of spores by attachment to soil arthropods (Mayfield et al., 1972), mixing of soil by soil animals or by the very nature of streptomycete growth leading to its re-distribution. The percolation of rainwater has been shown to result in the movement of viruses through soil columns, the movement being dependent on several variables, e.g. soil composition, virus type, frequency and amount of rainfall (Sobsey et al., 1980; Funderberg et al., 1981).
Several authors have investigated the effects and representation of

spatial heterogeneities of the insect parasitoids and their hosts (Griffiths & Holling, 1969; Rogers, 1972; Hassell & May, 1974; May, 1978). The simplest form of representation used was that of the negative binomial which included a clumping coefficient. However these models were based on non-random search with the predators spending more time in the densely populated areas. In the streptomycete-phage system the phage is unlikely to spend any more time in an area more densely populated with streptomycetes than in a less densely populated area due to its inability to actively move. Therefore, it appears that "a" can be altered by the effects of spatial heterogeneity, as "a" has previously been shown to be affected by low host densities for at least one phage concentration. If the host is completely separated from the phage, this will result in the occurrence of a refuge. Therefore, although heterogeneous distributions do occur in soil it is unlikely that they will be effective in the same way as in the insect-parasitoid system. However, some of the adaptations, e.g. representations of spatial heterogeneity, may still be useful for the further development of the model.

Another factor which could lead to the further stabilization of the interaction, but also to further complications, is the phenomenon of differing host susceptibility and its possible influence on phage production. The current model assumes that both germ tubes and mycelial tips are equally susceptible to phage infection and that both will provide numerically the same phage replication cycle. Age has been shown to affect phage production (Ritzi, Bradley & Jones, 1968; Rosner & Gutstein, 1980; Webb et al., 1982), and also phage adsorption and therefore the amount of infections occurring. The physiological condition, which alters with age, has also been shown to affect phage production (Delbrück, 1941; Thompson & Shafia, 1962; Tzagloff & Pratt,

1964; David et al., 1980). Hassell & Anderson (1984) considered host susceptibility as a component of insect-parasitoid systems and concluded that it was another manifestation of heterogeniety. They demonstrated with computer simulations that differential host susceptibility contributed to population stability, and that it was a result of the uneven exploitation of the host. The differential exploitation was due to either temporal asynchrony between the host and parasitoid (unlikely to occur in the streptomycete-phage system), or initially by some hosts being less exposed by virtue of their physical location. The extreme case was when some hosts were completely protected. It was also stated that if parasitoid density increases, the exploitation of susceptible hosts is very heavy and therefore "a" per individual is smaller than at lower parasitoid densities, a result of uneven exploitation.

The model deals with the simple two organisms in interaction, however the situation in the soil is more complicated as there can be more than one susceptible host per phage and even more than one type of phage per susceptible host. The effect of switching would be felt if the replication cycle parameters of a particular phage with different hosts differed to any great extent and may also result in an altered infection efficiency. The existence of another host may relieve the pressure of a phage on a particular host so allowing it to recover from any attacks made upon it. As previously stated, a lytic phage has a broader host range than temperate phage. If one host type was being attacked by a phage it may allow another host, relieved of the pressure of phage attack, to grow and act as an alternative host for when the initial host is reduced to low levels, thus allowing it to replenish its population.

It can be seen that the heterogeneous nature of soil can lead to the stabilizing of the streptomycete-phage system, many of the effects being

able to be classified as refuges. The spatial heterogeneous distribution of streptomycetes and phage, the occurrence of lysogeny, polyvalency and variations in host susceptibility will all lead to some degree of stability and co-existence of the streptomycete and phage in a soil. However, the chaotic interactions observed in the application of the simple model may still occur at individual sites, and these together with other interactions may contribute to the overall stability of the interaction, and therefore explain the coexistence of the streptomycete and phage populations in a soil.

The modified Nicholson-Bailey utilized here can therefore describe the interaction of streptomycetes and their phage in a soil system although it does have several limitations. However, other models have been used to simulate and describe the interaction of bacteria and their phage (Campbell, 1961; Paynter & Bungay, 1969; Levin et al., 1977; Gaspar et al., 1979). All proposed models are based on the interaction of unicellular bacteria and their phage, usually in batch culture. They were presented in differential equation form, and were very specific and rigid. Gaspar et al., (1979) dealt with E. coli + phage T2 & M2 in a batch culture. The model was utilized to determine individual parameters by comparing the resulting interaction of the model with that produced experimentally. It distinguished adsorption from infection, and was based on a much shorter time period than that used in the adapted Nicholson-Bailey model, justifying the use of differential equations. Levin et al. (1977) again dealt with E. coli but with T2. The model was based upon specific assumptions about the habitat which was a chemostat. These were the use of primary resources, the population growth and the nature of the interaction. They assumed that the encounters occurred randomly and at a frequency jointly proportional to the concentrations of host and phage, assumptions similar to those

in the Nicholson-Bailey model. Campbell (1961) presented a model as differential equations with logistic growth of the host, based on a simple predator-prey interaction. The Nicholson-Bailey model used here was selected for reasons already described. The interaction of streptomycetes and phage takes place over a long time period even under optimal conditions and therefore the application of differential equations are inappropriate. Therefore a very general model which could be adapted and point the way to further investigations and development was required.

An attempt has been made to simulate the interaction between streptomycetes and their phage in soil, with the aid of the adapted Nicholson-Bailey model for insect-parasitoid systems using data for the MX1 + ømx1 and MX8 + ømx8 systems. It has been shown that the phage can affect the host behaviour altering the oscillations, the extent and the nature of the interaction which is chiefly determined by the infection efficiency. It has also been shown that co-existence of the host and phage can initially be achieved by the alteration of the infection efficiency, at least for the MX8 + ømx8 system, although the co-existence is unstable. The introduction of a refuge, which can extend the bounds of the model from that of a microenvironment to a wider area/volume, has been shown to stabilize the interaction at high infection efficiencies similar to those obtained for soil systems.

The model was shown to have severe limitations due to its generality but these could be altered to reflect a more realistic system for future work. Other appropriate areas of alteration and investigation have been suggested. The comparison of the model predictions against an experimental system has not yet been performed. This is necessary to validate the model. Therefore this model is only a hypothesis of what may occur in the soil environment and already some alterations needed to reflect the

true situation have been suggested. In addition, more experimental work within the soil or simulated soil systems is required to provide more accurate data on the streptomycete-phage interaction and indeed other host-phage interactions in the natural environment.

CHAPTER 6

CONCLUDING COMMENTS

A study of the nature and extent of the interaction of streptomycetes and their phage in a soil system has been undertaken. Two approaches were made. Firstly the effect on both populations of adding streptomycete spores and a corresponding phage to a natural soil over a protracted period of time was determined. An interaction occurred between the two populations inoculated into the soil and also between them and the indigenous populations of phage and hosts. However this interaction was limited both in time and size. The second and major approach taken was the application of the Nicholson-Bailey model for insect-parasitoid systems to model the streptomycete-phage interaction in a soil environment using data derived from both broth and soil experiments. The model was used to examine, theoretically, the effect of logical alteration of individual parameters on the final outcome of the interaction, over a long period of time. It also provided information on which of the parameters would have the greatest effect on the outcome of the interaction and have the 'ability' to produce either a stable or unstable, sustained interaction.

The conditions which, by simulation, had the greatest control over the type and extent of the interaction were the infection efficiency ("a") which led to unstable but sustained interactions for one of the streptomycete-phage systems, and the introduction of a constant number refuge for the streptomycete host, which led to a sustained and stable interaction between the two organisms. The parameter "c", dealing with the phage production from successful infections, was interrelated to "a" and indeed its effect was dependent on "a" rather than on the value assigned to "c". The other parameters included in the model,

"K" & "r", also affected the outcome of the interaction of the streptomycete-phage system but the effect did not appear to be as great as that caused by the introduction of the refuge and the alteration in the infection efficiency. However, parameter "r" may assume a greater importance if the intrinsic rate of increased varied considerable within a single soil system and between cycles of replication for a particular streptomycete. The parameter "r" is obviously important because it affects the possible number of streptomycete mycelial tips and germ tubes which are available for infection.

The rate and frequency at which the phage progeny are produced in any interaction, is dependent on the size, availability and activity of its susceptible host. Thus the nutrient-poor environment of the soil and its heterogeneity will have an effect on the size of the phage population. Apart from adverse conditions, which may possibly result in the elimination of the phage population, the shortage of exogenous nutrients together with the effect of adverse conditions on the host could lead to a reduction of the fecundity of the streptomycete, reducing its true potential and thus limiting its availability. The availability of the host population to phage is determined not only by "r" but also by the presence of physical barriers within the soil which can result in the physical separation of streptomycete and their phage. This separation effect is enhanced by the concentration of streptomycete growth in localized areas which are usually associated with organic fragments (Mayfield et al., 1972; Ruddick & Williams, 1972).

The reliance of phage upon chance meetings with the streptomycete to form a successful infection could in part contribute to the limited interaction observed. The movement of the phage within the soil system is caused either be diffusion through the soil pore water system or by percolation through the soil possibly in rainwater (Duboise et al., 1978).

Sykes (1977) stated that the limited interaction of streptomycetes and their phages observed was not due to restricted virus mobility, which is observed when particles are introduced into an environment. He cited work by Anderson (1957) and Carlucci & Palmer (1960) who indicated that phage concentrations in aquatic environments were essentially no larger or smaller than those in soil environments. Primrose, Seeley & Logan (1982) quoted data which showed that phage infections occurred very infrequently in aquatic environments, but that the numbers detected suggested that the rate of infection was higher than that theoretically possible. The most likely explanation was that phage infection occurs at interfaces and on surfaces where host numbers are more concentrated. Colloidal surfaces could then serve as a concentrating surface for both the phage and host. Phage have been shown to adsorb to colloidal surfaces and to other soil particles (Marshall, 1974; Duboise et al., 1978; Sobsey et al., 1980) and to retain their infectivity when adsorbed to colloids (Sykes, Lanning & Williams, 1981), as too have streptomycete spores (Ruddick & Williams, 1972). Thus, as previously stated the effect of colloids must, at some later stage, be included into the model. The separation effect could also be overcome to a certain extent by the production of large numbers of phage hence increasing number and possibility of infections of susceptible host propagules.

Another reason for the assumed limited lytic interactions observed in the soil could be the occurence of lysogeny within the environment. As previously stated, Rautenstein (1970) stated that lysogeny in actinomycetes is widespread. Lysogeny enables the phage to perpetuate itself without harming its host, at least until it returns to its lytic cycle. Campbell (1961) indicated that the beneficial effects of the inclusion of a phage within the host genome must be weighed against

the cost of producing DNA which is 'dead weight'. However the phage DNA is not always dead weight, as lysogenization can cause specific character changes inseparably associated with the prophage. Among the best known examples are the conversion of avirulent diptheria bacilli to virulence, and some cases of antibiotic resistance and toxin production caused by genes borne on the prophage of temperate phage (Levin & Lenski, 1984).

Lysogeny not only fulfils the role of protecting the host and to a certain extent the phage, it can also play a part in the coevolution of the bacteria and phage thus leading to natural variation among isolates of both of the interacting populations and the persistance of the two populations within the same environment. Phage can serve as vehicles of genetic exchange and this is clearly more likely if the phage has initially been integrated into the host genome, as in lysogeny. When integrated into the host genome, the phage may undergo evolutionary changes along with the host; as stated by Levin & Lenski (1984), this coevolution may play a significant role in the overall evolution of the bacteria and phage. Obviously independent phage selection (that achieved without the aid of the integration into the host DNA) is antagonistic to that of the host, with the phage selecting for the properties (e.g. decreased latent periods) which will increase their ability to perform successful and more efficient infections. Bacterial selection would obviously be to reduce the rate of adsorption of the phage, possibly by altering the adsorption sites on the cell surface, and to increase fecundity. Campbell (1961) suggested that a simple model of virulent phage and sensitive host is not realistic (as already stated for the Nicholson-Bailey model) because hosts do mutate and are followed by mutations of the phage. He also suggested that it is extremely likely that these mutations are likely

to be cyclic with the host eventually mutating back to its original form, becoming sensitive to the original phage. Work by Barnett et al. (1981, 1984) with Plectonema boryanum and phage LPP DUN1 in continuous culture led to the development of at least two cyanobacterial variants and two phage types. Paynter & Bungay (1969) with E. coli + ØT2 also observed some phage and bacterial mutants under a prolonged incubation of the two organisms, although those cells which were phage sensitive showed an oscillating upward trend in numbers. Obviously, as stated by Levin et al.(1977) and Campbell (1961), if resistance evolved by evolutionary changes engenders some costs in the competitive performance of the bacteria, and if the sensitive cells are able to maintain a stable association with the phage, the evolution of resistance will not lead to the elimination of the phage from the habitat. Therefore, as already stated, the inclusion of the evolutionary development of the two interacting populations is necessary in order to produce a more complete picture of the situation occurring in the soil. Experimental work to investigate the alteration of the composition of the two populations is also desirable.

The initial model of the streptomycete-phage interaction has shown that in the simplest situation, with a virulent phage and sensitive host, a coexistence of the two populations could occur for long periods, as indeed it must to explain the presence of phage and streptomycetes in the same soil system. It should be noted that Hunter (1947) studying Streptococcus cremoris and a lytic phage demonstrated that the two populations could live in peaceful 'co-existence' although neither resistant mutants or lysogenic cultures were detected. One would expect that a multitude of interactions occur in the soil as it is reasonable to suppose that the individual parameters vary considerably in the soil

mass and within the individual micro-sites. These parameter variations would be due to the heterogeneous nature of the soil, physically, chemically and nutritionally. Therefore, the persistance of phage and streptomycetes together within a single habitat is not surprising considering the heterogeneous nature of the environment and the distribution of the two populations involved.

The application of the Nicholson-Bailey model, which is based upon the individual parameters remaining constant and no mutation within populations, gives only a limited insight into the interaction of the streptomycetes and their phage, and to the influence of the individual parameters in such a complex environment. Therefore, a model which allows for alteration in parameter perhaps based on probabilities, (a deterministic model) would probably give a more realistic insight into the interaction. However, this study was only an initial foray into the relatively unexplored region of streptomycete-phage interactions, it indicated the possible effect of individual parameters in the interaction, and thus highlighted those parameters which appear to be of greatest importance, i.e. "a" and the introduction of a refuge, and those needing more detailed investigation. Further developments needed to make a model which represents more realistically the situation in the soil have been discussed. One must remember that the aim of a modeller initially is to present a model which attempts to describe the system under investigation as simply as possible and then as further work is performed, either instigated by the model itself or from other sources, to develop models which will become more realistic and more complex. An initial model cannot include every individual aspect of the interaction and will, as does the streptomycete-phage model used here, have inherent assumptions which are not completely correct. These can

be rectified only as the model is developed. It must always be remembered that a model needs to be realistic, precise, general and simple but the extent to which any of these is achieved is always at the expense of the others.

An investigation then has been undertaken to examine the interaction of streptomycetes and their virulent phage, both experimentally and theoretically. A simple model, which was used to describe the interaction, has been presented and critically examined to assess its fitness to describe the interaction. Further developments of the model and experimental investigations have been suggested.

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APPENDIX I

Local stability analysis

Nicholson-Bailey model is:-

$$H_{t+1} = H_t \exp(r(1-H_t/K)-aP_t) \quad (1)$$

$$P_{t+1} = cH_t(1-\exp(-aP_t)) \quad (2)$$

At equilibrium:-

$$H_{t+1} = H_t = H^* \quad \& \quad P_{t+1} = P_t = P^*$$

therefore
$$H^* = H^*\exp(r(1-H^*/K)-aP^*) \quad (3)$$

$$P^* = cH^*(1-\exp(-aP^*)) \quad (4)$$

Now using the Taylors' theorem of linear expansion:-

$$F(H^*+h_t,\ P^*+p_t) = F(H^*,\ P^*) = h_t\frac{dF}{dH_t}\Big|_E + p_t\frac{dF}{dP_t}\Big|_E + O(h_t^2,\ p_t^2,\ \ldots\ldots\ldots)$$

$$G(H^*+h_t,\ P^*+p_t) = G(H^*,\ P^*) = h_t\frac{dG}{dH_t}\Big|_E + p_t\frac{dG}{dP_t}\Big|_E + O(h_t^2,\ p_t^2,\ \ldots\ldots\ldots)$$

Now using compact notation for partial derivatives and ignoring second order and above terms, at equilibriums:-

$$\frac{dF}{dH_t} = F_H \ , \quad \frac{dF}{dP_t} = F_P \ , \quad \frac{dG}{dH_t} = G_H \ , \quad \frac{dG}{dP_t} = G_P$$

Using the Cayley-Hamilton theorem:-

$$M^2 - (\text{Trace } M)M + (\det M)I = 0 \quad (5)$$

Where $\text{Trace } M = F_h + G_p$

$\det M = F_H G_P - F_P G_H$

Application of this method to equations (3) and (4) leads to:-

$$\lambda^2 - (1+Q-r)\lambda + (1-rq)Q = r^2q(1-q) = 0$$

where $q = (1-H/K)$

where $Q = \dfrac{r(1-q)}{1-\exp(-r(1-q))}$

and M and I have been replaced by the symbol λ. This is the same solution as obtained by Beddington *et al.* (1975). The subsequent analysis of the behaviour of the roots of this quadratic is available from work by Maynard-Smith (1968):-

If $b^2 < 4ac$ then if $c > 1$ divergent oscillations will occur.

and if $\sqrt{c} < 1$ damped oscillations will occur.

If $b^2 > 4ac$ then complex roots Y_1 and Y_2 exist and will dictate stability observed.

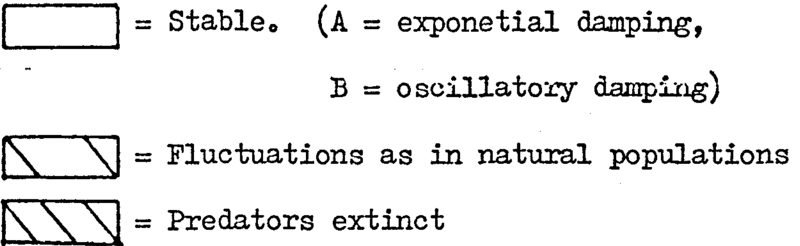
$$Y_1 = \frac{-b + b^2 - 4ac}{2a} \qquad \& \qquad Y_2 = \frac{-b - b^2 - 4ac}{2a}$$

If $Y_1 > 1$ then the predator will increase without limit and therefore the result will be chaotic (extinction)

If $Y_2 < 0$ then the system will oscillate

If $Y_2 < -1$ then oscillations will be divergent resulting in a chaotic interaction.

The results can ben be plotted graphically by examining the behaviour of the root at varying values of r and q (H*/K). A typical stability diagram is shown in Fig. 41.



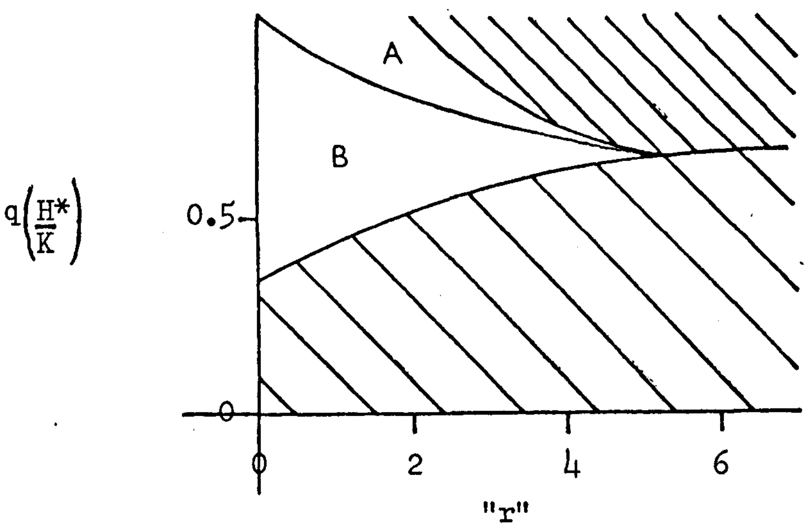


Fig. 41:- Stability boundaries for the original Nicholson-Bailey model

(After Beddington, Free and Lawton, 1975)