

LABORATORY STUDIES OF COMPETITION IN TWO SPECIES
OF CELLULAR SLIME MOLDS; DICTYOSTELIUM DISCOIDEUM
AND POLYSPHONDYLIUM PALLIDUM

by

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ABSTRACT

The mechanics of a short term interspecific competitive situation and some of the consequences of long term interspecific competition were studied in the laboratory using two species of cellular slime mold; Dictyostelium discoideum and Polysphondylium pallidum. The mechanics of competition were studied using a components method in which the process was divided into component parts. These were assessed experimentally, modelled mathematically, and linked together to form a computer model, the predictions of which were tested in the laboratory.

Five major components contributed to the competitive situation. These were: exploitation, toxic interference, the effect of physical factors or external forces, the availability of resources, and the number of potential competitors engaged in exploitation and interference. The exploitation component depended upon all of the sub-components which contributed to the life cycle of the cellular slime mold species. These were: the time required for spore germination, the rate and form of amoeba colony expansion, the time required for fruiting body production, and the rate and form of fruiting body colony expansion. Both species interfered with the other's ability to form fruiting bodies. In mixed cultures, D. discoideum amoebae divided and consumed food between 9° and 27°C but D. discoideum fruiting did not occur above

about 24°C. In mixed cultures, P. pallidum amoebae divided and consumed food between 18° and 37°C but P. pallidum fruiting bodies did not form below about 24°C. In both cases interference was mediated by temperature and competitor numbers. Temperature, the representative external force, altered the parameter values of all the sub-components contributing to exploitation and interference.

When all of the components were assessed they were incorporated into a computer model which was used to predict the area occupied by the fruiting bodies of both species. The simulation was tested 324 times and was accurate in 90.1% of the cases.

The long term experimental studies of the consequences of continued competition revealed that after a period of continued competition P. pallidum overcame the effects of D. discoideum inhibition and fruited in the presence of D. discoideum. When grown alone P. pallidum fruited from 18° to 37°C and D. discoideum fruited from 9° to 27°C. In mixed cultures, before competition, P. pallidum fruited from about 24° to 37°C and D. discoideum from about 9° to 24°C. In mixed cultures, after continued competition, P. pallidum fruited from about 20° to 37°C and D. discoideum from about 9° to 24°C.

Apparently P. pallidum converged towards D. discoideum, and at the same time D. discoideum increased its rate of resource use, and diverged away from P. pallidum. The data

suggested that interference was related to the production of chemicals during the aggregation stage. It is possible that acrasin, the chemical which attracts amoebae to aggregation centers is involved. Experimental evidence also suggested that the change experienced by P. pallidum might have resulted from para-sexuality. This entails the production of diploid spores, the recombination of alleles, and chromosome loss, all of which tend to protect recessive and less fit characteristics.

PREFACE

The work presented in this thesis is directed towards the study of competition between two species of cellular slime mold grown under laboratory conditions. It was anticipated that the mechanics of competition could be studied over short time periods and that some of the results of competition could be studied during long periods of competitive exposure. It was also hoped that some of the laboratory findings from this study could be related to existing competitive theory to obtain an overview of competition that might be applicable to field studies.

The first results section on competitive mechanics describes the way in which the two species exploit their environment, beginning with the germination of spores and ending with the production of fruiting bodies which contain spores. As the experimental evidence is presented, diagrams have been used to follow the build-up of information and to describe the way in which the various pieces of evidence link together. A similar procedure is followed during the development of the second section dealing with the way in which the two species interfere with one another. Since it was possible to express the action of the various components mathematically, a computer model was constructed to simulate the way in which the two species exploited their environment and interfered with one another. The results of this simulation are presented at the end of the first section on competitive mechanics.

In the second results section which deals with continued competition, hypotheses relating competitive pressure, ecological convergence, and ecological divergence are tested and some of the data obtained are used in the simulation model.

The discussion is presented in two sections. The first considers findings relevant to general cellular slime mold biology. The second deals with the ecological aspects of the study.

There are four appendices. In the first, nine computer programs and their explanatory write-ups are presented. The second contains several figures which are indirectly referred to in the results sections. The third is concerned with the generality and goodness of fit, of the equation which describes the way in which amoebae and fruiting body colonies expand. The fourth appendix contains an explanation of the tests used to statistically determine the descriptive ability of the various rate and lag equations developed in the results section.

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INTRODUCTION

The process of animal competition has fascinated a great many ecologists over the years; and their interest has yielded a large number of descriptive, experimental, and theoretical studies. It has also resulted in a certain amount of confusion over the exact meaning of the term competition. Milne (1961) and Birch (1957) have devoted entire papers to the subject, and have noted that definitions ranged from broad to strict coverage. For example, Odum (1959) includes any process involving the common use of space, food and light; or waste material action, or mutual predation, or susceptibility to carnivores and disease. Clements and Shelford (1939) provide a strict definition. In their words: "... the process may be defined inclusively as a more or less active demand in excess of the immediate supply of material or condition on the part of two or more organisms".

The term has also been sub-divided. Nicholson (1954) used the terms "contest" competition (the competitors contest for their requisites "... and all of the governing requisite collectively secured by animals is used effectively in maintaining the population.") and "scramble" competition ("... some, and at times all, of the requisite secured by the competing animals takes no part in sustaining the population being dissipated by individuals which obtain insufficient for survival."). Allee et al (1949) have identified "co-operative competition" (beneficial effect) and "dis-operative

competition" (harmful effect).

The one idea that runs throughout all the definitions and subdivisions is that animals compete when they use a resource that is in short supply. Also, both Nicholson (1954) and Park (1954) discuss the importance of physical factors, such as temperature. Finally, Park (1954) points out that exploitation (the common use of a resource in short supply) and interference (the direct interactions between competitors) can play active roles in the determination of competitive outcomes. As a starting point for this study the role of exploitation for resources in short supply, the effects of physical factors, and the occurrence of interference, will all be considered.

To this end, the components method will be used. The biological relevance of the method has been demonstrated by Holling (1963, 1964, 1965) during the construction of a predation model, and has been discussed in a series of papers edited by Watt (1966). Six basic steps are involved. A process is broken into what appear to be experimentally tractable components. The effect of a component is demonstrated experimentally and modelled mathematically. The sub-model is then used to make predictions which are tested independently. Failure of the sub-model initiates a reassessment of the component and starts the cycle back at the first step. Sub-models are linked together as they are formulated and the resulting model is periodically tested against

independent data.

The use of the components method is possible only because past work on the mechanisms which mediate competitive interactions has advanced significantly through use of experimental techniques in both the laboratory (Park 1954, 1965) and the field (Ulllyett 1950, Connell 1961a, b). Studies of the consequences of continued competition have progressed at a different level. For the most part they have been of a theoretical nature and have centered around the Lotka-Volterra model of interspecies competition (Gause 1934, Kostitzin 1939, Slobodkin 1961, Larkin 1963). Through the years this model has been clarified and slightly altered but the four original predictions regarding the outcome of competitive situations have remained intact.

The predictions of exclusion (competitor population "a" excludes "b", or the reverse) have been demonstrated in the laboratory (Gause 1934) and in the field (Elton et al 1946, Brian 1952). The predictions of stable equilibrium (both competitor populations stabilize irrespective of the initial numbers) and unstable equilibrium (stability depends upon initial numbers, under some initial number conditions both populations stabilize and persist; under others, one population excludes the other) remain untested and undemonstrated in nature because the parameters required by the model are difficult to assess. Recognizing this fact, MacArthur and Levins (1964, 1967) have restricted initial

conditions and theoretically derived relationships between competition and coexistence. They demonstrated that when three populations compete so that 1 and 3 interact with 2 in the same way (α) and with each other in the same way (β), population 2 can do one of three things. When α is large 2 can be excluded or can converge with 1 or 3. When α is small 2 can diverge from 1 and 3 towards a phenotype intermediate between the two. In this situation competition is a selective force, convergence or divergence are directions of movement with respect to selection, and coexistence is the end result.

There is also some rather circumstantial field evidence (Keast 1968, Ficken et al 1968) and at least one laboratory study (Seaton and Antonovics 1967) linking competition, divergence, and coexistence. And there are laboratory studies (Miller 1964a, b, 1967) in which Drosophila melanogaster and D. simulans have been used to demonstrate a relationship between convergence and coexistence.

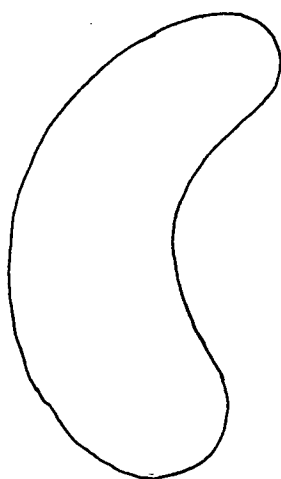
In general, however, the link between competition, convergence or divergence, and coexistence is tenuous and in need of further experimental work. Since this study was conducted in the laboratory under restricted conditions it should be possible to test the hypothesis that competition can result in coexistence. By noting the changes experienced by the competing populations it might also be possible to assess the magnitude and the direction of selective forces

involved, and to determine some of the conditions under which convergence or divergence might occur.

THE ANIMALS

The Acrasiales or "cellular slime molds" were first identified in 1880 by Van Tieghem. Their life cycle begins in the spore stage which germinates to produce vegetative amoebae which divide by mitosis, consume bacteria and aggregate to produce fruiting bodies.

The size, shape, and colour of P. pallidum Salvador and D. discoideum VC4 and V12 spores are different. D. discoideum VC4 spores are about 18 μ long and 8 μ wide. They are kidney shaped and have a distinctive green-gold hue. P. pallidum Salvador spores are about half this size, ellipsoid, and pale yellow.



D. discoideum



P. pallidum

The size difference suggests that the D. discoideum VC4 strain is typically "diploid" and has 14 chromosomes while the P. pallidum Salvador strain is "haploid".

Upon germination the spores produce amoebae, which in most cases, are very similar. Both species produce amoebae, which are small "with a number of filose pseudopods" (Bonner 1967). The amoebae are capable of consuming at least 93 different strains of bacteria (Singh 1946) although some bacteria appear to inhibit rather than support growth. Raper (1937) found that Gram-negative, non-slimy bacteria like Escherichia coli were the most suitable strain for laboratory culture of cellular slime molds. The amoebae divide by mitosis and when food becomes scarce a substance which has been called acrasin (and which remains largely unidentified) is produced by founder cells. This substance attracts other amoebae which move towards the aggregation center producing acrasin themselves. In D. discoideum the chemical gradient along which the cells move, is shortened by the production of phosphodiesterase which breaks the acrasin down.

The exact mechanics of this procedure vary from species to species. In the case of D. discoideum, aggregation occurs in pulses and the aggregating amoebae form loosely integrated streams flowing towards the aggregation center (Shafter 1956). As aggregation advances the streams become more dense and the amoebae adhere strongly to one another. The aggregating streams produce a mass of amoebae or

pseudoplasmodium which migrates. During this process the amoebae begin to differentiate to form pre-spore and pre-stock cells. Not so much is known about aggregation in P. pallidum, but it has been observed during the course of this study that pseudoplasmodia migrate in response to light as they do in D. discoideum. Konijn et al (1969) also reports that P. pallidum does not produce a chemical to break down acrasin.

Following the aggregation stage the cells begin to differentiate to form stock and spore cells. In D. discoideum about 20% of the cells are involved in stock formation. The stock cells build up the stock with the spore cells following behind. When stock formation is complete the spores are at the top of the stock, in the case of D. discoideum, or distributed at intervals along the stock, in the case of P. pallidum. The two species used in the present study are easily distinguished at the fruiting body stage. P. pallidum fruiting bodies are distinct when formed at about 24°C (Fig. 1) but become disorganized at higher temperatures (Fig. 2). D. discoideum fruiting bodies remain much the same over its entire growth range (Fig. 3). When mixed, the co-fruiting strains produce well organized and distinct fruiting bodies (Fig. 4).

Cellular slime mold species react to several environmental characteristics. Their pH tolerance ranges from about 4.0 to 7.8 (Cavender 1963) and humidity (Whittingham and Raper 1957) and light (Bonner 1950) are

important in some cases. The two strains used had well defined temperature tolerances with D. discoideum growing from 9.0° to 26.5° and P. pallidum from 18.0° to 37.5° centigrade. P. pallidum is also greatly influenced by richness of the medium and amount of food available. For this reason a simple medium was used and food concentration was closely controlled.

Finally, the genetics of the Acrasiales are still very much a mystery. It is generally assumed that 14 chromosome amoebae are diploid and that 7 chromosome amoebae are haploid. Sussman et al (1962) report that some strains are diploid and that others are haploid and that others contain both haploids and diploids. Loomis (pers. comm.) adds that amoebae coalesce about one in a thousand times during aggregation to form diploids which lose chromosomes on subsequent mitotic divisions so that a population could have members with from 7 to 14 chromosomes. It is generally agreed that meiosis does not occur. Sussman (1956) met with no success when trying to demonstrate the occurrence of recombination and Sussman et al (1961) reported one recombination which has not been repeated.

Figure 1

P. pallidum fruiting body grown at 24° centigrade.

0.25mm

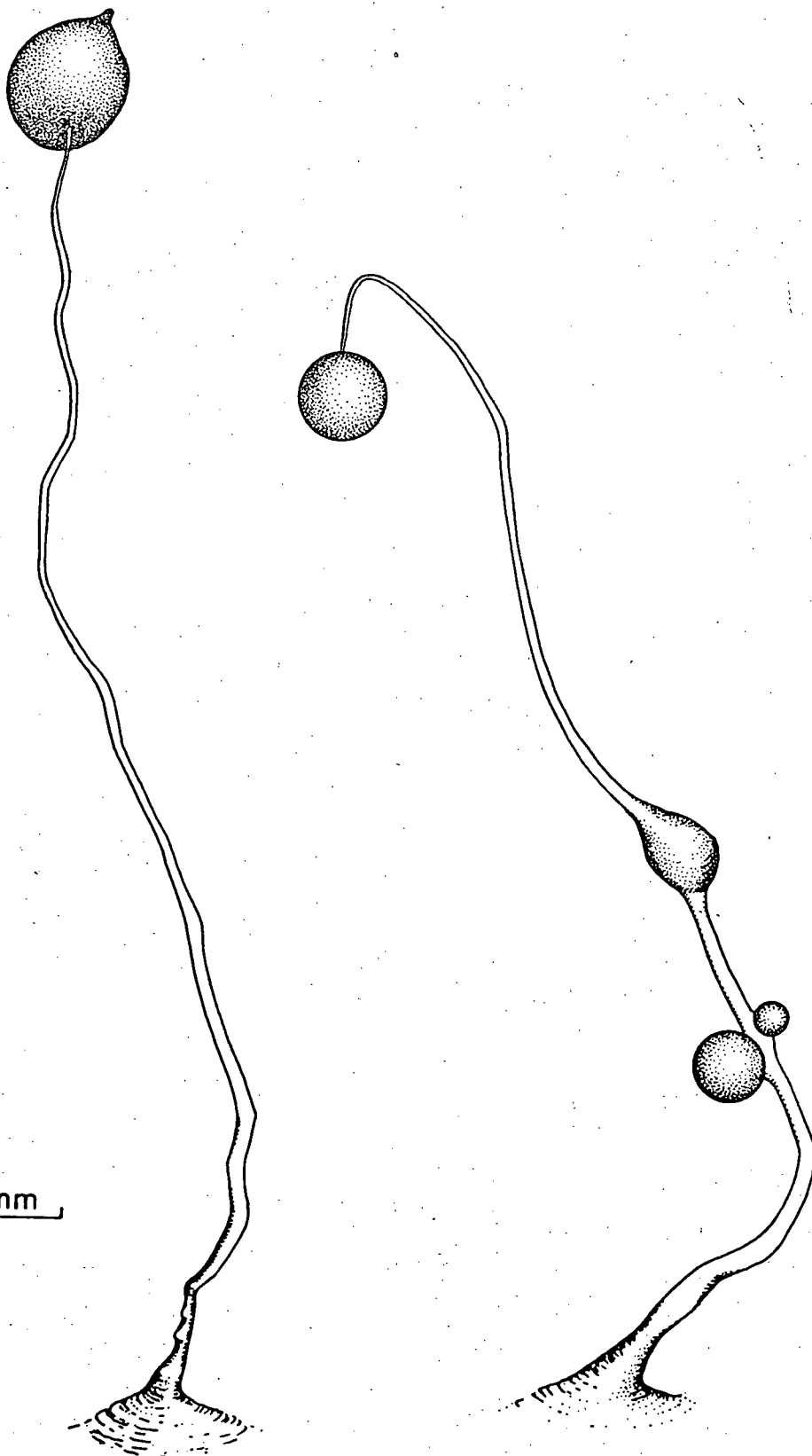
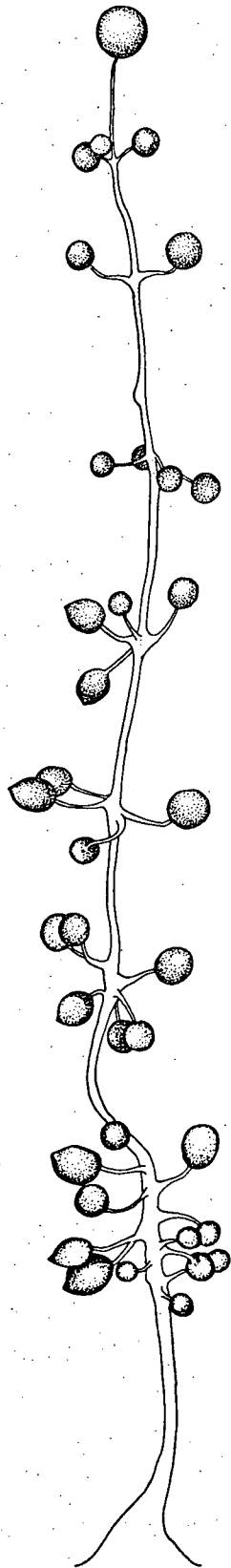


Figure 2

P. pallidum fruiting body grown at 36° centigrade.



0.25mm

Figure 3

D. discoideum fruiting bodies grown at 20° centigrade.

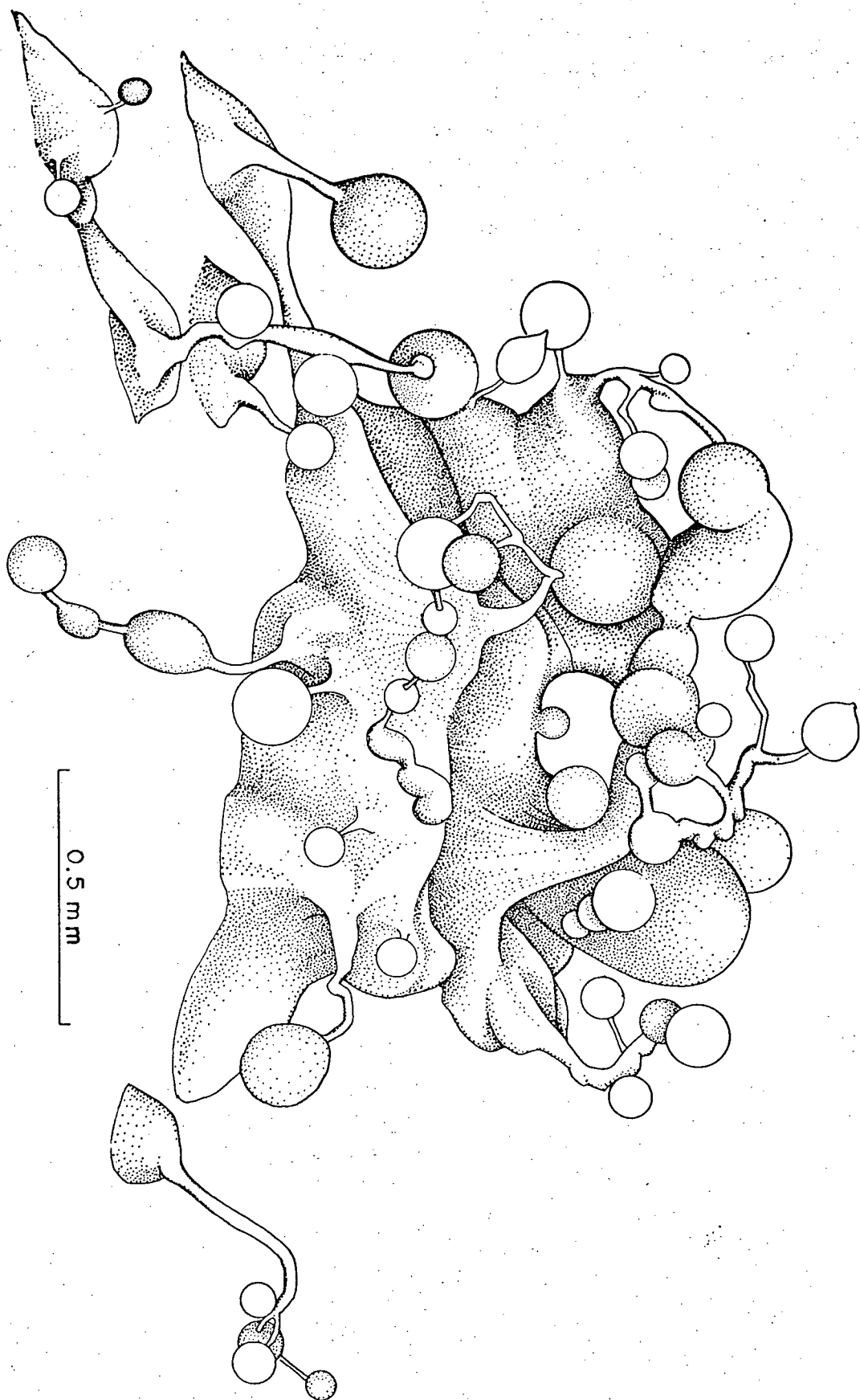
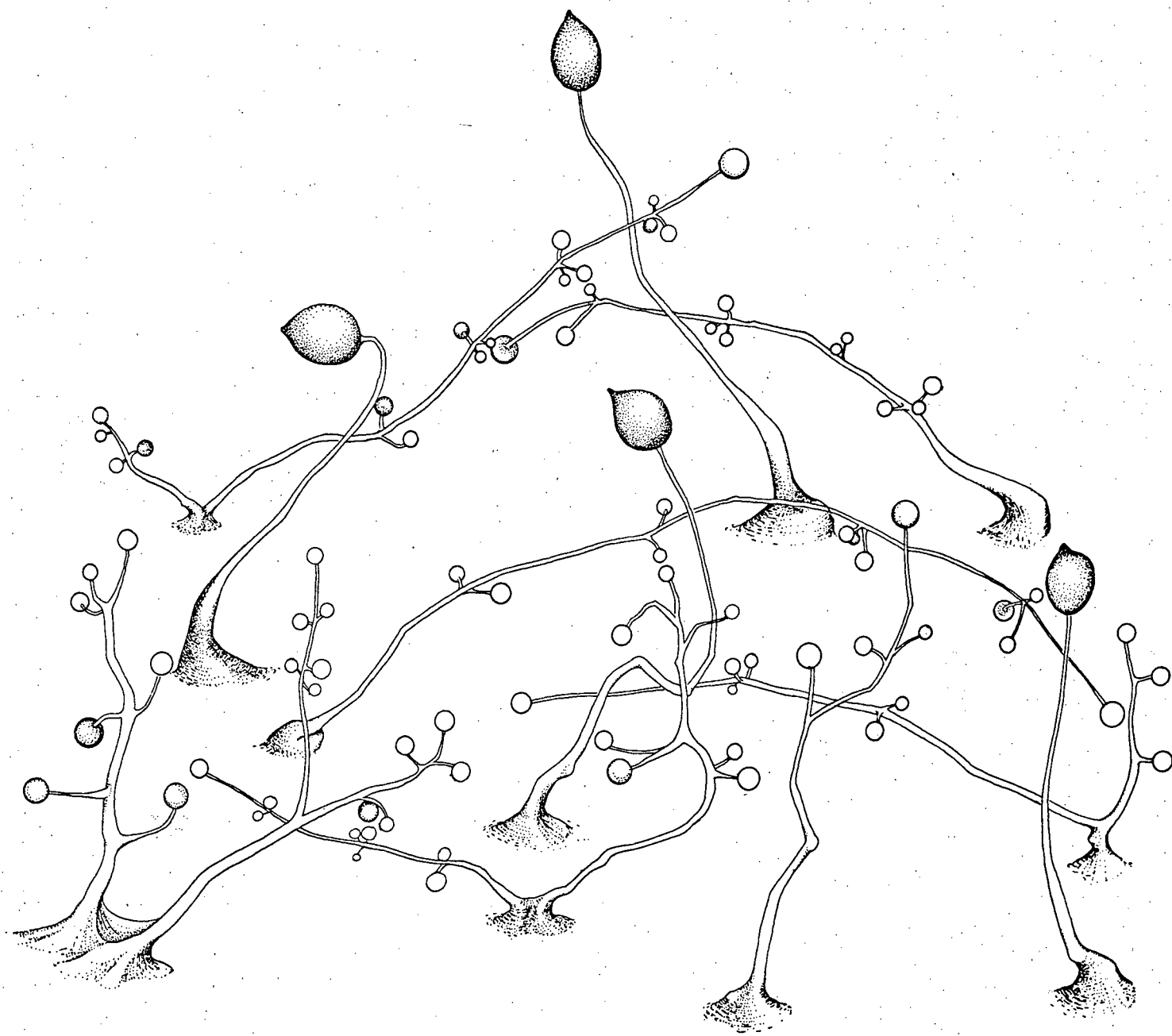


Figure 4

D. discoideum and P. pallidum co-fruiting at about 23.0° centigrade.



0 0.25mm

MATERIALS AND METHODS

Laboratory Methods

The bacteria (Escherichia coli 281) used as a food source were obtained from Dr. K.B. Raper, Department of Bacteriology, The University of Wisconsin. They were maintained by a series of bi-weekly serial transfers. The medium used was prepared from 1000 cc of water, 15 g of Difco-Bacto Agar, 5 g of yeast extract, 5 g of triptose, and 1 g of dextrose. The constituents were dissolved in 100 cc of cold water and the solution made up to 1000 cc with boiling distilled water. The medium was autoclaved for 20 minutes at 15 pounds per square inch and 257°F, and poured into sterile petri dishes. When the agar had set, bacteria were streaked on the surface and allowed to grow for two days at room temperature, then removed from the surface with a spatula. A mixture of 0.6 ml of bacteria and 4.4 ml of sterile distilled water was placed in a 5 cc syringe and was then ready for use in the cellular slime mold culture dishes.

The cellular slime mold medium was prepared by heating 5 g of hay in 1000 cc of distilled water for 15 minutes. The hay was then removed, 20 g of Difco-Bacto Agar, and 1 g of dextrose were added, the solution was autoclaved for 20 minutes at 15 pounds per square inch and 257°F, and was poured into sterile growth chambers (the type of chamber depending upon the experiment being conducted). The chambers were then placed in a refrigerator at about 4°C for seven days.

The chambers were then removed from the refrigerator and allowed to achieve room temperature. The bacterial food was then added.

For every 2000 mm² of surface area on the culture dish, 0.2 ml of standard bacterial-water solution was added. The aliquot of bacterial solution was placed in the center of the growth surface and was spread over the surface by revolving the chamber at a 45° angle. The chambers were then allowed to sit for two hours during which time the moisture in the bacterial-water solution was absorbed by the agar. This procedure produced a dry agar surface covered with a homogeneous "lawn" of bacteria.

During the course of the study several different experimental set-ups were used. The three major variations are mentioned here and any minor changes are described with the results.

Method 1 was used to grow single species and mixed species from point source inoculations. The culture chambers were 60 mm plastic petri dishes with approximately 2000 mm² surface area. The dishes were half filled with cellular slime mold agar, allowed to sit for one week, and covered with 0.2 ml of standard food solution. Cellular slime mold spores from either species were picked from the fruiting bodies of stock cultures, suspended in water, agitated to break up clumps, and counted using a haemocytometer. At least four and usually six or eight counts were made. The mean spore number

per ml of spore solution was calculated, and the solution was diluted to the appropriate spore concentration. A known number of spores suspended in .001 ml of water was then placed in the center of the petri dish. The dish was placed in an incubator and the spores were allowed to germinate and produce amoebae which produced a colony. The area covered by the colony at various periods of time was observed under a binocular dissecting microscope.

Method 2 was used to grow single species and mixed species from many points of inoculation. The culture chambers were 60 mm petri dishes which were half filled with cellular slime mold medium and placed in a refrigerator for one week. The spores were counted as in Method 1, and appropriate mixtures of the two species were made up. The spore mixtures were then mixed with the bacteria-water solution which was added to the surface of the plate in the usual manner. In this way spores were dispersed over the surface of the growth plate. The plates were incubated and the appearance of fruiting bodies was noted.

Method 3 was used to grow mixed species cultures over a continuous temperature range. The culture chambers were long stainless steel troughs with glass lids and containing about 11,000 mm² surface area. They were filled with agar and placed in the refrigerator. Spore mixtures were added with the 1.2 ml aliquot of bacterial food. The chambers were then placed on a temperature gradient and allowed to incubate

for 7 or 14 days. At the end of the incubation period the area covered by fruiting bodies was noted.

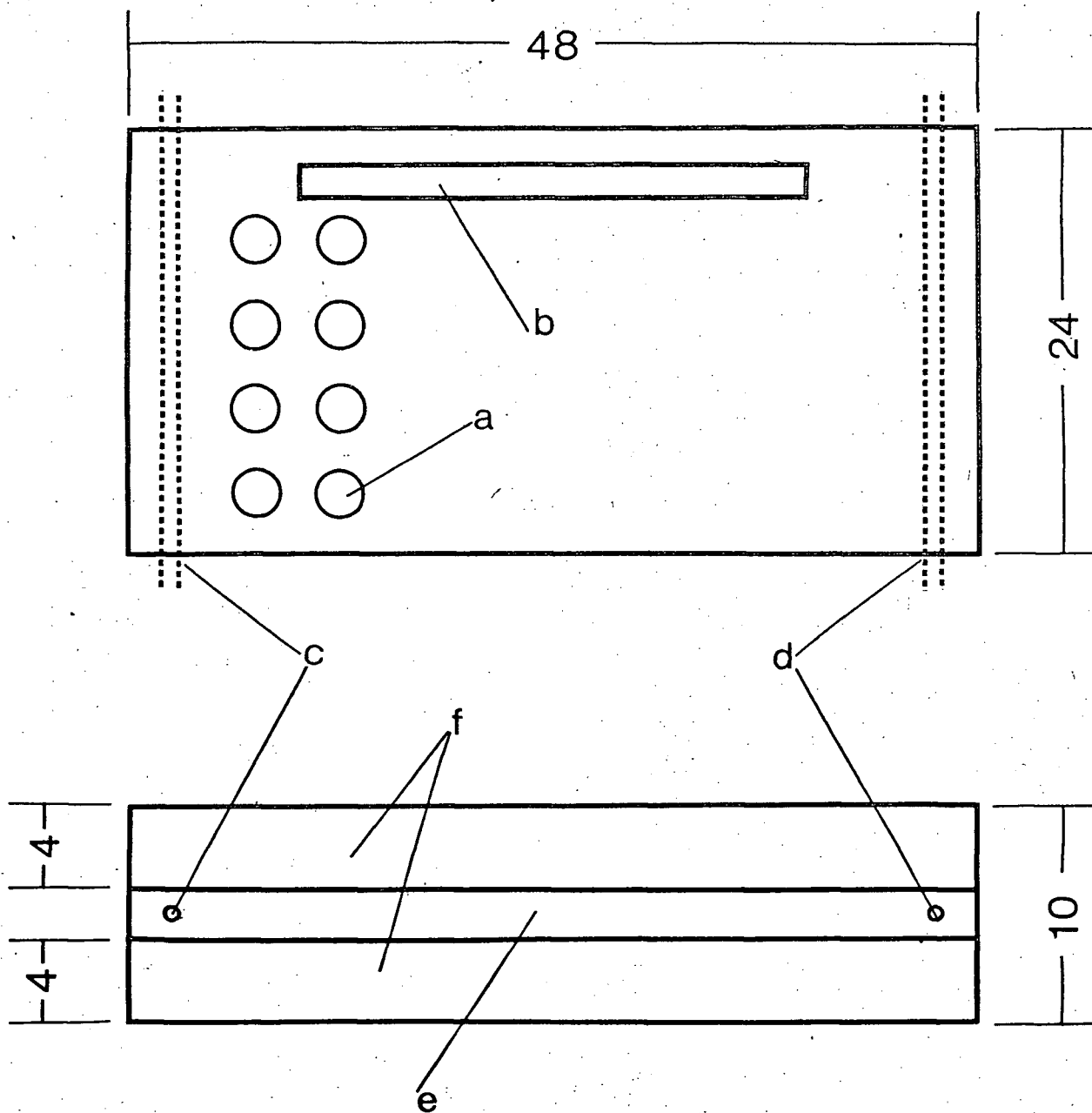
Most of the incubation was conducted in a temperature gradient device which was constructed for this study. A 200 pound aluminum block heated at one end and cooled at the other, was insulated with poly-urethane. Culture dishes were placed in small holes in the insulation next to the block (Fig. 5).

Two temperature gradients were used. The main gradient was heated by a HAAKE model FS constant temperature circulator capable of controlling temperature to within $\pm .004^{\circ}$ centigrade; the cold end was maintained by a PARMETIC compressor, cooling water in a 100 l vat and controlled with a HONEYWELL thermostat model T6 75 A1011, capable of 0.1° centigrade accuracy. The secondary gradient had the same cold water control but the hot end was maintained using a HAAKE model ED UNITHERM which was accurate to within 0.01°C . For both arrangements, the gradient could be shortened or lengthened by changing water temperature at either end. Temperature was monitored and recorded continuously at seven positions along the length of the gradients using a YSI TELETHERMOMETER and YSI MODEL 80 RECORDER.

Throughout the study one strain of P. pallidum was used. It was maintained at 34°C from April 1968 to May 1969, and at 29°C from May 1969 to April 1970. Cultures were maintained with a series of weekly or bi-weekly serial transfers and care was taken to mix the spores from each replicate

Figure 5

Plan (top) and side (bottom) view of the temperature gradient constructed for this study. The letter "a" represents petri dish chambers, "b" represents the culture gradient chamber, "c" represents the cold water line, "d" represents the hot water line, "e" represents the aluminum block in side view, and "f" the urathane insulation. All measurements are in inches.



before establishing new stock cultures. Separate stocks were maintained at other temperatures for varied lengths of time as checks for experiments in progress.

Three separate strains of D. discoideum were used. D. discoideum V12 was maintained from February 1968 to February 1969 when it was destroyed by an incubator failure. D. discoideum VC4 was used as a replacement and kept from March 1968 to April 1970 at 20°C. D. discoideum DF was used for a short period from March 1968 to June 1968. The VC4 stock was lyophilized to avoid further stock loss. Throughout the study stocks have been identified and data from one stock has not been used to simulate outputs from another.

Experimental Error

The experimental methods outlined on the proceeding pages involved several sources of error which were constant throughout the study.

The temperature equipment was accurate to within 0.5°C for the first year and after improvement, accuracy was increased to at least $\pm 0.3^\circ\text{C}$. Whenever a temperature is mentioned in the results section a 0.3°C variation is implied unless otherwise stated.

The medium used for cellular slime mold growth was kept homogeneous by using the same hay for a period of two years from the beginning to end of the study. The hay was

kept in a sealed plastic bag at all times and although there was a breakdown of chlorophyll no other observable changes occurred.

The bacteria were grown at room temperature throughout the study. During the two year experiment period the strain may have undergone some media conditioning or mutational changes. In an attempt to guard against this, Dr. D. Francis (who was also using the E. coli 281 stock) made dilute spreads, and picked up 281 plaques on two occasions (during the summer of 1968 and the spring of 1969). Cellular slime mold growth on the E. coli 281 food source was also checked during the study period.

The bacterial "lawn" on the surface of the culture dishes was assumed to be homogeneous in thickness. However, visual checks with a binocular dissecting microscope suggested there probably were some areas where the lawn was thicker, even though care was taken to spread the bacteria in a standard way. When spores were mixed and spread with the bacteria they too were subject to possible non-homogeneous spreading. Since there was no way to check exact spore distribution it was assumed that they were distributed on the agar surface randomly.

Spore counts prior to inoculation were made using a haemocytometer. Confidence limits (95%) were calculated for every mean count when the work first began, but it was soon discovered that in most cases six counts yielded optimum results considering the work involved in counting and the accuracy attained. The cases in which confidence limits were calculated suggested that the mean count may deviate from the

actual count by as much as 20%. But since accuracy increases in proportion to the reciprocal of the number of counts this was deemed acceptable.

Mathematics

A mathematical description of each sub-component could have been achieved by actually describing the mechanics of the process in mathematical terms, or by fitting a polynomial equation to the observed relationship. In all cases an attempt was made to implement the former approach. This attempt met with success during the description of the form of amoebae and fruiting body expansion (equation 1c) and during the description of the relationship between competitor numbers, temperature, and P. pallidum inhibition (Program VIII).

However, the lag equations (2b) and the expansion rate equations (3c, 4b) were not strictly mechanistic. The mechanisms involved in these processes were undoubtedly complex and probably involved enzyme kinetics, dispersal mechanics, and feeding mechanics. Rather than treat each of these processes independently, their total effect was considered with respect to temperature. This meant that the lag and rate equations were not mechanistic, nor were they deterministic. All of the parameters in the equations were both meaningful and measurable in biological terms.

In all cases, once the experimental data had been gathered, appropriate equations were formulated. The best

set of parameter values was then chosen by an iterative fitting procedure and by the calculation of the sum of squares of the deviations between the observed and calculated relationships. The descriptive power of the relationship chosen by this procedure was then tested by calculating the correlation coefficient.

RESULTS SECTION I

MECHANICS OF COMPETITION

Before any actual competitive experiments were conducted it was important to describe both the way in which, and the rates at which, the two species consumed the resources of food and space in separate cultures. It was known from the literature, and confirmed by observation, that the cellular slime mold life cycle went from spore, to vegetative amoebae, to aggregation, to fruiting body, to spore. It was also known that only the vegetative amoebae actually used up food and space, but their action depended upon all the other steps in the life cycle. Therefore, to describe adequately the rate of use of food and space, all the stages in the life cycle had to be investigated.

Spore Germination

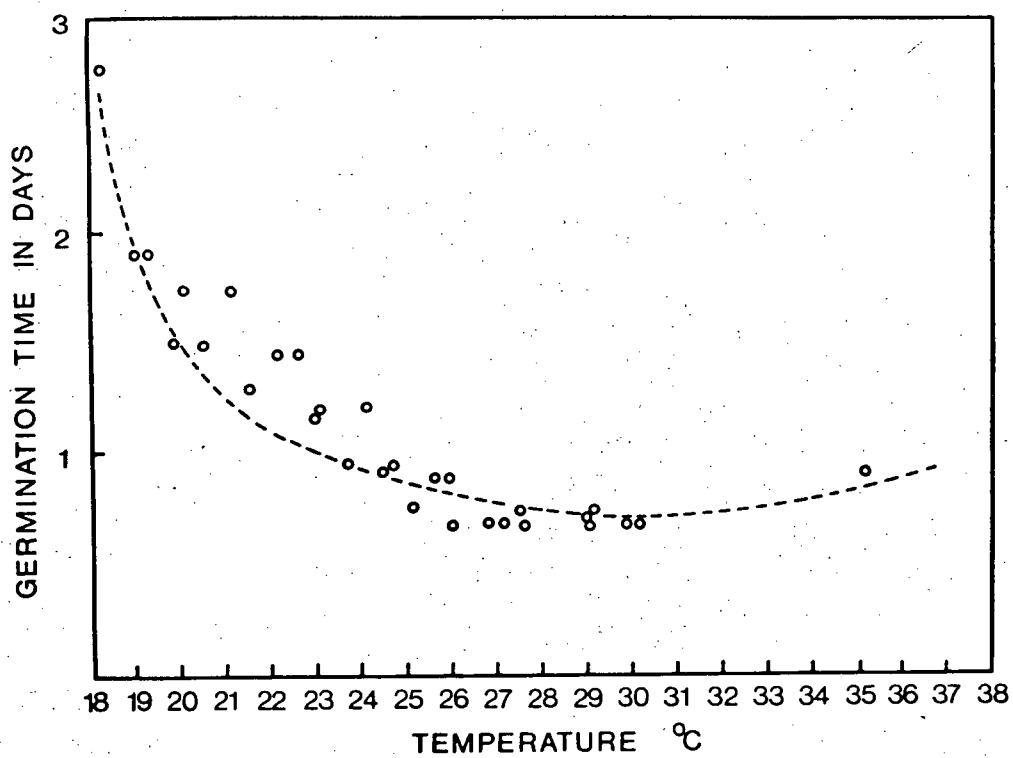
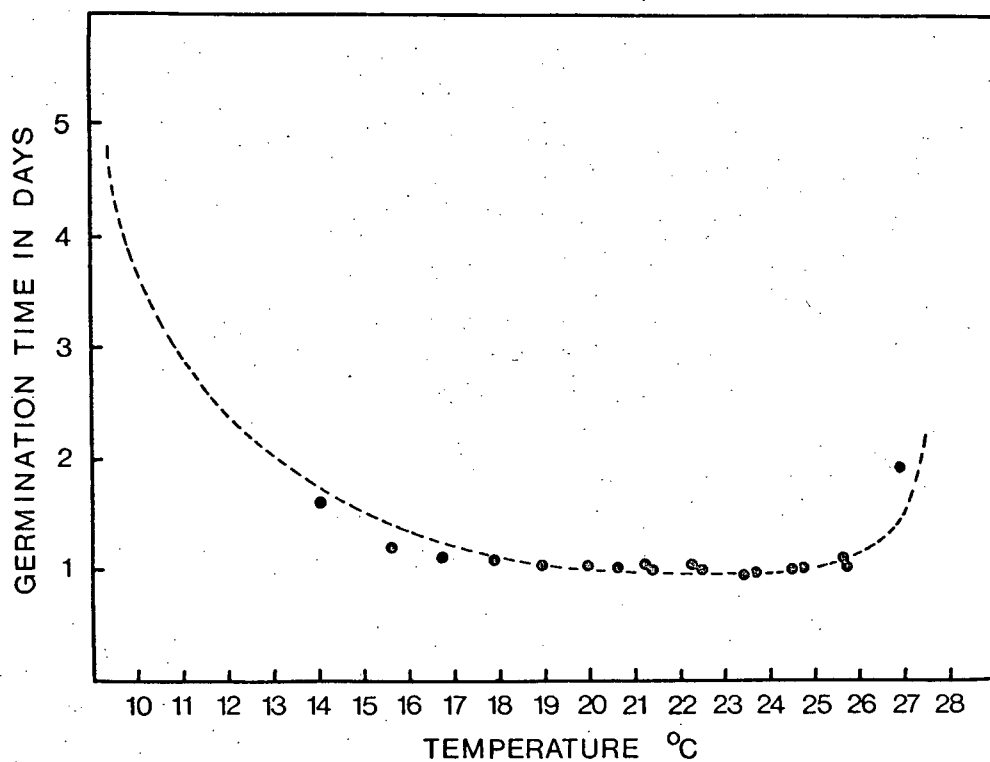
When spores were placed on an agar surface in the presence of bacterial food they required a certain period of time to germinate and produce amoebae. This time period might be called the spore germination lag. It was further observed that as the temperature changed the spore germination lag also changed. To quantify these observations, data were collected for both species by placing a known number of spores in the center of an agar filled petri dish and observing the time required for spore germination. The data for D. discoideum (Fig. 6) and P. pallidum (Fig. 7) suggested that the germination

Figure 6

The time necessary for D. discoideum VC4 spore germination is plotted against temperature in degrees centigrade. The points are data, the line is fitted from equation (2b). $T_H = 27.5$, $T_L = 9.0$, $T_O = 23.0$, $K = 1.60137$, $C = 4.74403$.

Figure 7

The time necessary for P. pallidum spore germination is plotted against temperature in degrees centigrade. The points are data, the line is fitted from equation (2b). $T_H = 37.0$, $T_L = 18.0$, $T_O = 31.0$, $K = 0.81132$, $C = 2.59356$.



time decreased as temperature increased, reached a minimum at some optimum temperature and then increased with further increases in temperature.

These observations form the basis of three assumptions which must be met by any equation which describes the relationship between lag and temperature. The assumptions are: (1) the spores do not germinate below some temperature T_L , (2) spores do not germinate above some temperature T_H , (3) the germination time is optimized at some optimum temperature T_O .

The assumptions demand that the curves have a slope of minus infinity at T_L and T_H and have a slope of zero at T_O (Fig. 8). The absence of any other restrictions on the curve implies that T_O may occur anywhere between T_L and T_H and that the curve may move to any position on the time axis.

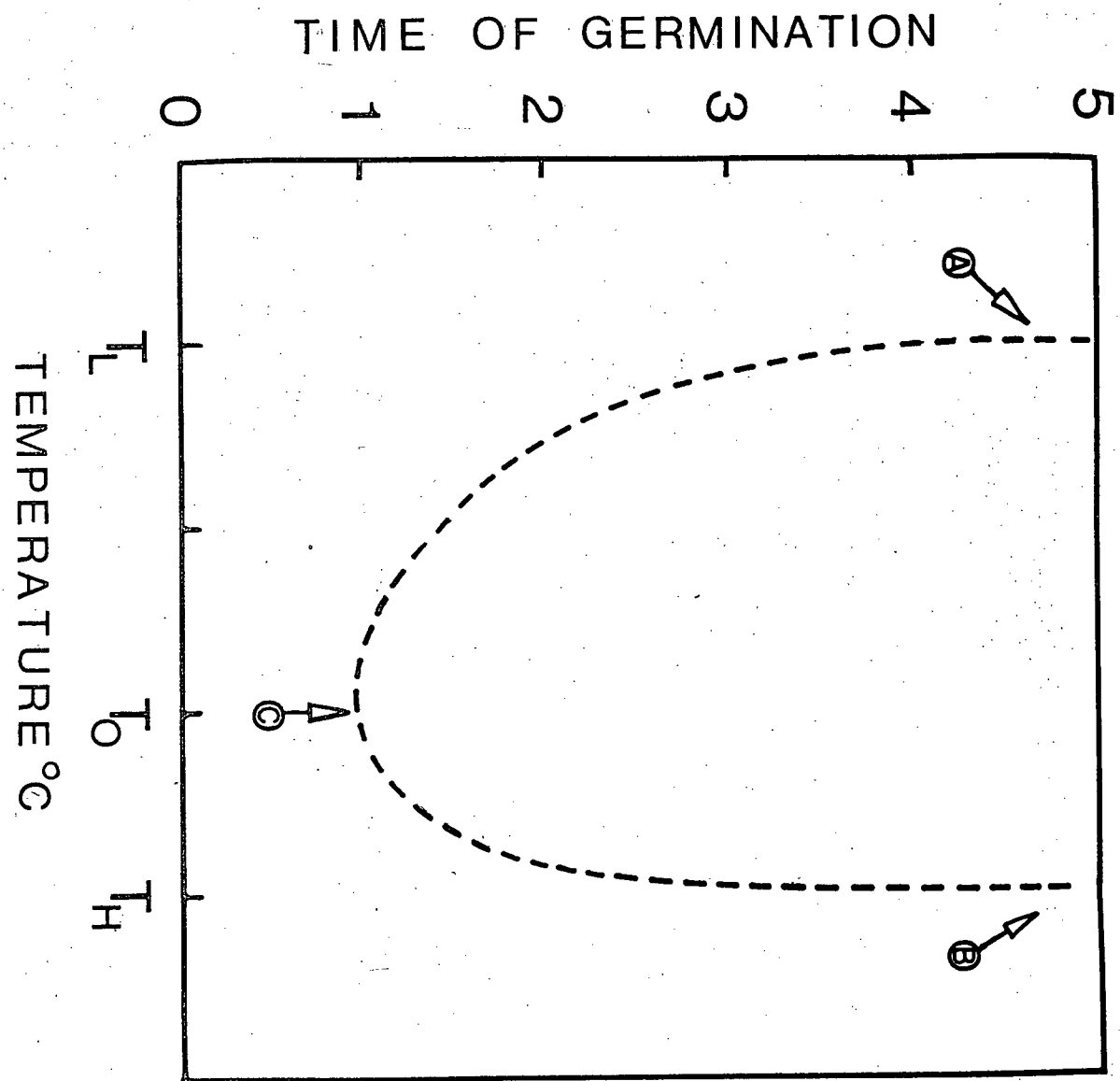
A family of curves which incorporates the three assumptions is represented by the following equation:

$$\frac{dL}{dT} = K \left[\frac{T - T_O}{(-1)T^2 + T \cdot T_H + T \cdot T_L - T_H \cdot T_L} \right] \quad (2a)$$

where dL/dT is the rate of change of germination time with respect to temperature, T is temperature, T_O is temperature optimum, T_L is temperature low (below which no germination occurs), T_H is temperature high (above which no germination occurs), and K is a constant. This equation may be integrated

Figure 8

A representative curve from the family of curves which have infinite slopes at $T_L(A)$ and $T_H(B)$, and a slope of zero at $T_O(C)$.



by making use of the following identities (Dwight 1947):

$$\int \frac{dx}{X} = \frac{1}{a(p-q)} \log \left| \frac{x-p}{x-q} \right|$$

$$\int \frac{xdx}{X} = \frac{1}{2a} \log |x| - \frac{b}{2a} \int \frac{dx}{X}$$

where $X = ax^2 + bx + c$ and where p and q are roots of the quadratic. The integration yields the following general equation:

$$L = K \left[(-.5 \log |(-1)T^2 + T(T_H + T_L) - T_H \cdot T_L|) - \left[\left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right] \right] + \quad (2b)$$

$$K \cdot T_O \left[\left[\frac{1}{T_H - T_L} \right] \cdot \log \left| \frac{T - T_H}{T - T_L} \right| \right] + C$$

where L is the spore germination lag and C is a constant of integration. All the other terms were defined for (2a).

This equation, and all those that follow, is not just a curve of "best fit". It is based upon realistic biological facts and assumptions and contains only measurable variables. There are no unknown variables, or variables which take up unknown sources of error. The equation does contain two constants K and C . But these depend directly upon L_{min} and T_O , and remain constant once fit to a set of data.

To test the descriptive ability of equation (2b) the data (Fig. 6, Fig. 7) were fit using Program I. A complete listing of the program and a description of the curve fitting procedure is presented in Appendix I.

The line fitted to the D. discoideum VC4 data explains 75% of the variation and the correlation coefficient (Table XIV - Appendix IV) is "significant" at $\alpha = 0.025$. The line fitted to the P. pallidum data accounts for 88% of the variation and is also "significant" at $\alpha = 0.025$ (Table XIV - Appendix IV). This leads to the acceptance of the curve as an adequate description of the relationship between spore lag and temperature.

It is possible to begin building the first stages of a descriptive components model. The information considered in this section deals with components 1 to 4 in Figure 22 (page 67). The spores (SPORE) experience a germination lag (GERM LAG) which is temperature (TEMP) mediated and the first group of amoebae (AMOEBAE PRESENT) are formed.

The effect of initial spore concentration on lag times was also considered. Paired and replicated cultures were grown at several temperatures, and the initial concentration was varied. Lag time was regressed (using the standard IBM 1130 regression package) against area so that the constant C in the equation $Y = C + BX$ approximated the lag time due to germination. The results for both D. discoideum V12 (Table I) and P. pallidum (Table II) suggest that initial

TABLE I

D. discoideum V12. A comparison of the lag times for cultures inoculated with varied spore concentrations. Actual lags are compared to lags calculated by regression.

TEMPERATURE	INOCULUM CON. SPORES PER ml	REGRESSED LAG	5% CON LIMIT	ACTUAL LAG
18.2	20000	1.27	$\pm$ 0.28	1.55
	5000	0.89	$\pm$ 20.07	
19.5	32000	1.43	$\pm$ 0.34	1.15
	80000	1.12	$\pm$ 0.44	
20.7	32000	0.65	$\pm$ 0.46	1.00
	80000	0.52	$\pm$ 0.70	
21.9	32000	0.82	$\pm$ 2.29	0.95
	80000	0.65	$\pm$ 0.22	
21.9	20000	0.94	$\pm$ 0.18	0.95
	5000	0.94	$\pm$ 0.33	
23.4	20000	0.91	$\pm$ 0.74	1.10
	5000	0.67	$\pm$ 0.58	

TABLE II

P. pallidum. A comparison of the lag times for cultures inoculated with varied spore concentrations. Actual lags are compared to the lags calculated by regression.

TEMPERATURE	INOCULUM CON. SPORES PER ml	REGRESSED LAG	5% CON. LIMITS	ACTUAL LAG
20.1	100000	3.05	$\pm$ 1.03	1.20
	50000	1.96	$\pm$ 0.31	
20.7	20000	1.88	$\pm$ 0.97	1.18
	5000	1.54	$\pm$ 1.24	
21.9	20000	2.43	$\pm$ 1.90	1.10
	5000	1.86	$\pm$ 0.39	
22.6	100000	1.34	$\pm$ 0.56	1.02
	50000	1.56	$\pm$ 0.58	
25.5	100000	1.08	$\pm$ 0.47	0.80
	50000	1.29	$\pm$ 0.24	
28.7	100000	1.16	$\pm$ 0.42	0.75
	50000	0.61	$\pm$ 0.52	

concentration does not alter lag time. It might be also noted that the lags generated in this way agree with those that were actually measured and approximated with equation (2b).

These data agree with the findings of Russell and Bonner (1960) and Cohen and Ceccarini (1967) who observed that only very high concentrations of D. discoideum caused some spore inhibition. In their work germination success dropped from about 99% at low concentrations to about 70% at spore concentrations of 2000 spores per mm^2 . In the present work concentrations varied from about 0.1 to 3.0 spores per mm^2 , and therefore little inhibition was expected.

Amoeba Colony Expansion

After the spores germinate the amoebae begin to divide, disperse, and use food. In the culture dishes these processes were observed together as the amoebae front moved over the bacterial lawn removing the food. Rather than divide this rather complex process into its component parts, both the rate and the form of colony expansion were assessed and modelled as one component.

The Form of Colony Expansion

When spores were placed at one point in a 20 x 60 mm agar filled petri dish in the presence of bacteria, the colony expanded in a relatively uniform circle away from the

point of inoculation. The amoebae divided and moved into new areas so that the area covered by the entire colony grew slowly at first and then increased until the rate of acquisition became almost constant (if the area became infinitely large the rate of acquisition would become constant, because the growth essentially would be along a straight line front). Horn (1969) has observed that movement along a front is constant. As the amoebae moved over the surface of the agar they consumed all the food, thereby securing that area from invasion by other cellular slime mold species.

These observations suggested that it was only the amoebae on the perimeter of the colony which expanded the colony. In mathematical terms, the rate of increase in the area covered by a colony is proportional to the circumference of the colony. This is:

$$\frac{dA}{dt} = g \cdot 2 \cdot \pi \cdot r \quad (1a)$$

where dA/dt is the rate of change of area covered, g is a constant, and r is the radius of the colony. Since $\pi \cdot r^2$ equals Area (denoted by A), then $r = (A/\pi)^{1/2}$. Incorporating this substitution into (1a):

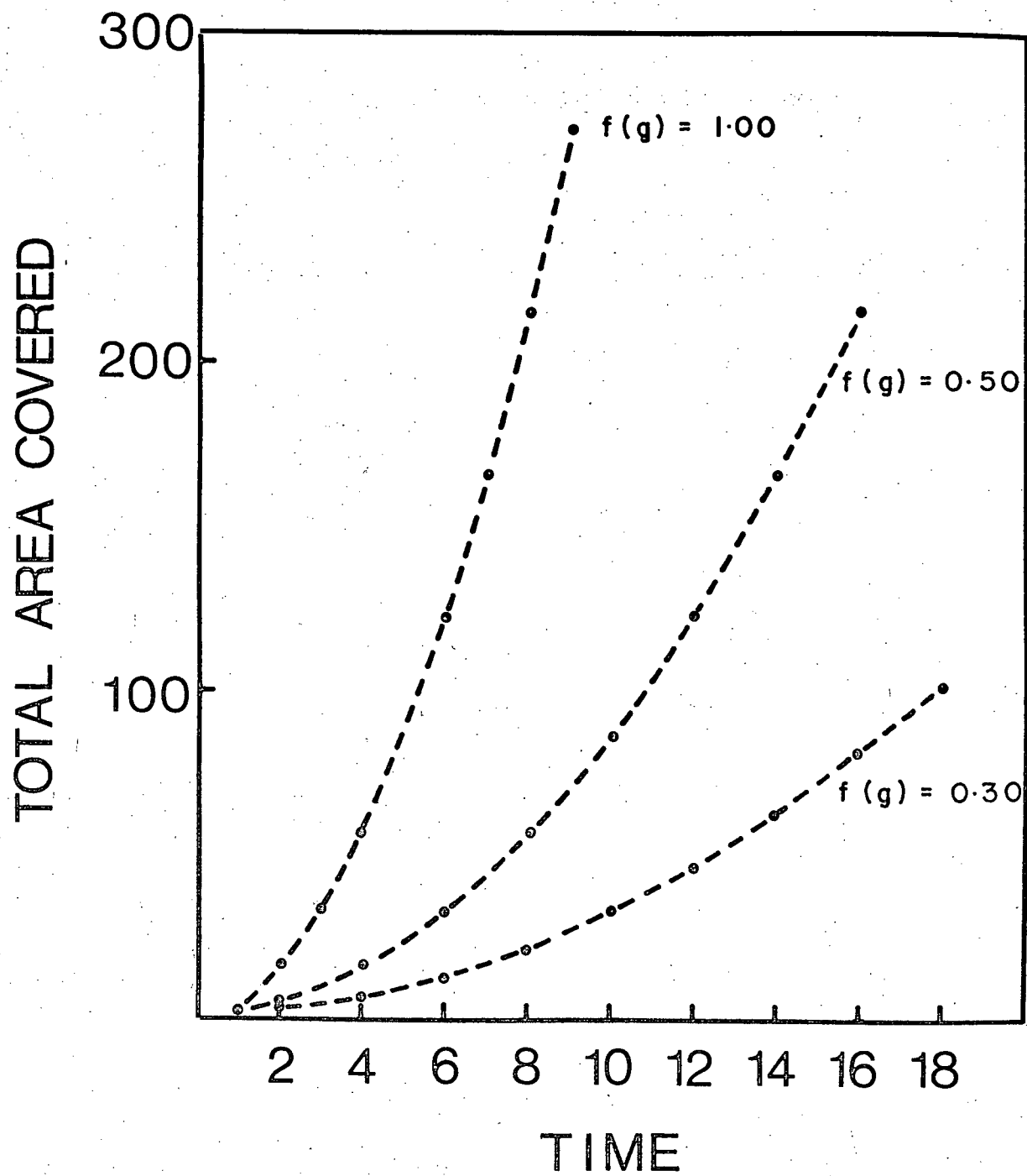
$$\frac{dA}{dt} = [g \cdot 2 \cdot \pi] \cdot (A/\pi)^{1/2} \quad (1b)$$

rearranging and integrating:

$$A = \left[\frac{g \cdot 2 \cdot \pi^{1/2} \cdot t + C}{2} \right]^2 \quad (1c)$$

Figure 9

Areas covered by cellular slime mold cultures growing from point sources as calculated by Program II and equation (1d). Both the time and area units are arbitrary.



The relation between A and t at various values of g was examined using Program II (Appendix I), and the output (Fig. 9) was compared to the actual growth of cellular slime mold cultures. The actual cultures expanded their areas slowly at first, and then moved quickly, with the rate of area acquisition tending towards a constant. This general process is well described by the family of curves generated by equation (1c) and drawn in Figure 9.

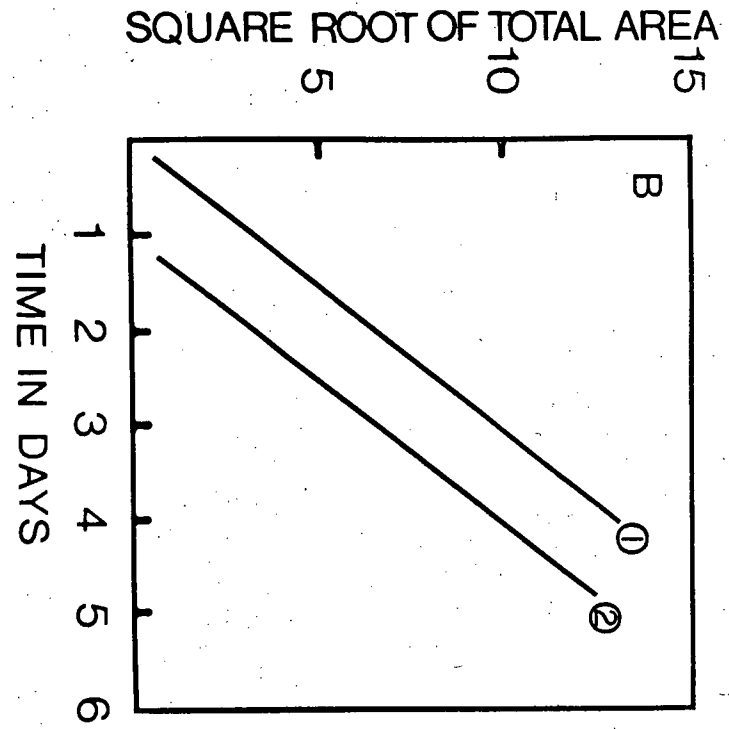
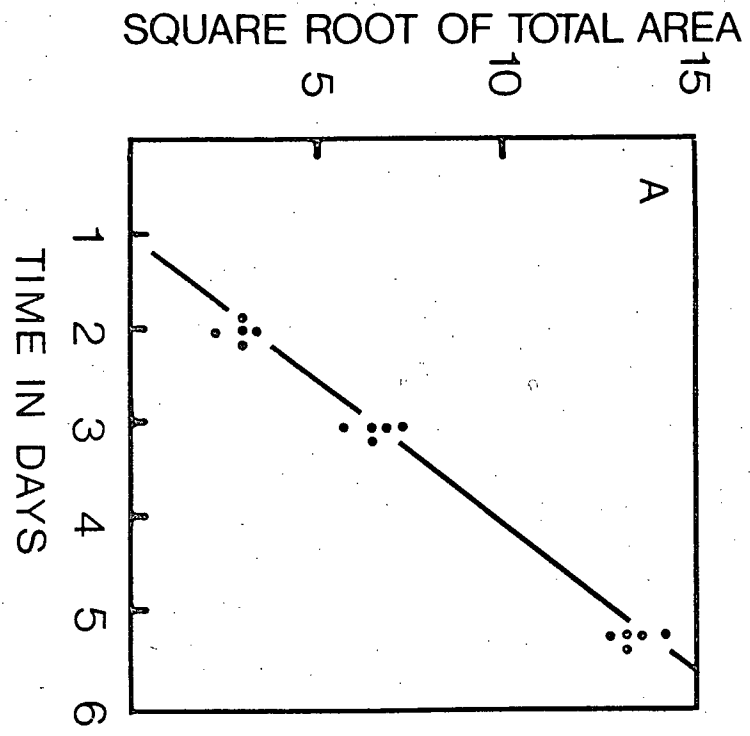
A quantitative comparison is also possible. If the square root of the area calculated from equation (1c) is plotted against time, a straight line should result. To test this hypothesis, several D. discoideum V12 cultures were established with a point source inoculation at a temperature of $18.9 \pm 0.2^{\circ}\text{C}$. This simple experiment yielded two very important pieces of information. When plotted against time, the square root of area did fall along a straight line (Fig. 10-A). Subsequent work has reaffirmed this observation many times. The straight line through the data points (Fig. 10-A) passed through the x-axis at 1.0 days, but the straight line of the same slope predicted by equation (1c) passed through 0.0 days (Fig. 10-B) suggesting that the spore germination lag described in the previous section must be accounted for.

The lag can be incorporated by letting the lag equation (2b) equal a function of temperature $L(T)$. This can then be substituted into (1c) to yield:

Figure 10

Fig.10-A: The square roots of the areas covered by four cultures of D. discoideum V12 are plotted against time in days. The regression line obtained is $Y = -3.73 + 3.61X$.

Fig.10-B: The square roots of the areas generated by equation (1c) with $g = 3.61$ are plotted to form line one. Line two is the actual regression line depicted in Fig. 9-A.



$$A_t = \left[1.7728 g (t - L(T)) + C \right]^2 \quad (1d)$$

The Rate of Amoeba Colony Expansion

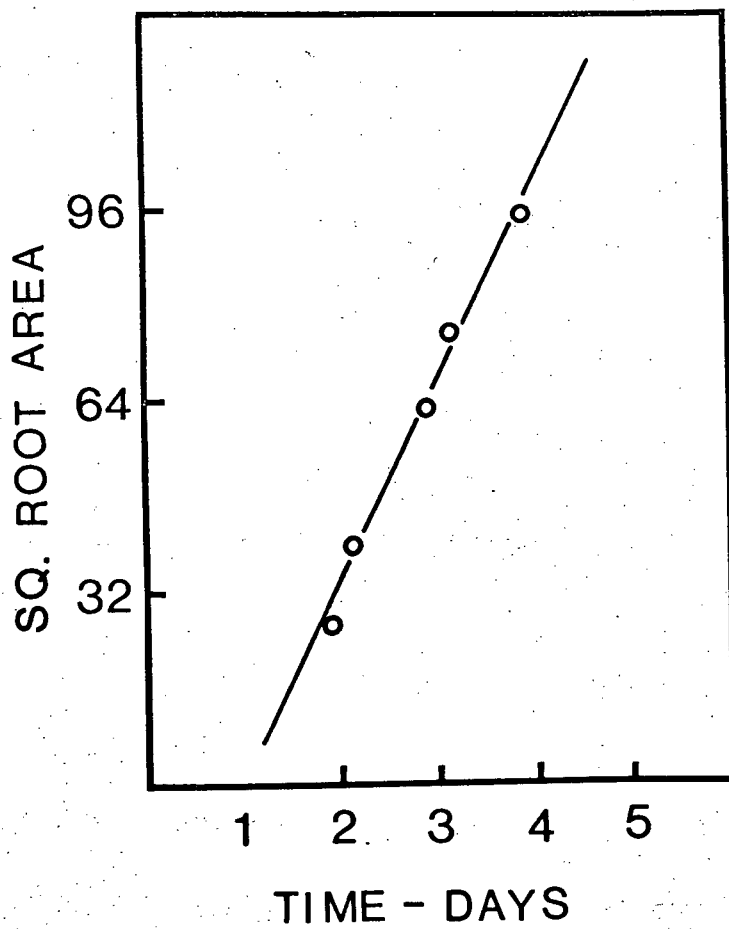
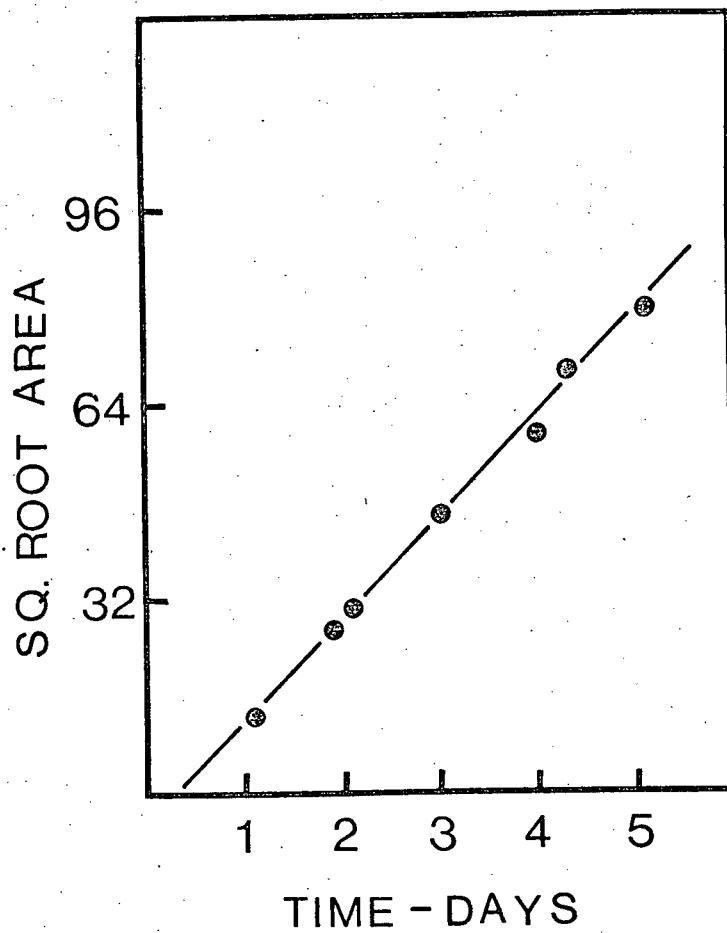
Both D. discoideum and P. pallidum show variations in the rate of colony expansion with variations in temperature. In view of this, the constant g in equation (1d) must be modified to a function of temperature $g(T)$.

To quantify this relationship, cultures were grown at temperatures ranging from 9° to 37.5°C. Two observations were made: (1) There was a straight line relationship between the square root of area and time. Representative cultures are plotted in Figure 11. (2) Both species used all the food and space available. These observations were made in 100% of the 400 - 500 cultures run, but it might be argued that because a small number of data points were available from each culture the straight line relationships observed might be due to a lack of data (it is not difficult to fit a straight line to only 4 or 5 data points). To prove that this hypothesis was incorrect data from many temperatures were transformed and plotted together. A straight line relationship resulted (Fig. 53 - Appendix III) demonstrating that the individual observations and equation (1c) are valid.

Since many cultures were run at each temperature, Program III (Appendix I) was used to calculate the slopes of individual cultures, to group the data to form mean slopes at various temperature intervals, and to plot the individual

Figure 11

The straight line relationship between the square root of area (measured in cm^2) and time is demonstrated for *D. discoideum* VC4 (top) grown at 24.5° and for *P. pallidum* (bottom) grown at 30.5°C .



slopes and mean slopes.

The slope data obtained from the above procedure was then plotted against temperature. The data from D. discoideum V12 (Fig. 12), P. pallidum (Fig. 13), and D. discoideum VC4 (Fig. 14) suggested that the rate of colony expansion increased with increased temperature, reached a maximum at some optimum temperature, and decreased with further temperature increases. The confidence limits (95%) around the individual P. pallidum data points were rather large due to unrecognized bacterial food contamination. However, despite the fact that the source of error was discovered, it was impossible to do the experiments again because the stock had experienced media conditioning.

On the basis of the foregoing data an equation was formulated to describe the relationship between colony expansion rate and temperature. The equation had to meet three conditions: (1) that the rate of expansion be zero at some low temperature T_L , (2) that the rate of expansion be zero at some high temperature T_H , (3) that the rate of colony expansion be optimized at some optimum temperature T_O between T_H and T_L . An example of a curve that meets these conditions is presented in Figure 15. At T_L the expansion rate is zero. From this point the slope (Fig. 15) gradually decreases, reaching zero at $T = T_O$. As T increases beyond T_O the slope decreases rapidly becoming negatively infinite at $T_s = T_H$.

Figure 12

The relationship between growth index and temperature for D. discoideum V12. The points are mean data points $\pm$ 5% confidence limits on the means. The growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from equation (3c) with $T_H = 26.5^\circ$, $T_L = 13.0^\circ$, $T_O = 22.5^\circ$, $G_{max} = 4.5$, $K^H = 0.97009$, and $C = 2.99964$.

Figure 13

The relationship between growth index and temperature for P. pallidum. The points are mean data points $\pm$ 5% confidence limits on the means. The growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from equation (3c) with $T_H = 37.0$, $T_L = 18.0$, $T_O = 30.0$, $G_{max} = 8.3$, $K = 1.71712$ and $C = -4.77034$.

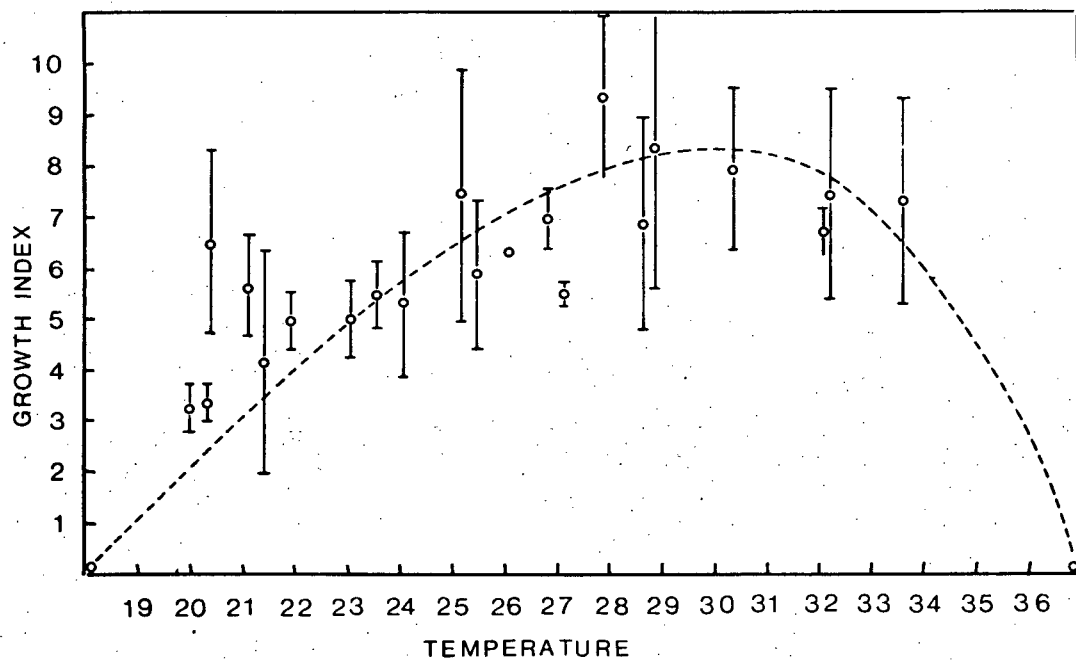
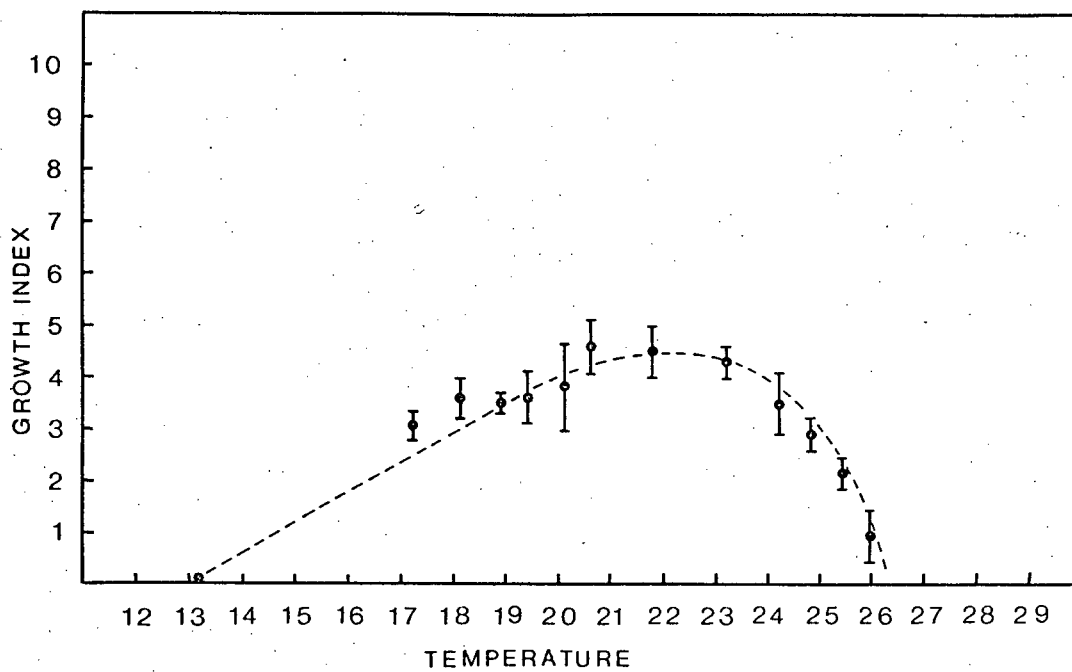
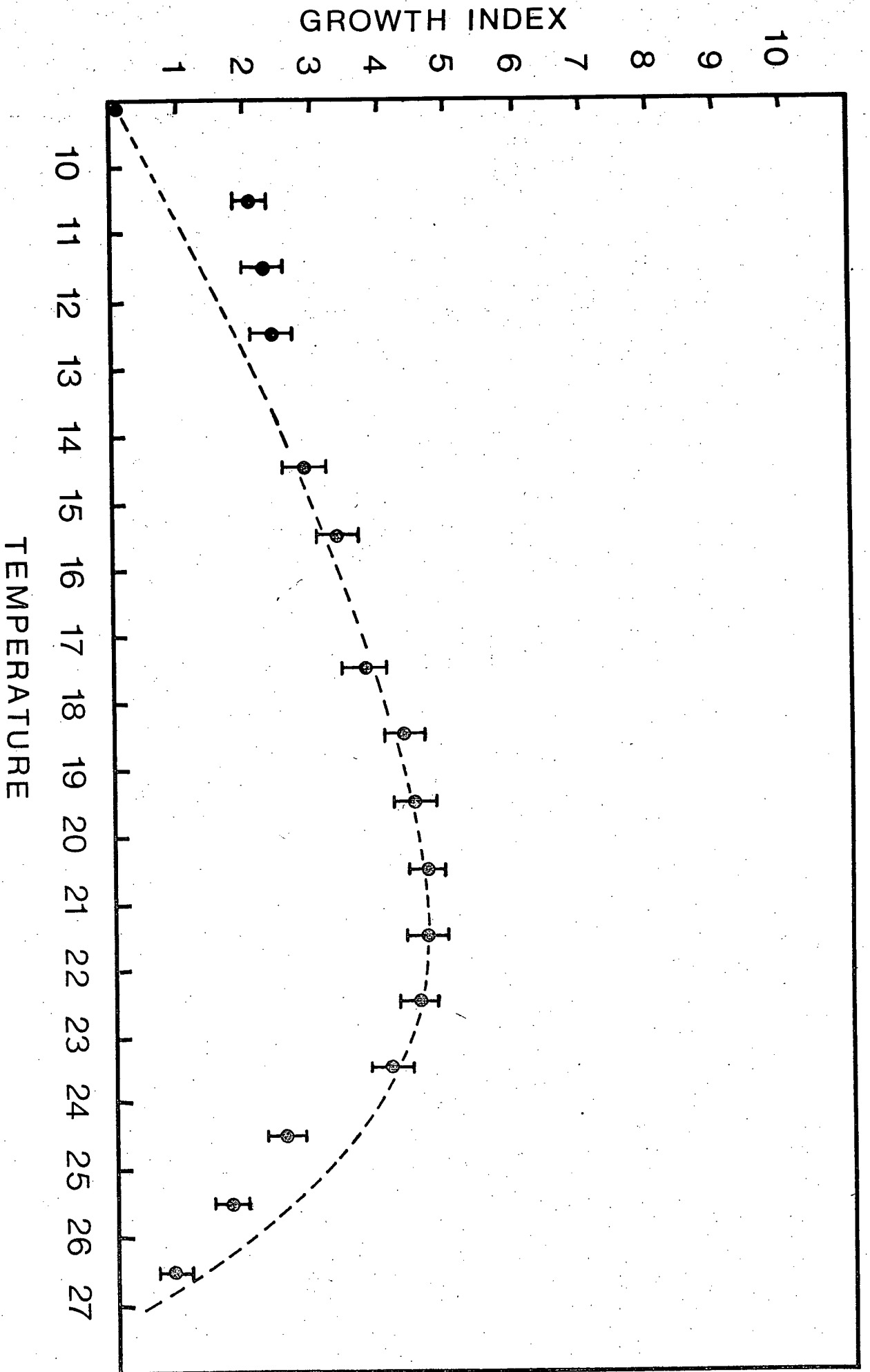


Figure 14

The relationship between growth index and temperature for D. discoideum VC4. The points are mean data points $\pm$ 5% confidence limits on the means. The growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from equation (3c) with $T_H = 27.5^\circ$, $T_L = 9.0^\circ$, $T_0 = 21.5^\circ$, $G_{max} = 4.6$, $K = 0.80084$, and $C = 0.79554$.



One equation which represents this type of response and which fulfils the three requirements stated above is the following:

$$\frac{dG}{dT} = K \left[\frac{T_O - T}{T_H - T} \right] \quad (3a)$$

where dG/dT is the rate of change of area acquisition with respect to temperature, and K is a constant. This equation must be integrated by parts:

$$\int dG = K \cdot T_O \int \frac{dT}{T_H - T} - K \int \frac{T}{T_H - T} dT \quad (3b)$$

Dwight (1947) gives the following identities of integration:

$$\int \frac{dx}{X} = \frac{1}{b} \log |X|$$

$$\int \frac{x \cdot dx}{X} = \frac{1}{b^2} [X - a \log |X|]$$

where $X = a + bx$. Integrating (2b) it is found that:

$$G = [\log |T_H - T|] [K \cdot (T_H - T_O)] - K \cdot T_H + K \cdot T + C \quad (3c)$$

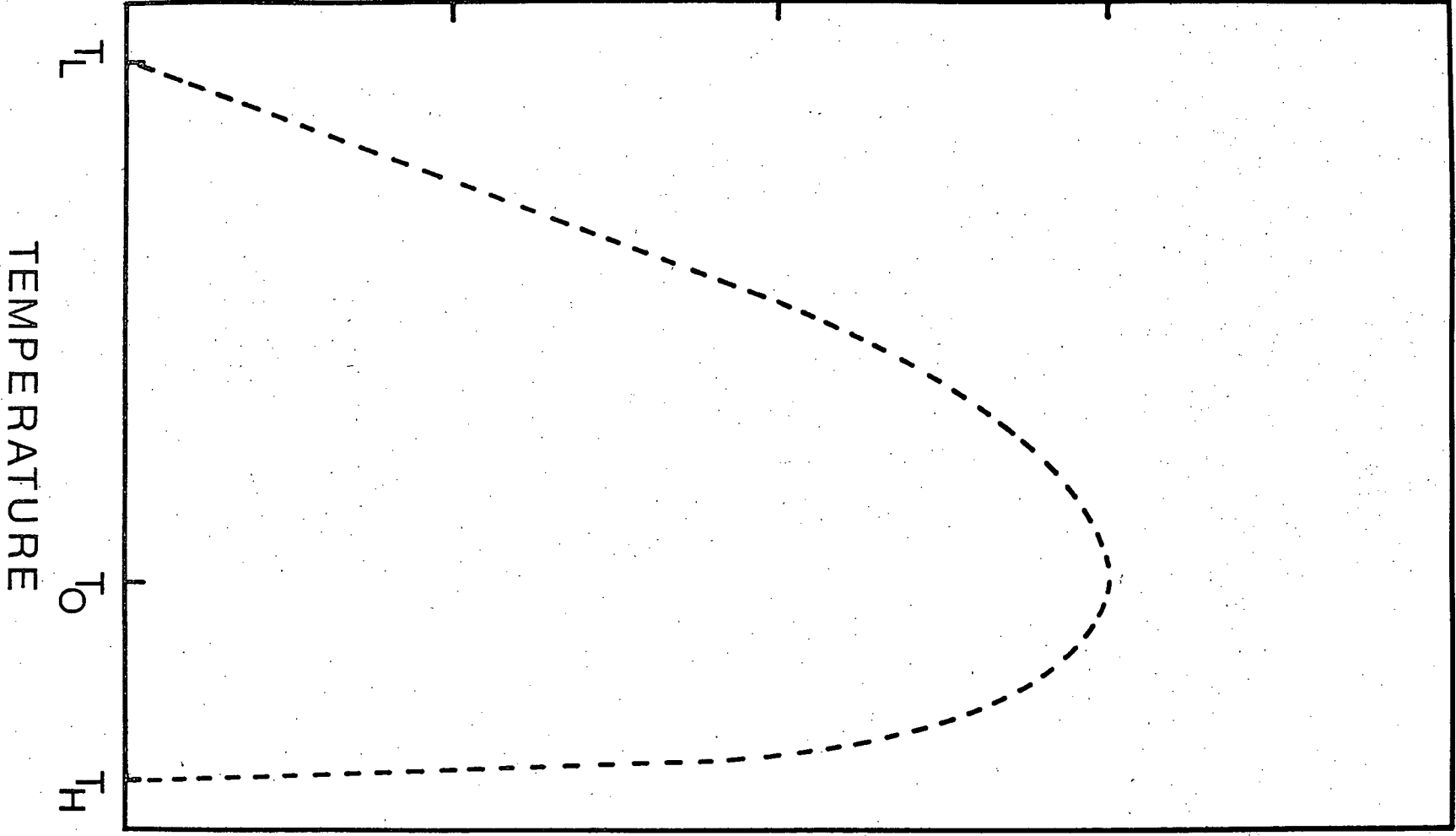
where G is the rate of area acquisition and C is a constant.

But this equation and its assumptions are only hypothetical. It must be proven that it describes the

Figure 15

One of a family of curves designed to describe the relationship between the rate of colony expansion and temperature. At T_L and T_H the rate is zero and T_O the rate is optimum.

GROWTH RATE



relationship between area acquisition and temperature.

To test the descriptive ability of equation (3c) Program IV (Appendix I) was used to fit one curve from the family described by (3c) to each set of data. The line fitted to the D. discoideum V12 data (Fig. 12) explains 94% of the variation. The line fitted to the P. pallidum data (Fig. 13) explains 56% of the variation. The line fitted to the D. discoideum VC4 data (Fig. 14) explains 95% of the variation. All three are "significant" at $\alpha = 0.025$ (Table XIV - Appendix IV). The descriptive power of equation (3c) was accepted in all cases.

With the information provided by equations (1d) and (3c) it was possible to add steps 5, 6 and 7 to the components model outlined in Figure 22 (page 67). After the first amoebae emerge from the spores (AMOEBAE PRESENT) they begin to expand the colony in the form described by equation (1d) and at the rate described by (3c) (COLONY EXP.). This process is temperature dependent. At any point in time the colony size (COLONY SIZE) is known and the amount of food and space (FOOD-SPACE) used can be calculated.

It is also possible to modify equation (1d) to account for the temperature dependence of the colony expansion rate. When $g(T)$ (a summarized form of equation (3c)) is set in place of g in equation (1d) the following equation results:

$$A_t = [1.7728 g(T) (t - L(T)) + c]^2 \quad (1e)$$

TABLE III

D. discoideum V12 - a comparison of growth indexes from cultures inoculated with various spore concentrations.

TEMPERATURE	SPORE CON NUM/ML	MEAN GROWTH INDEX	5% CON LIMIT
17.0	32 000	2.82	$\pm$ 0.22
	80 000	2.66	$\pm$ 0.69
18.2	32 000	3.16	$\pm$ 0.76
	80 000	3.63	$\pm$ 0.46
	20 000	3.79	$\pm$ 0.34
	5 000	2.33	$\pm$ 3.44
19.5	32 000	3.54	$\pm$ 0.45
	80 000	2.79	$\pm$ 0.45
20.7	32 000	4.12	$\pm$ 0.97
	80 000	4.23	$\pm$ 0.78
21.9	32 000	4.92	$\pm$ 3.88
	80 000	5.05	$\pm$ 0.38
	20 000	4.89	$\pm$ 0.35
	5 000	4.83	$\pm$ 0.64
23.4	32 000	4.17	$\pm$ 0.37
	80 000	4.26	$\pm$ 0.29
	20 000	4.70	$\pm$ 1.37
	5 000	4.20	$\pm$ 0.87
24.8	32 000	2.89	$\pm$ 0.31
	80 000	2.80	$\pm$ 0.25

TABLE IV

P. pallidum - a comparison of growth indexes from cultures inoculated with various spore concentrations.

TEMPERATURE	SPORE CON NUM/ML	MEAN GROWTH INDEX	5% CON LIMIT
20.1	100 000	3.50	$\pm$ 2.26
	50 000	2.74	$\pm$ 0.33
20.7	20 000	4.16	$\pm$ 2.10
	5 000	2.96	$\pm$ 1.83
21.9	20 000	5.33	$\pm$ 5.60
	5 000	4.72	$\pm$ 0.95
25.5	100 000	7.10	$\pm$ 2.61
	50 000	5.60	$\pm$ 0.98
28.7	100 000	8.66	$\pm$ 3.16
	50 000	7.67	$\pm$ 0.74

where $g(T)$ is colony expansion rate mediated by temperature.

The hypothesis that initial spore concentration alters the expansion rate must also be tested. This test was conducted by inoculating plates with various spore concentrations, observing and recording the areas covered by the colony, and using Program III to calculate the mean growth index $\pm$ 5% confidence limits. No growth inhibition was detected at the concentrations used, for D. discoideum V12 (Table III) and P. pallidum (Table IV).

Fruiting Body Lag

To this point the development of a cellular slime mold colony has been considered from the spore stage, through the spore germination lag to the amoebae stage, through the development of a colony, to the colony stage. But, as the amoebae reproduce and disperse using up food and space, the amoebae in the center of the colony find themselves in an area without food. Acrasin is then produced and aggregation and fruiting body production begins. These processes require a period of time that might be referred to as the fruiting body lag. This period of time includes the spore germination lag and the time required for aggregation after the amoebae appear.

To quantify the relationship between the fruiting body lag and temperature, data was collected by inoculating agar filled and bacteria covered petri dishes with a known

number of spores, incubating at various temperatures, and noting the time between inoculation and the formation of the first fruiting body. For both D. discoideum VC4 (Fig. 16) and P. pallidum (Fig. 17) the lag decreased with increased temperature reached a minimum at some optimum temperature and increased with further temperature increases.

It appeared that the assumptions pertaining to the model constructed for the germination lag might also be applicable to the fruiting body lag. These assumptions demand that the curve relating fruiting body lag and temperature have an infinitely negative slope at T_L (temperature low) and an infinite slope at T_H (temperature high) and that the slope be zero at T_O (temperature optimum) (Fig. 8). Equation (2b) describes this relationship.

To test the descriptive power of equation (2b) Program I (Appendix I) was used to fit a line to the data. The line relating fruiting body lag and temperature for D. discoideum VC4 explained 96% of the total variability (Fig. 16). The line for P. pallidum explained 97% of the variation. In both cases the fit was "significant" at $\alpha = 0.025$ (Table XIV - Appendix IV). Equation (2b) apparently can be used to describe the relationship between fruiting body lag and temperature.

This new information can be used to expand the components model through the addition of steps 8 and 9 (Fig. 22, page 67). After the first amoebae are present

Figure 16

The time necessary for D. discoideum fruiting body formation is plotted against temperature measured in degrees centigrade. The black dots are data points, the dotted line is fitted from equation (2b).
 $T_H = 27.5$, $T_L = 9.0$, $T_O = 24.0$, $K = 2.47681$,
 $C = 7.62542$.

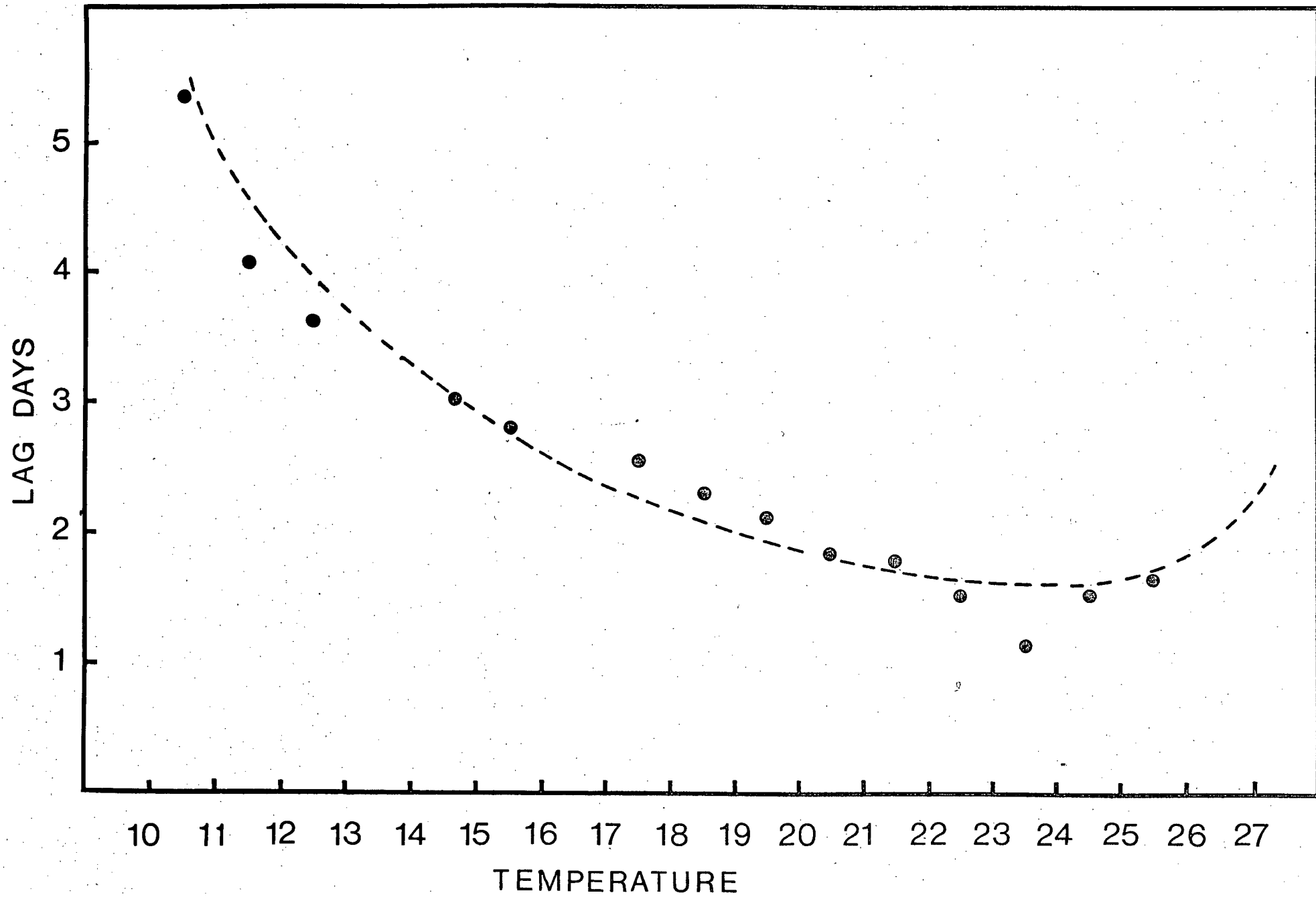
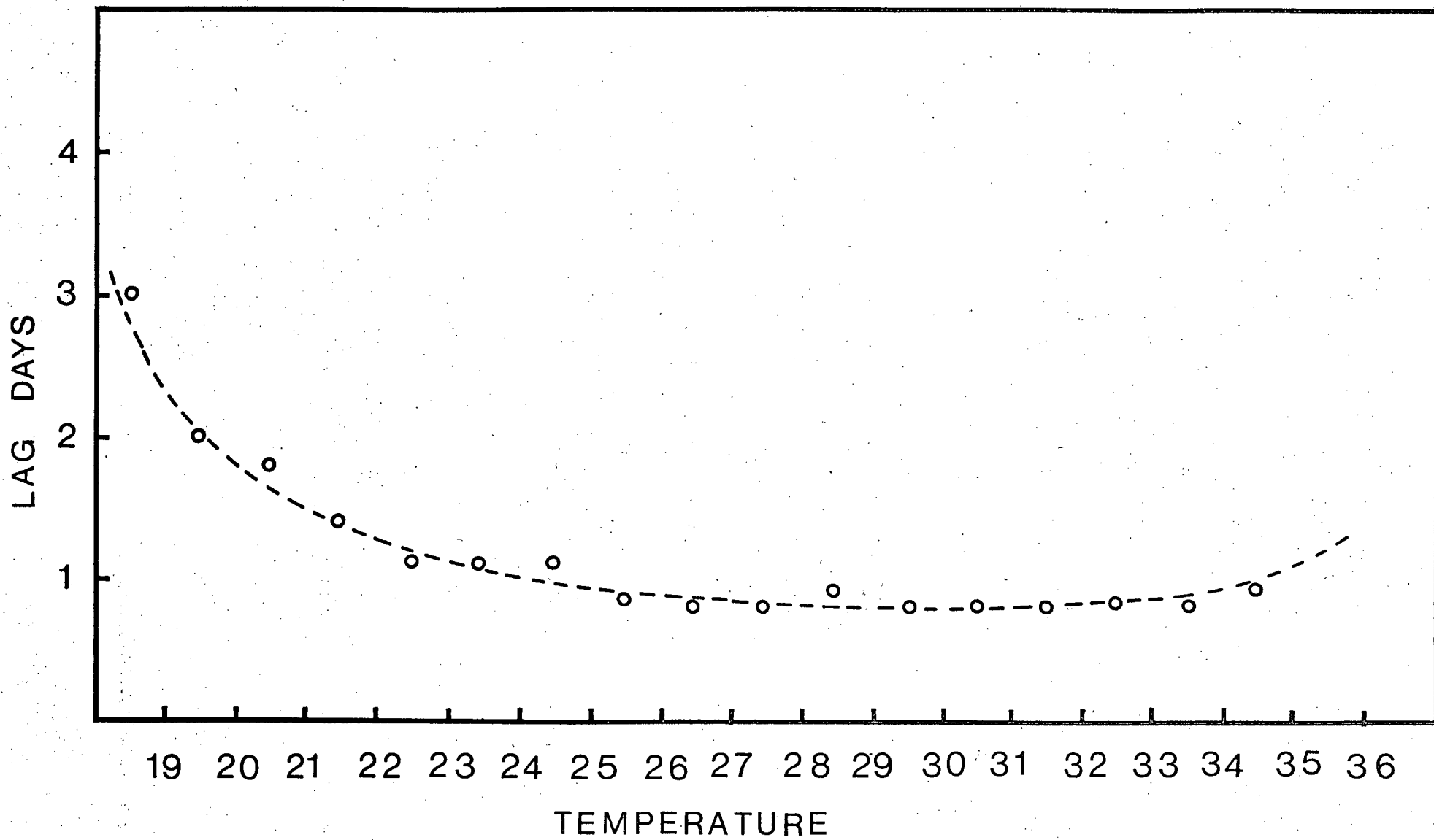


Figure 17

The time for P. pallidum fruiting body formation is plotted against temperature measured in degrees centigrade. The open circles are data points, the dotted line is fitted from equation (2b). $T_H = 37.0$, $T_L = 18.0$, $T_O = 30.0$, $K = 1.28527$, $C = 3.76145$.



there is a temperature dependent lag period (F.B. LAG) before the first fruiting bodies are produced (F.B. PRESENT).

Fruiting Body Formation

Once the first fruiting bodies have formed the number increases as more amoebae aggregate. Both the way in which this process occurs (the form), and the rate at which it occurs (the rate), must be described.

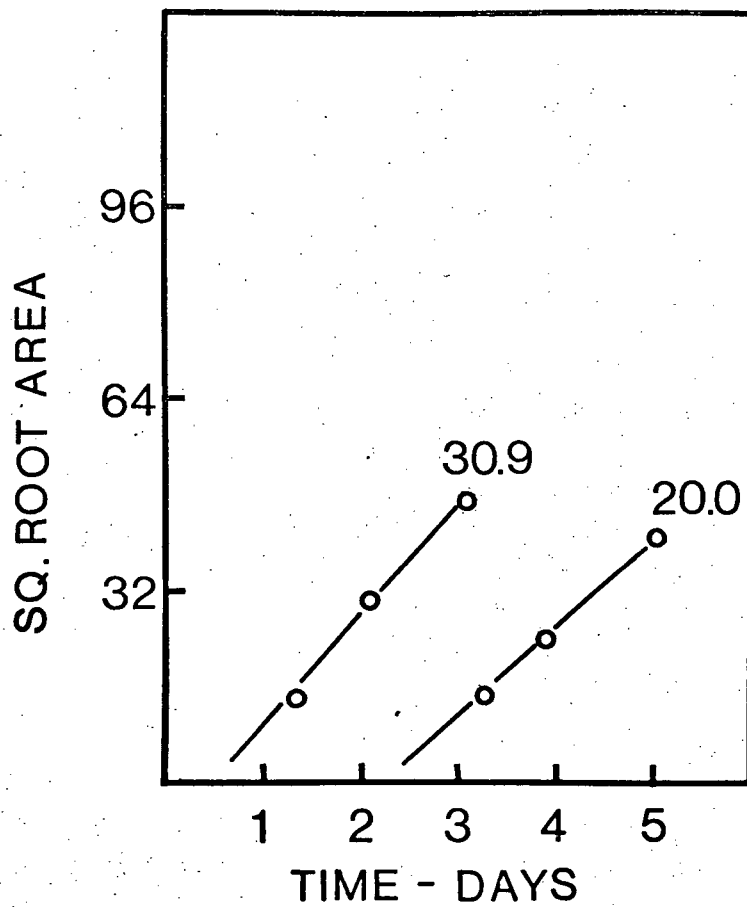
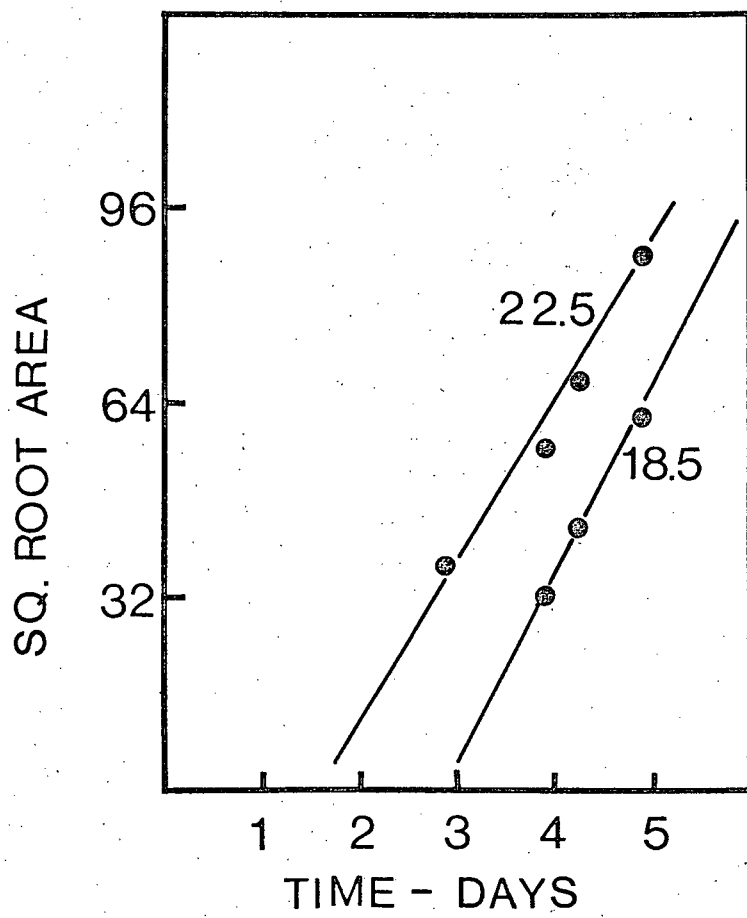
The Form of Fruiting Body Colony Expansion

Fruiting body colonies expand slowly at first and then increase their expansion rates as the area occupied increases. It appeared possible that expansion rate might be directly related to perimeter size just as it was for the amoeba colony expansion. In this case the form of colony expansion should be described by equation (1d).

To test this hypothesis cultures were grown at a number of temperatures and the fruiting body colony area was noted at various intervals of time. The square root of area was then plotted against time and straight line relationships resulted for both D. discoideum VC4 (Fig. 18) and P. pallidum (Fig. 18). These experiments were repeated several hundred times and in every case straight line relationships were found. But, because only a few data points were used for each straight line relationship it might be hypothesized that the straight lines resulted from a lack of data. To test

Figure 18

The straight line relationship between the square root of area (measured in cm^2) and time is demonstrated for D. discoideum VC4 (top) and P. pallidum (bottom). The temperatures at which these cultures were grown are noted on the graph.



this hypothesis, data from D. discoideum VC4 and P. pallidum fruiting body expansions were transformed and plotted together (Fig. 54 - Appendix III). A straight line relationship resulted, demonstrating that the square root of area plotted against time does yield a straight line, and that equation (1c) can be used to describe this relationship.

Two other observations resulted from these experiments. (1) D. discoideum fruiting bodies covered all of the area occupied by the amoeba colony (Fig. 18), and (2) the P. pallidum fruiting body colonies stopped expanding long before they occupied the area used by the amoeba colonies (Fig. 18). In the case of P. pallidum Salvador the amoebae apparently used up all of the food on the plate and then retraced their route back to the center of the plate where one large disorganized fruiting body colony formed. The response is peculiar to this strain of P. pallidum and has been observed by both Dr. Raper (pers. comm.) and myself. The maximum area occupied by P. pallidum fruiting bodies may be expressed as a percentage of the maximum area occupied by the amoeba colonies. When 40 experiments were run it was found that P. pallidum fruiting bodies occupied 14.08 ± 0.85 percent of the maximum area occupied by the amoeba colony (the data are given as the mean $\pm$ the 95% confidence limit around the mean). Also, there was no significant change in the maximum area attained by P. pallidum over the full range of temperature at which this species grew (Table V). All of

TABLE V

The change in maximum area with temperature for P. pallidum.

TEMPERATURE	NUM. REP.	MEAN AREA	95% CON LIMIT
20.5	3	41.0	± 17.2
21.5	5	38.3	± 11.2
22.5	4	39.3	± 5.7
23.5	4	40.5	± 16.1
24.5	4	38.5	± 11.6
25.5	5	38.2	± 12.0
27.5	5	38.5	± 8.3
28.5	6	41.8	± 9.6
29.5	5	39.0	± 11.2
30.5	7	44.7	± 7.0
31.5	5	45.5	± 7.1
32.5	4	44.2	± 16.2
33.5	6	54.1	± 5.3
35.5	5	44.5	± 11.3

the 95% confidence limits, excepting the interval around the mean calculated for 33.5° , overlapped, suggesting that area did not change with temperature (Table V).

In summary, D. discoideum fruiting body colonies cover all of the area covered by the amoeba colony while P. pallidum fruiting body colonies are limited to about 14.08% of the area covered by the amoeba colony. Equation (1c) can be used to describe the form of colony expansion for both.

The Rate of Fruiting Body Colony Expansion

Both D. discoideum and P. pallidum show variations in the rate of fruiting body colony expansion with variations in temperature. Therefore, the constant g in the "form" equation (1c) must be modified to become a function of temperature $g(T)$.

To quantify this relationship data were collected by noting the area occupied by fruiting bodies at various intervals of time. Program III (Appendix I) was used to group the data with respect to temperature, and to calculate mean slopes $\pm$ 95% confidence intervals. The mean slopes and confidence intervals were plotted against temperature. For both D. discoideum VC4 (Fig. 19) and P. pallidum (Fig. 20) the fruiting body expansion rate increased with increased temperature, reached a maximum at some optimum temperature

and decreased with further temperature increase. Apparently equation (3c), which was used to describe the relationship between amoeba colony expansion and temperature, might also be applicable to this situation.

To test this hypothesis curves were fit from equation (3c) using Program IV (Appendix I). For D. discoideum VC4 the fitted curve accounted for 69% of the total variation and the fit was "significant" at $\alpha = 0.025$. For P. pallidum (Fig. 20) the fit was not "significant" at $\alpha = 0.025$ (Table XIV - Appendix IV).

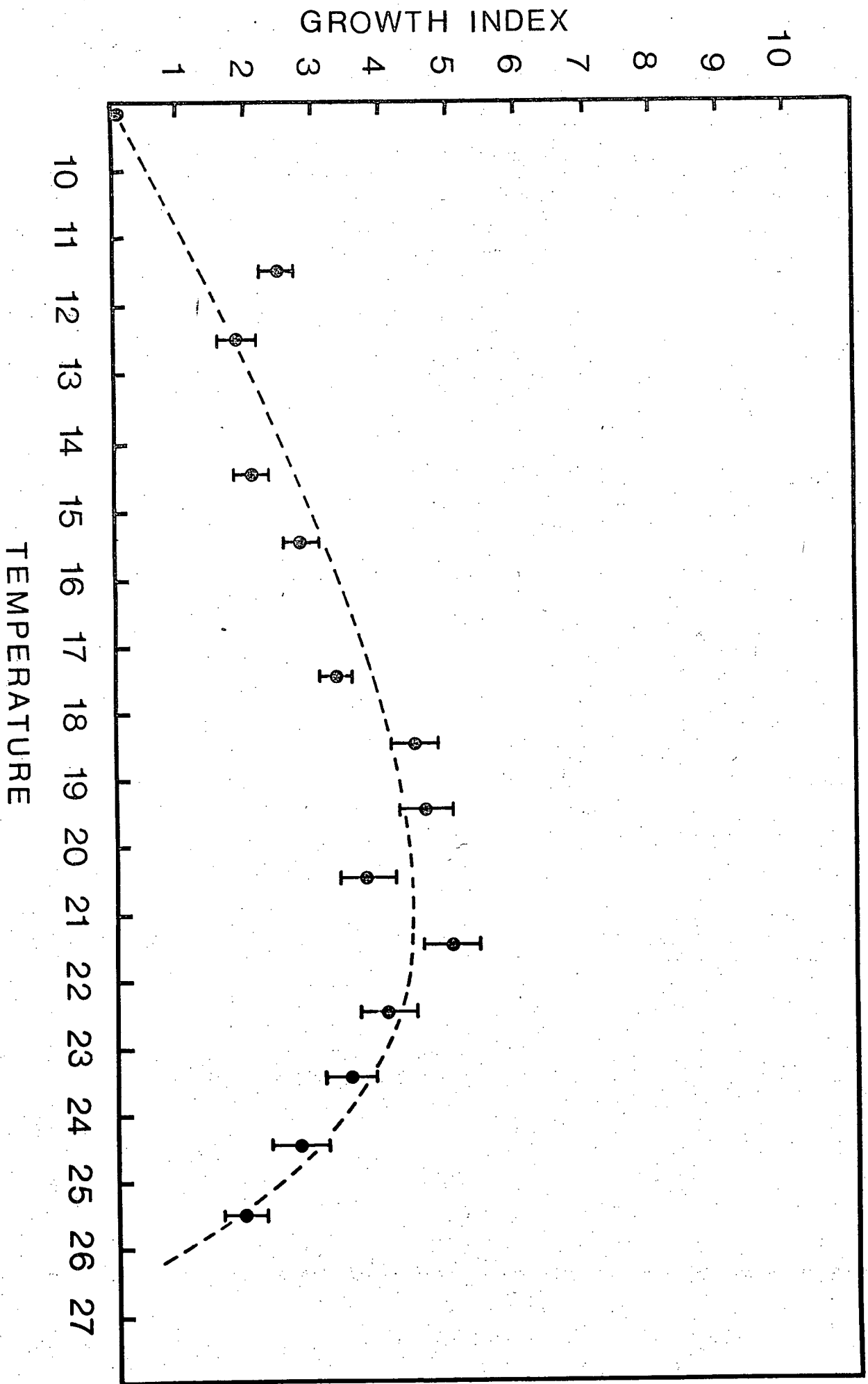
Equation (3c) demands that the expansion rate increases gradually with increased temperature, reaches a maximum at temperature-optimum, and decreases rapidly to negative infinity at some high temperature. However, the P. pallidum data suggests that the rate of colony expansion increases rapidly at T_L , remains approximately constant between T_L and T_H , and decreases rapidly to zero at T_H . In view of these findings, a new equation was developed which meets the special criteria imposed by P. pallidum fruiting body formation. Such an equation is:

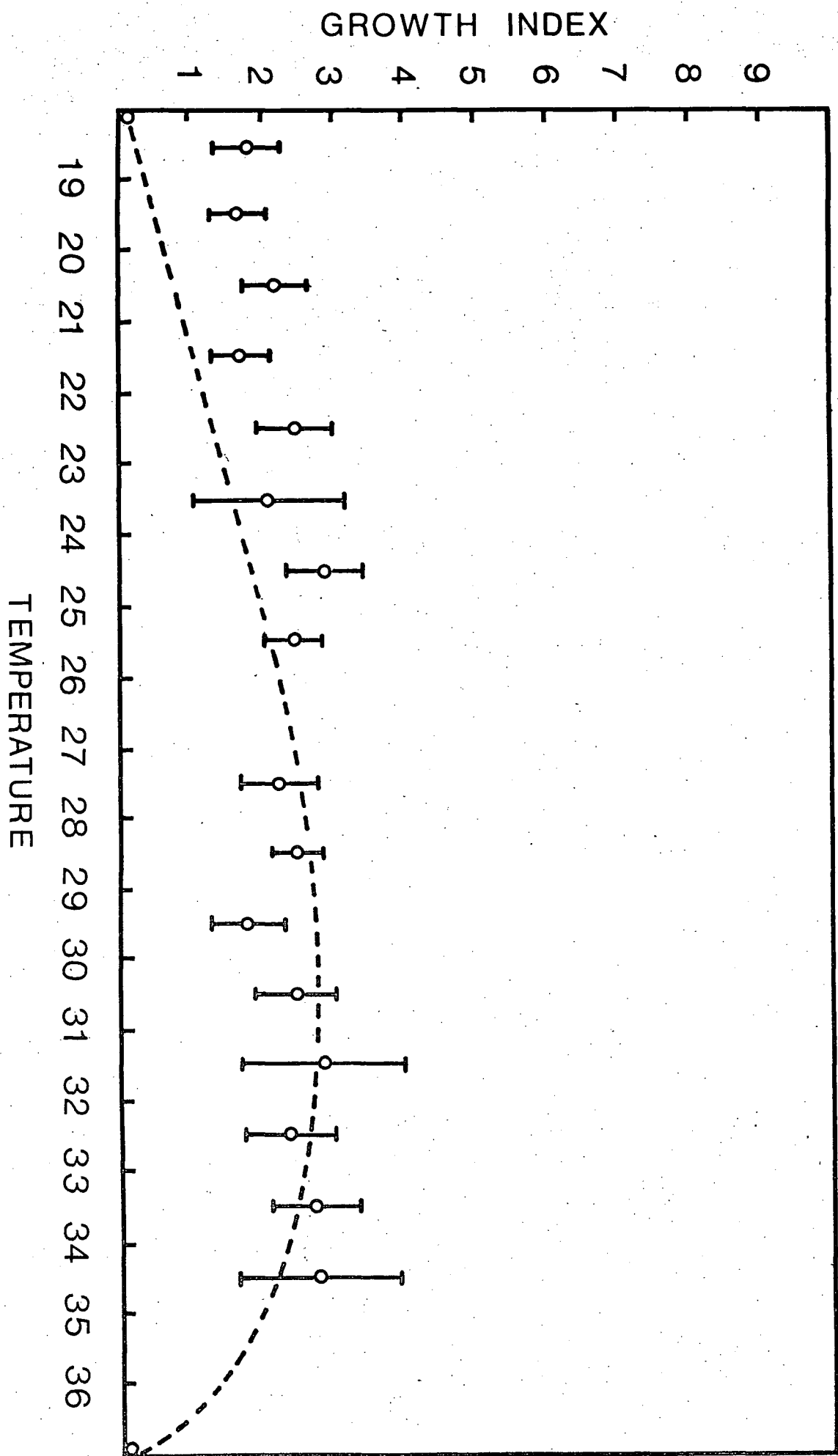
$$\frac{dG}{dT} = K \left[\frac{T_o - T}{(-1)T^2 + T \cdot T_H + T \cdot T_L - T_H \cdot T_L} \right] \quad (4a)$$

where dG/dT is the rate of change of colony expansion with respect to temperature, T is temperature, T_o is temperature

Figure 19

D. discoideum VC4; fruiting body expansion rates with respect to temperature. The points are mean data points $\pm$ 5% confidence limits on the means. The growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from equation (2c) with $T_H = 27.5$, $T_L = 9.0$, $T_O = 21.0$, $G_{max} = 4.4$, $K = 0.86318$, and $C = -0.65368$.





optimum, T_L is temperature low, T_H is temperature high, and K is a constant.

Using two identities of integration (Dwight 1947) this equation can be integrated to yield:

$$G = -K \left[(-.5 \log |(-1)T^2 + T(T_H + T_L) - T_H \cdot T_L|) - \left[\left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right] \right] + \quad (4b)$$

$$-K \cdot T_0 \left[\left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right] + C$$

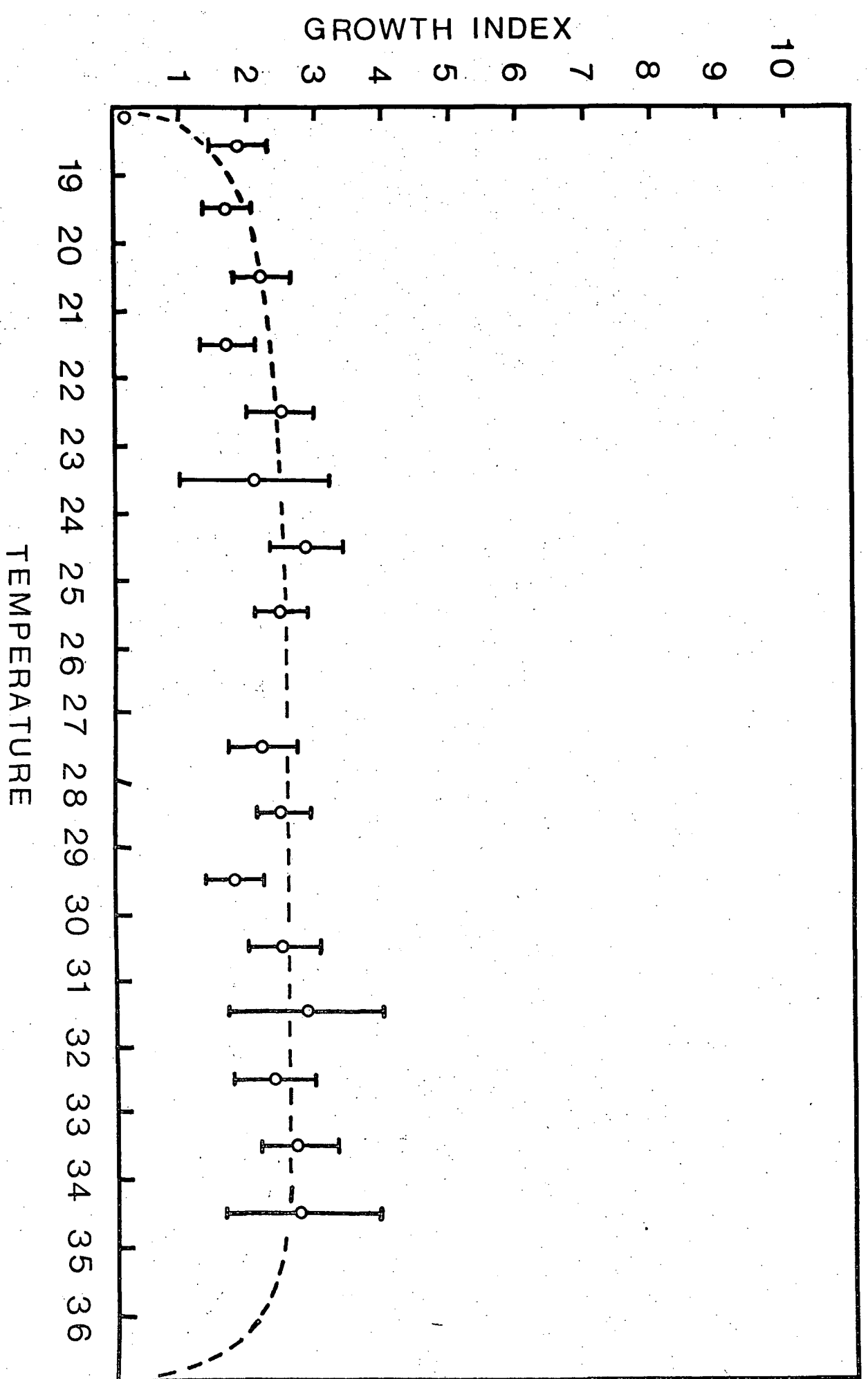
where G is the expansion coefficient and C is a constant of integration.

This equation was fit to the data (Fig. 21) using Program V (Appendix I). The line accounted for 75% of the total variation and the fit was "significant" at $\alpha = 0.025$ (Table XIV - Appendix IV). Therefore, equation (4b) was accepted as an adequate description of the relationship between P. pallidum fruiting body expansion rate and temperature.

The information considered in this section can be incorporated into the components diagram (Fig. 22, page 67) as steps 10, 11, 12 and 13. After the first fruiting bodies are formed (F.B. PRESENT) the colony expands (F.B. EXP) with

Figure 21

P. pallidum fruiting body expansion rates with respect to temperature. The points are mean data points $\pm$ 5% confidence limits on the means. The growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from equation (4b) with $T_H = 37.5$, $T_L = 18.0$, $T_O = 31.0$, $K = 0.78172$, and $C = 0.97552$.



the rate of expansion mediated by temperature. At any point in time the area occupied by fruiting bodies is known (F.B. AREA) and it is possible to convert area to spore numbers (S. PER AREA). For D. discoideum VC4, $6.4 \text{ mm}^2 = 47000 \pm 18000$ spores and for P. pallidum, $6.4 \text{ mm}^2 = 300000 \pm 62000$ spores (mean $\pm$ 95% confidence limit). The spores can then produce new amoebae in a food renewed situation. The thirteenth component (LIMIT), limits the area covered by fruiting bodies. D. discoideum fruiting bodies can cover 100% of the area occupied by the amoebae, P. pallidum fruiting bodies can occupy 14.08% of the area occupied by amoebae.

Testing the Exploitation Models

Both species have been described by models which should predict the amount of food and space taken up by the amoebae and fruiting body colonies at any point in time. Before these models can be used it must be proven that they actually mimic the expansion of a cellular slime mold colony.

The predictive power of the two models was tested by simulating the growth of both species (Program VI - Appendix I) and comparing the output to independent experimental data. Data were collected at eleven temperatures for D. discoideum VC4 amoebae and in every case there was no significant difference (at the 95% level) between the observed and the predicted (Table VI). Data were collected at eleven

TABLE VI

D. discoideum VC4 amoebae. The output from Program VI is compared with independently collected area data. A star indicates a significant difference (95% level) between the data and the output.

TEMP.	TIME	REP. NO.	95% CON AROUND MEAN AREA	PREDICTED AREA
14.5	5.0	6	138.4 - 168.2	120
15.5	4.0	6	95.9 - 135.3	97
16.5	4.0	6	84.5 - 184.4	126
17.5	4.0	6	153.5 - 217.4	158
18.5	4.0	6	181.4 - 239.2	190
19.5	4.0	7	203.2 - 263.6	218
20.5	3.3	6	115.3 - 196.6	151
21.5	3.3	6	131.0 - 214.6	160
22.5	3.3	6	124.0 - 233.2	146
23.5	3.3	5	73.7 - 185.8	142
24.5	4.0	4	110.7 - 197.7	169

TABLE VII

P. pallidum amoebae. The output from Program VI is compared with independently collected data. A star indicates a significant difference (95% level) between the output and the data.

TEMP.	TIME	REP. NO.	95% CON AROUND MEAN AREA	PREDICTED AREA
18.5	5.0	6	26 - 67	10.6*
19.5	3.9	7	23 - 62	25.4
20.5	3.9	6	34 - 107	57.1
21.5	3.9	9	95 - 178	102.7
22.5	3.9	3	134.8 - 257.8	160.8
23.5	3.3	5	124.7 - 212.4	154
24.5	3.2	4	154.4 - 275.5	192
25.5	3.2	3	159.4 - 285.2	243
27.5	3.0	4	223.4 - 294.5	294
28.5	2.3	6	162.1 - 201.8	173
29.5	2.4	3	21.4 - 289.9	209
30.5	2.2	5	98.1 - 272.2	176
32.5	2.2	4	235.2 - 261.7	167*
33.5	2.2	7	136.7 - 230.6	146

TABLE VIII

D. discoideum VC4 fruiting body. The output from Program VI is compared with independently collected area data. A star indicates a significant difference (95% level) between the output and the data.

TEMP.	TIME	REP. NO.	95% CON AROUND MEAN AREA	PREDICTED AREA
14.5	4.8	6	17.3 - 22.3	20.5
15.5	4.8	6	25.6 - 43.9	37.7
16.5	4.8	6	28.2 - 60.4	60
17.5	5.0	6	78.6 - 123.0	99
18.5	5.0	6	106.4 - 185.2	130
19.5	4.1	7	77.8 - 107.8	79
20.5	4.1	6	48.4 - 113.5	93
21.5	4.1	6	130.7 - 160.2	101*
22.5	4.1	6	74.8 - 167.4	99
23.5	4.1	5	64.3 - 163.6	83
24.5	4.0	4	0.0 - 72.6	49

TABLE IX

P. pallidum fruiting body. The output from Program VI is compared with independently collected area data. A star indicates a significant difference (95% level) between the output and the data.

TEMP.	TIME	REP. NO.	95% CON AROUND MEAN AREA	PREDICTED AREA
19.5	5.0	3	11.8 - 26.8	32*
20.5	3.7	5	13.9 - 20.4	19
21.5	3.8	5	19.9 - 30.8	30
22.5	3.9	4	33.5 - 44.9	41
24.5	3.2	3	24.8 - 57.1	32
25.5	3.2	3	13.6 - 70.3	36
27.5	3.2	3	37.7 - 46.5	41
28.5	3.2	5	37.4 - 52.1	43
29.5	3.2	3	13.0 - 57.6	44
30.5	3.2	7	37.7 - 51.6	44
32.5	3.3	6	36.7 - 53.8	45

temperatures for D. discoideum VC4 fruiting bodies and in ten of the eleven cases there was no significant difference between the observed and the predicted (Table VII). Data were collected at fourteen temperatures for P. pallidum amoebae, and in twelve of the fourteen cases there was no difference between the observed and the predicted (Table VIII). Data were collected at eleven temperatures for P. pallidum fruiting bodies and in ten of eleven cases there was no difference between the observed and the predicted (Table IX).

These data seem to support the contention that the two exploitation models do describe both the form and the rate of colony expansion for both species over the entire range of temperature used.

Summary: Exploitation

- (1) The cellular slime mold life cycle is composed of five steps: spores germinate to produce amoebae which divide, disperse, and consume food, the amoebae aggregate, fruiting bodies form, and the fruiting bodies contain spores.
- (2) Since food is the resource in short supply, only this step in the life cycle is directly relevant to the study of exploitation.
- (3) But the rate of food use depends upon all the other steps if any more than one generation is considered, therefore all the steps were modelled.

- (4) The spore germination lags, and the fruiting body production lags were both temperature dependent and were described by equation (2b).
- (5) The form of amoeba colony expansion and fruiting body colony expansion was described by equation (1c) because the rate of expansion was directly proportional to the perimeter of the colony.
- (6) The rate of amoeba and fruiting body colony expansion was temperature dependent. Amoebae colony expansion rates for both species, and D. discoideum fruiting body expansion rates were described by equation (3c). P. pallidum fruiting body expansion rates were described by equation (4b).
- (7) The components were linked together (Fig. 22) to form exploitation models which were tested against independent data. The model proved to be accurate in at least 90% of the cases.

The Exploitation - Competition Simulation

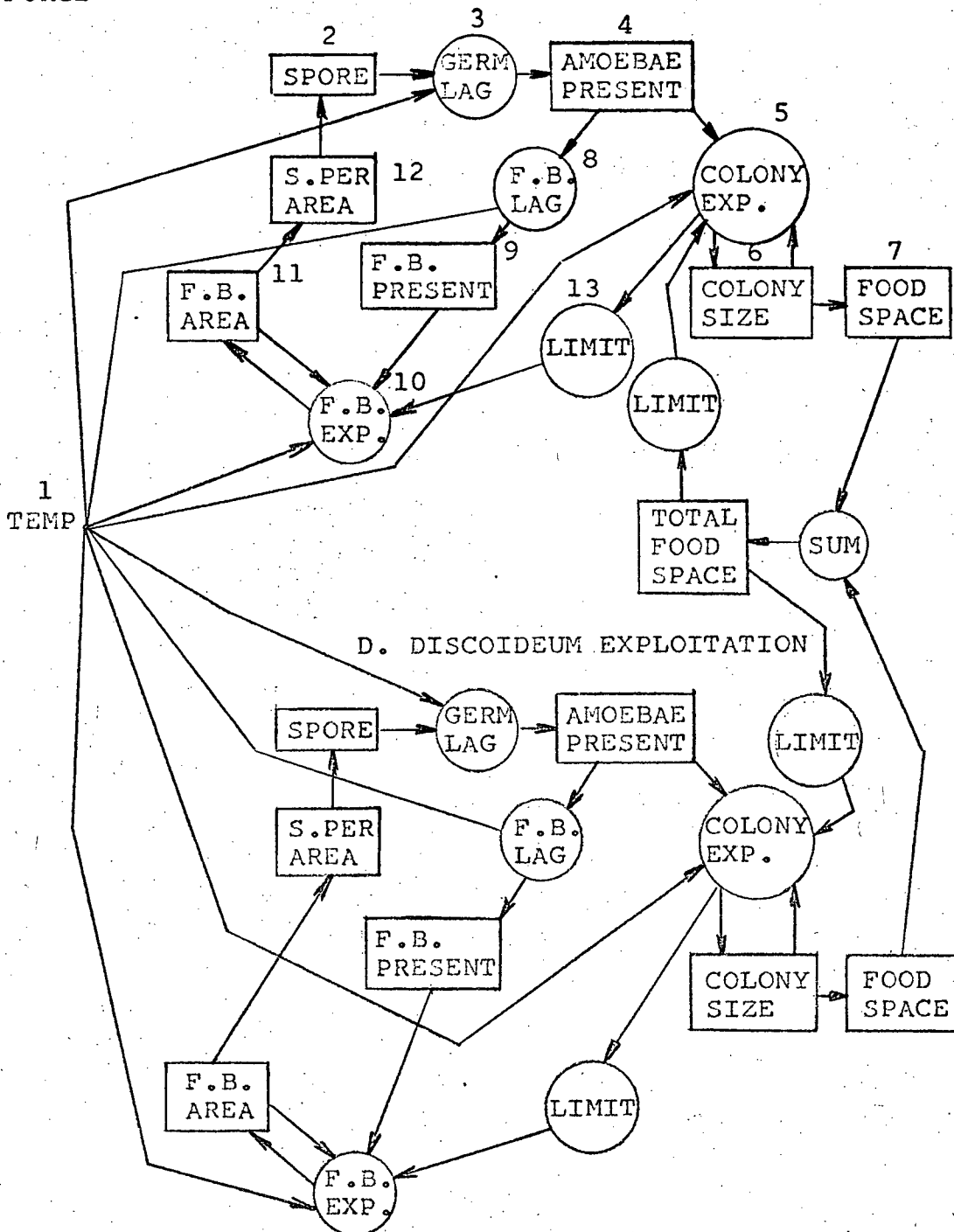
If the two species, when grown together, only exploit their environment (in this case: compete for food and space) it should be possible to predict exactly what the two species will do when put together, on the basis of the independently derived exploitation models. If the two exploitation models fail to predict the outcome of a real

Figure 22

Diagrammatic representation of the exploitation model simulated in Program VII. The abbreviations are explained in the text.

EXTERNAL
FORCE

P. PALLIDUM EXPLOITATION



competitive situation then it must be assumed that the competitors are directly interfering with one another.

A competitive situation for cellular slime mold species in the laboratory was simulated by joining the two independent exploitation models together in Program VII (Appendix I). Figure 22 illustrates the system used. All but four components were identified in previous sections. The "SUM" component sums the food and space used by both species. It outputs the "TOTAL FOOD-SPACE" used by both species. This quantity inputs to two "LIMIT" components which stop the "COLONY EXPANSION" components when all the food and space has been used.

The results of the exploitation competition simulation can be assessed at two levels: (1) the presence or absence of P. pallidum and D. discoideum at any temperature, and (2) the amount of food and space used by amoebae and fruiting bodies at any time and temperature.

At level (1) the model predicts that from 9.0° to 18.0°C D. discoideum should be the only species able to grow and consume food (Fig. 23). From 18.1° to 26.5°C both species should be capable of growing and using food (D. discoideum becomes less fit as the temperature increases towards 26.5°C and P. pallidum becomes fit). Beyond 26.5°C and up to 37.5°C P. pallidum is the only species that should be able to consume food and fruit (Fig. 23). At level (2) the various areas occupied by fruiting bodies and amoebae of

both species were output by the simulation at intervals of 2.4 hours (Fig. 23).

The predictions were tested by growing the two species together. Tests were conducted at the presence or absence level (level 1) by inoculating bacteria covered plates with single drops of water containing known numbers of spores. Both species did not fruit between 18.0° and 26.5°C as the exploitation-competition simulation predicted (Fig. 24, Fig. 25). These data also indicated that D. discoideum V12 never fruits above 24.3°C and that the probability of P. pallidum fruiting below 24.3°C depends upon the initial P. pallidum concentration. It should be noted that the data are such that only the presence or absence of fruiting bodies is detected. Amoebae may be present on non-fruiting body plates but since the two species are indistinguishable at the amoebae stage, it is impossible to determine which amoebae occupy any given plate. This shortcoming was circumvented by experiments which will be presented later. Now it is enough to know that the model predicts that both D. discoideum and P. pallidum should fruit between 18.0° and 26.5°C when in fact D. discoideum V12 never fruits above 24.3°C and P. pallidum fruits below 24.0°C only under conditions of high spore concentration.

In short, the exploitation - competition model does not completely describe the competitive interaction, and the two species must directly interfere with one another.

Figure 23

Output from the exploitation - competition model. The results over five successive days are shown. Area covered (proportional to the amount of food used) is plotted on the y-axis. Temperature along the x-axis. Four quantities are represented:

- (1) Area covered by D. discoideum amoebae (solid line).
- (2) Area covered by D. discoideum fruiting bodies (dotted line).
- (3) Area covered by P. pallidum amoebae (dashed line).
- (4) Area covered by P. pallidum fruiting bodies (dotted and dashed line).

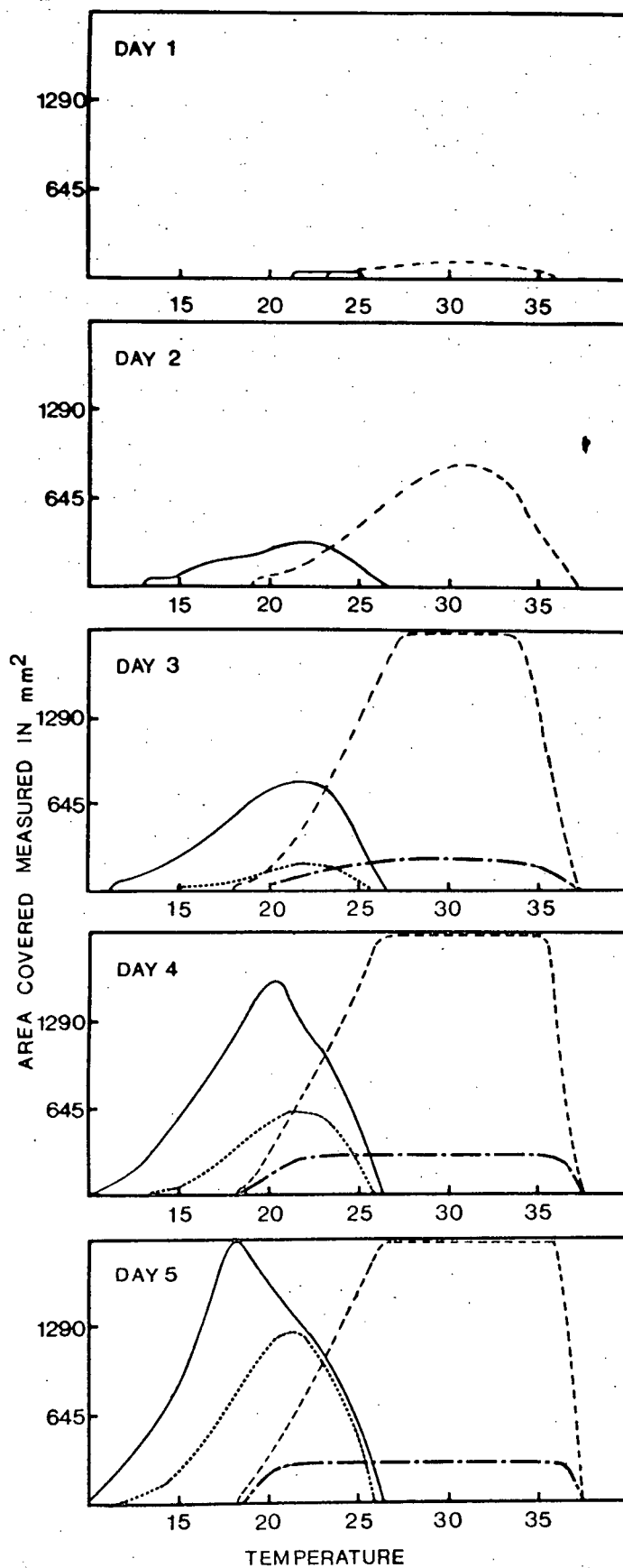


Figure 24

The presence or absence of D. discoideum and P. pallidum fruiting bodies is noted with respect to (1) temperature - x-axis, (2) relative spore concentration - y-axis, (3) absolute spore concentration. A black dot indicates that D. discoideum V12 fruited at any temperature and relative concentration. An open circle indicates that P. pallidum fruited. The figure is divided into four sections to denote the various absolute spore concentrations used: (a) 8800 spores per plate, (b) 4400 spores per plate, (c) 2200 spores per plate, (d) 1100 spores per plate. An example: If a culture were set up with a total spore concentration of 8800 (part a), and this total was composed of 4400 D. discoideum V12 and 4400 P. pallidum (5/5), and if this culture were grown at 22.3° then only D. discoideum V12 would fruit.

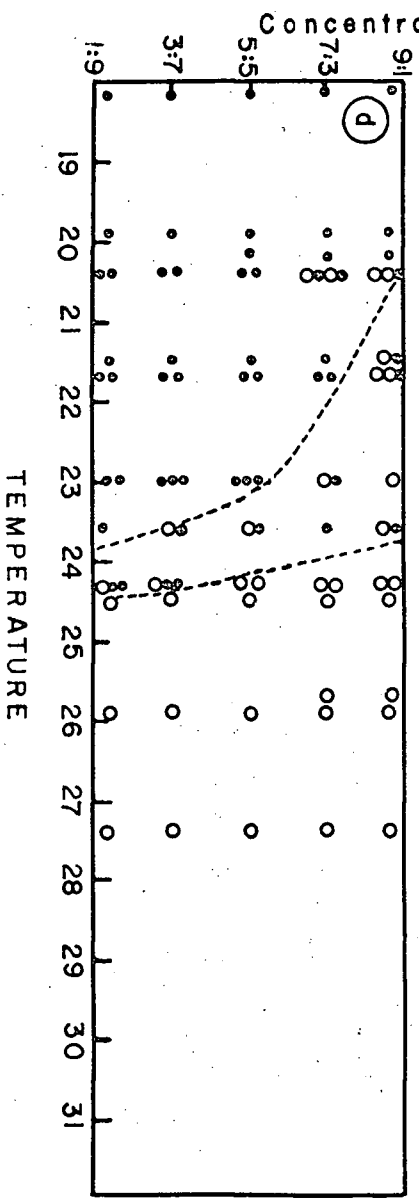
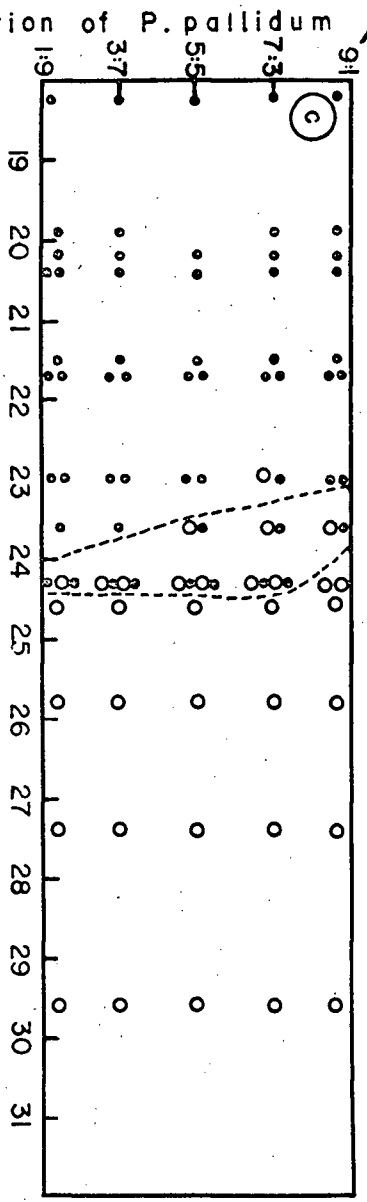
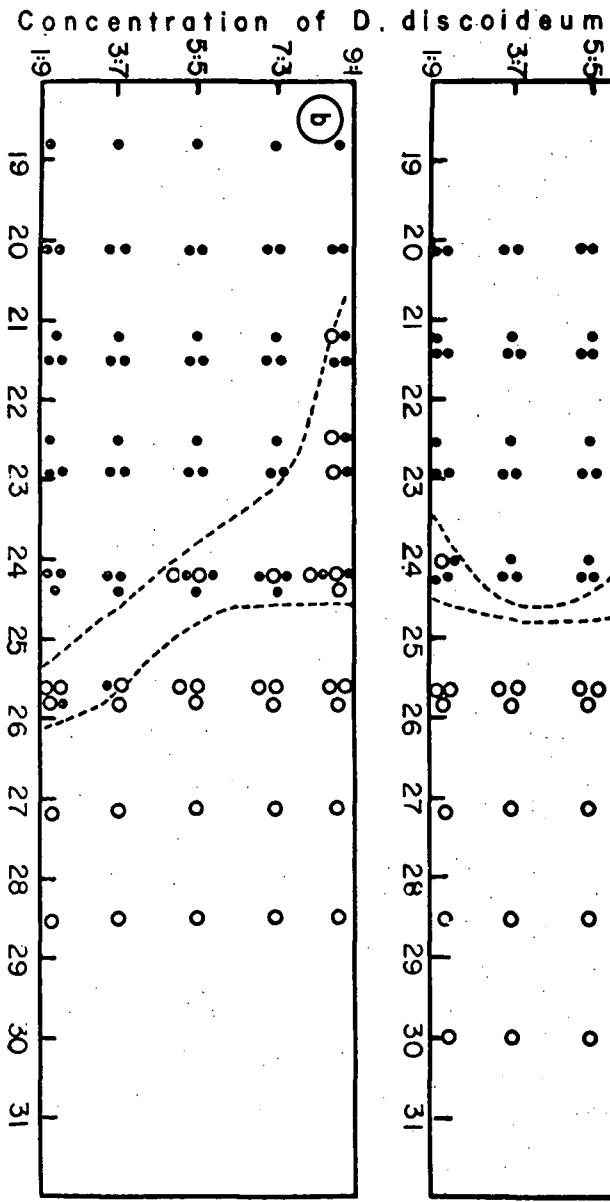
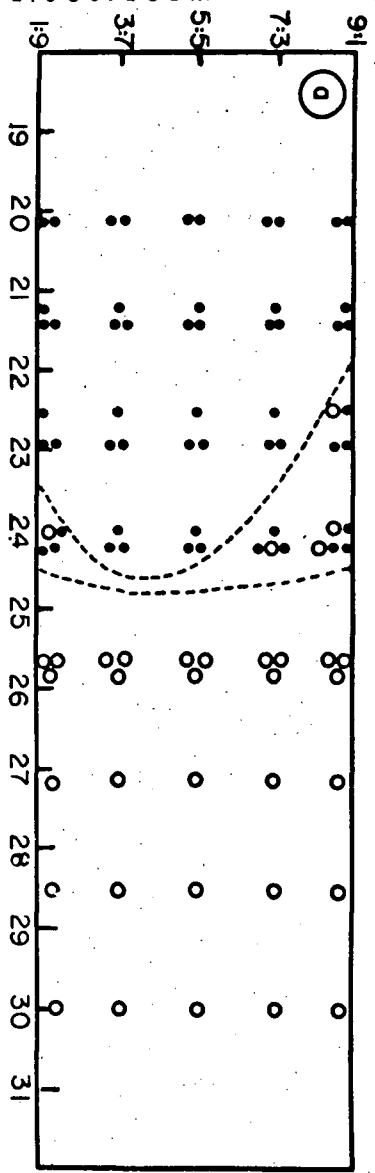
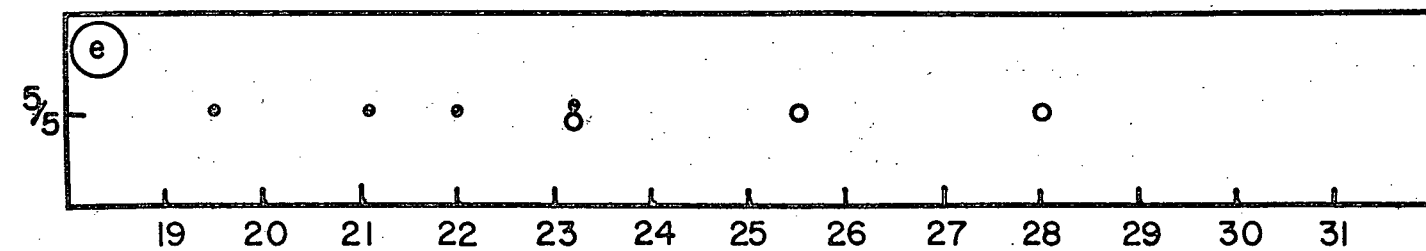
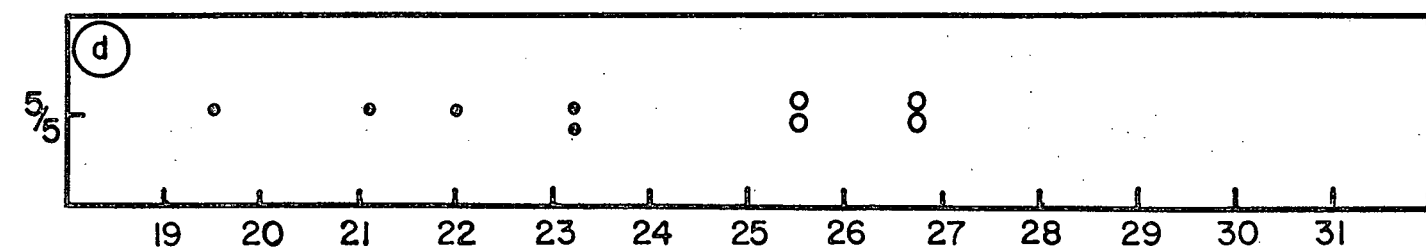
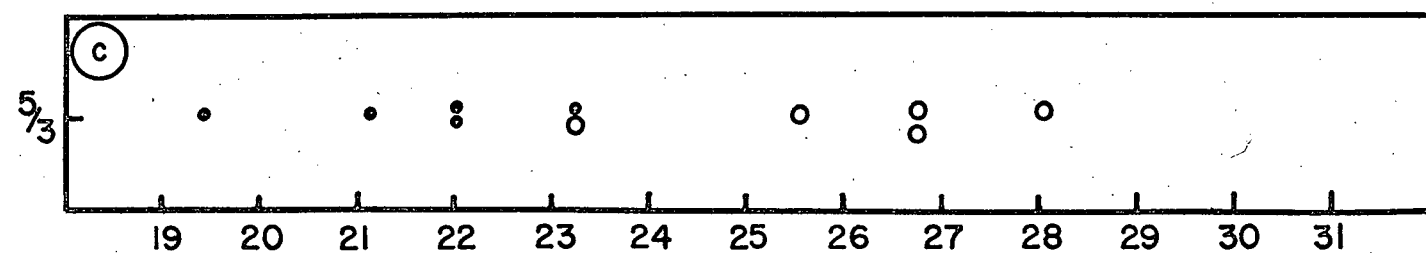
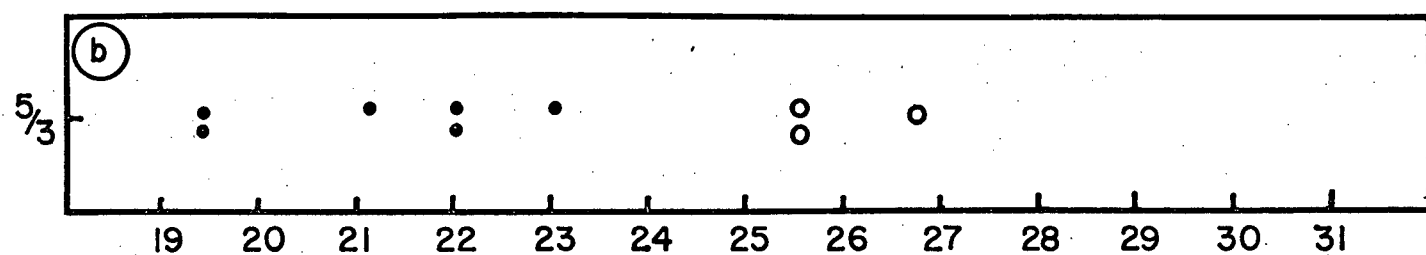
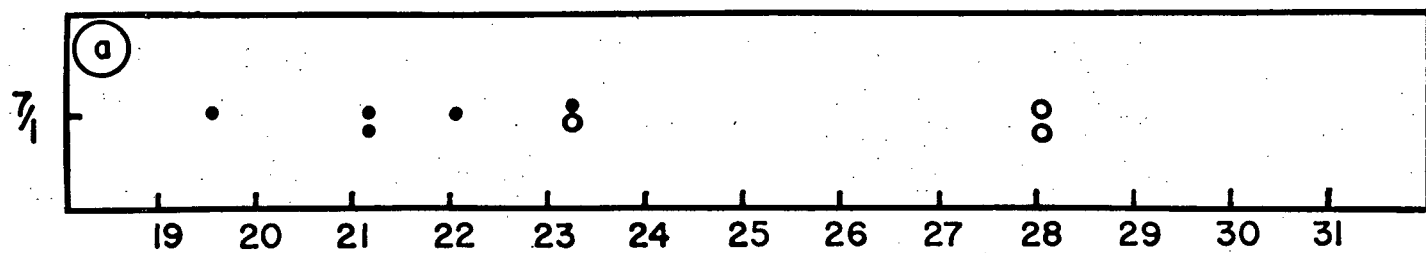


Figure 25

The presence or absence of D. discoideum V12 and P. pallidum fruiting bodies is noted with respect to (1) temperature - x-axis, (2) relative spore concentration, (3) absolute spore concentration. A black dot denotes that D. discoideum V12 fruited. An open circle denotes that P. pallidum fruited. The figure is divided into five parts to denote the various spore concentrations used: (a) 6500, (b) 8000, (c) 5000, (d) 4400, (e) 2000 spores per plate.



TEMPERATURE °C

Interference

The discrepancy between the simulated competitive outcome (Fig. 23) and the actual outcome (Fig. 24, Fig. 25) suggests that exploitation alone cannot explain the mechanics of competition for food between D. discoideum and P. pallidum. Interference must be playing some part in the process. At this point four characteristics of the interference process are known: (1) both species did not fruit between 18° and 26° as the exploitation - competition simulation predicted, (2) the probability of P. pallidum fruiting at any temperature depended upon the initial concentration of P. pallidum spores. As the spore number increased fruiting occurred at lower and lower temperatures (Fig. 24), (3) D. discoideum V12 apparently prevented P. pallidum from fruiting over most of the range extending from 18° to 24°C, (4) P. pallidum prevented D. discoideum V12 from fruiting in the temperature range extending from 24.3° to 26.0°C (Fig. 24, 25).

From these observations it can be hypothesized that two distinct types of interference occur:

(1) D. discoideum V12 interferes with P. pallidum below 24°C and, (2) P. pallidum interferes with D. discoideum V12 above 24.3°C. These two processes were treated separately.

Inhibition of P. pallidum

At the outset it can be stated that P. pallidum is

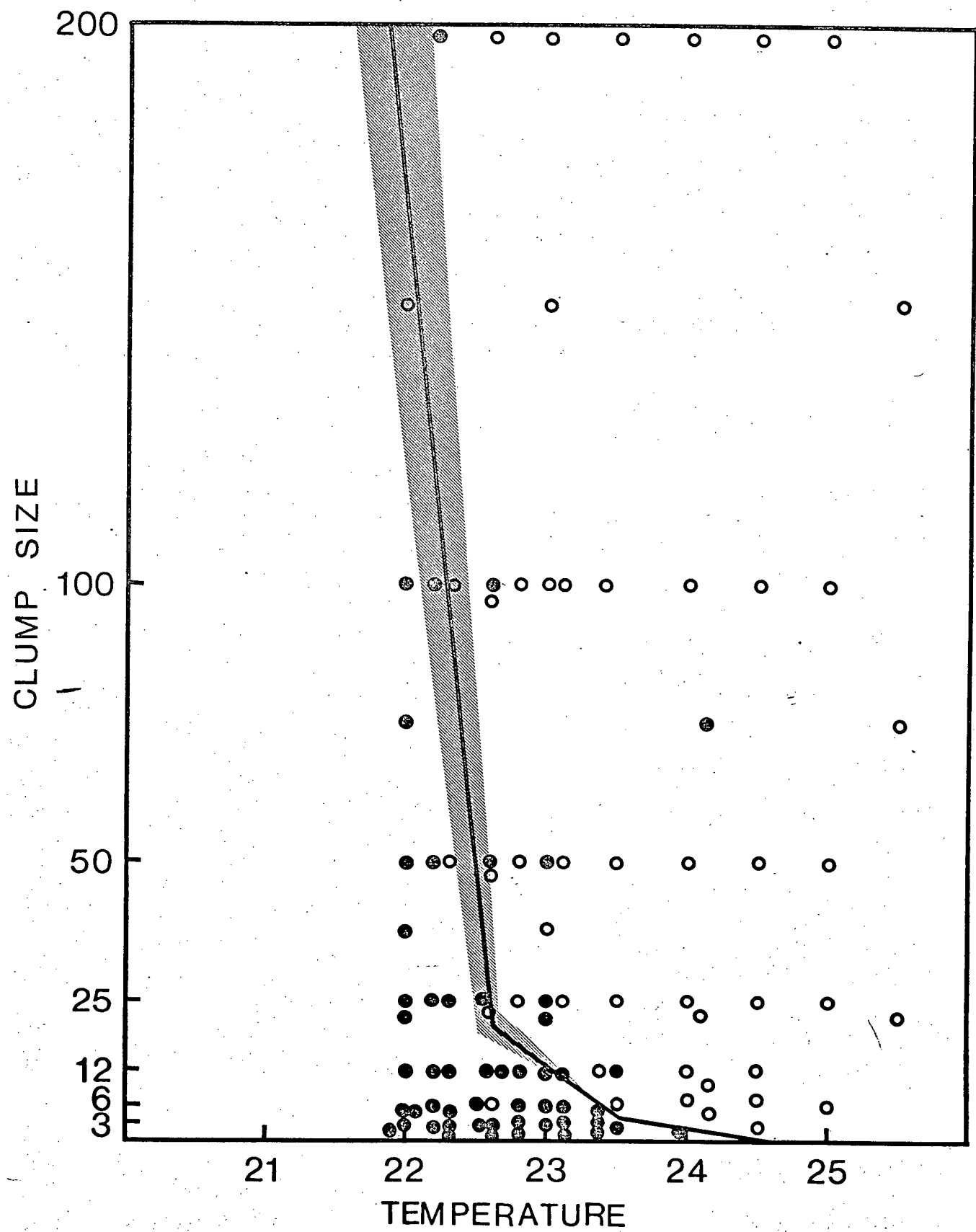
inhibited at the fruiting body formation stage. The evidence for this statement will be more logically presented later (Fig. 33), but P. pallidum spores do germinate in the presence of D. discoideum and the vegetative amoebae do consume food and divide but are unable to aggregate to produce fruiting bodies. Presumably D. discoideum interferes with this process, possibly with the production of a chemical inhibitor.

Since P. pallidum is able to fruit in the presence of D. discoideum under conditions of high P. pallidum concentration it can be hypothesized that small clumps of P. pallidum spores might be able to overcome D. discoideum and produce fruiting bodies. The higher the P. pallidum spore concentration the greater the likelihood of spore clump formation.

If this hypothesis is to be tested, the term "spore clump" must first be defined. It was observed that when 2 spores were suspended in .001 ml of water and placed on a culture dish containing bacteria and D. discoideum spores (concentrated from 1000 to 5000 spores per plate), they were able to influence the use of food in an area of about 16 to 22 mm². In this area D. discoideum was unable to use the food for a period of at least three days. Eventually, the time depending upon the temperature, D. discoideum overcame P. pallidum and used the food preventing P. pallidum from fruiting.

Figure 26

The relationship between P. pallidum clump size and temperature. Single black dots denote the occurrence of D. discoideum VC4 fruiting bodies only. Open circles denote the occurrence of both P. pallidum and D. discoideum VC4 fruiting bodies. The line is filled by eye and the shaded area denotes other positions that the line could have taken. In all cases the plates were inoculated with a random spread of 1000 D. discoideum VC4 spores. Appropriate numbers of P. pallidum spores were inoculated in .001 ml of water.



In view of these data a "clump of spores" could be the spores occupying each 19 mm^2 area unit on the surface of a petri dish. Each clump area constitutes about 1/100th of the surface area of a 60 mm petri dish. Therefore, if it were possible to divide a petri dish into 100 sections the spores in each could represent one "clump".

This procedure was followed in the first section of Program VIII (Appendix I) where a plate was divided into 100 equal sections, the spores were spread at random, and the clump containing the greatest number of spores was identified. From the output of Program VIII it was found that as the number of P. pallidum spores used to inoculate a plate increased the size of the maximum clump increased. Because the hypothesis stated that only one clump of a particular size would be required to overcome the inhibitory effect of D. discoideum only the largest clump was of interest.

To recapitulate: (1) For lack of any information to the contrary it is assumed that when spores are spread on the surface of a 60 mm petri dish they were distributed at random. (2) A random distribution function mimics this occurrence. (3) The plate is divided into 100 equal area units or spheres of influence. (4) The maximum clump size for any spore concentration is calculated. (5) This clump size will be the "available" clump size for any particular spore concentration.

This information is summarized in a components

diagram (Fig. 29, page 87) by steps 1, 2, 3, and 4. Where the number of P. pallidum spores (SPORE PP), the size of the area controlled by a spore clump (SPHERE INF), and the total area of the plate (TOTAL AREA); all act as inputs to a poisson distribution (POISSON) which divides the plate into appropriate area units, distributes the spores at random, and chooses the area unit containing the most spores (AVAILABLE CLUMP SIZE).

Having found the "available clump size" for any spore concentration of P. pallidum the clump size necessary for fruiting in the presence of D. discoideum grown at any temperature must be derived.

To find the "necessary clump size" bacteria covered petri dishes containing 1000 D. discoideum spores were established. Various numbers of P. pallidum spores were suspended in distilled water and inoculated onto the plates in .001 ml suspensions. The plates were incubated at various temperatures and the presence or absence of P. pallidum and D. discoideum fruiting bodies were noted after seven days growth. It was found that as the concentration of P. pallidum spores increased fruiting occurred at lower and lower temperatures (Fig. 26). Due to the nature of this experimental technique it was difficult to judge the precise temperature at which P. pallidum was unable to fruit. Therefore, the boundary line in Figure 26 was fitted by eye and the shaded area indicates other possible positions that the line could

have assumed. In Figure 26 the area on the right side of the line is the area in which P. pallidum can fruit. The area on the left is the area in which P. pallidum cannot fruit.

The boundary line is composed of three straight lines which were described as an array and incorporated into the second section of Program VIII (Appendix I).

In terms of the components diagram (Fig. 29, page 87) this information is summarized by components 5, 6, and 7. The array describing the relationship between clump size and temperature (PP DATA INPUT), and temperature (TEMP), act as inputs to a function which selects the necessary clump size which allows fruiting (NECESSARY CLUMP SIZE).

In the foregoing section it has been assumed that P. pallidum fruiting ability is independent of D. discoideum background concentration. This assumption is probably not absolutely correct, however, the variation is so small that it cannot be detected using the methods employed in this study. The evidence for this statement comes from two sources of data.

Data source 1: Plates were established with random distributions of various numbers of D. discoideum V12 and P. pallidum spores. If experimental sets inoculated with equal numbers of P. pallidum spores are compared it could be hypothesized that if D. discoideum background is important

then P. pallidum should be able to fruit at lower temperatures on the plates with low D. discoideum background concentrations. Comparisons are made in the following table:

EXPERIMENTAL SET NO. 1			EXPERIMENTAL SET NO. 2		
PP CON	Dd. CON	LOWEST PP. F.B.	PP CON	Dd. CON	LOWEST PP. F.B.
Figure 42-a			Figure 43-c		
90	10	24.3°	66	600	24.3° *
Figure 42-a			Figure 44-d		
600	66	21.8°	798	1862	25.0°
Figure 43-d			Figure 44-e		
200	466	24.3°	266	2394	24.3° *
Figure 44-b			Figure 45-e		
1862	798	24.3°	1600	14400	23.0° *

One star in the extreme right hand column indicates that the comparison refuted the hypothesis that D. discoideum concentration alters P. pallidum's ability to fruit. The hypothesis was refuted three out of four times, suggesting that P. pallidum fruiting is independent of D. discoideum concentration.

Data Source 2: A more direct test of the hypothesis that D. discoideum background altered P. pallidum fruiting was conducted by establishing plates containing 1000 P. pallidum spores and six different D. discoideum VC4 concentrations. P. pallidum might have been able to fruit at a slightly lower temperature (Fig. 27) as D. discoideum background decreased. But this trend was very weak if it existed at all.

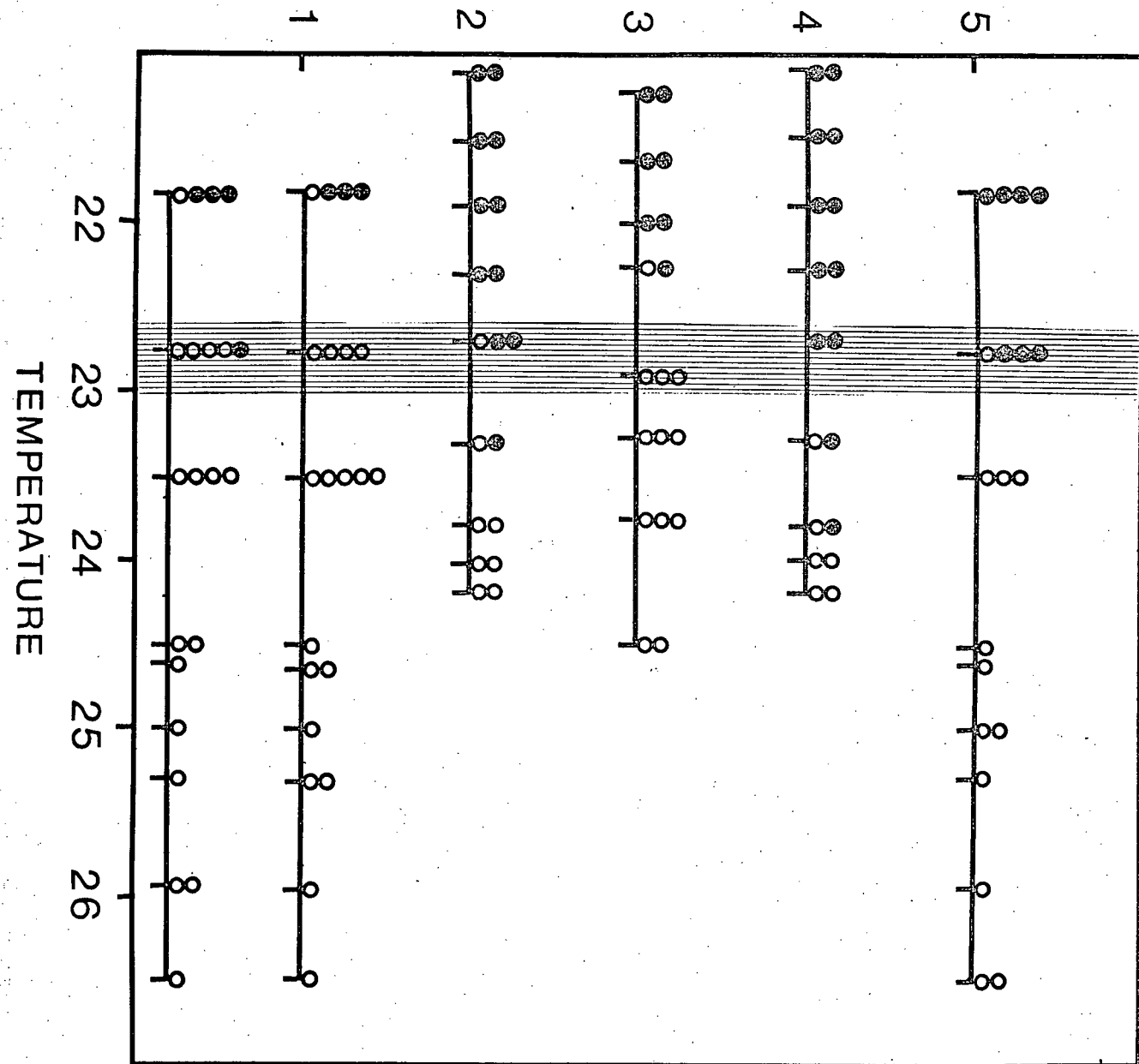
In both cases the data failed to show any real trend in P. pallidum fruiting ability with respect to changes in D. discoideum concentration. When considered with the fact that the boundary line (Fig. 26) may be imprecise and the temperature measurements may be inaccurate by as much as 0.3°C , it is apparent that the trend, if it exists, is smaller than the experimental error. Therefore, the experimental method precludes inclusion of any interaction between P. pallidum fruiting ability and D. discoideum concentration.

Returning to the main body of the interference section it has been shown that both "necessary clump size" and "available clump size" are calculable quantities. Therefore, under any conditions of temperature and P. pallidum spore concentration it should be possible to link these two quantities together and make a decision about whether or not P. pallidum will fruit. This procedure was carried out using the finished version of Program VIII (Appendix I).

Figure 27

P. pallidum concentrated at 1000 spores per plate was grown with D. discoideum concentrated at 200, 1000, 2000, 3000, 4000, and 5000 spores per plate. Temperature in degrees centigrade is measured along the x-axis and D. discoideum spore concentration is measured along the y-axis. An open circle denotes the presence of P. pallidum fruiting bodies in a culture dish, and a black dot represents a dish in which P. pallidum fruiting bodies did not appear. The limit line $\pm$ 95% confidence limit is drawn from Program VIII.

D. DISC. CONCENTRATION $\times 10^3$



The interactive components were also added to the components diagram (Fig. 29, page 87) as steps 8 and 9. The COMPARISON function compares the available and necessary clump sizes. If the available clump size exceeds the necessary clump size then fruiting occurs. If not, then P. pallidum does not fruit.

The predictive ability of this model was tested using the data in Figures 42, 43, 44 (Appendix II) and Figure 27. In the figures a star appears at points where the model fails to predict the outcome. The following table lists the results of comparing expected with observed.

FIGURE NUMBER	NUMBER CULTURES RUN	NUMBER FAILURES	% FAILURE
Fig. 42	45	3	6.6%
Fig. 43	39	1	2.5%
Fig. 44	45	3	6.6%
Fig. 27	115	6	5.2%
TOTAL	244	13	5.3%

Out of the 244 cultures run the model failed to predict the correct outcome 5.3% of the time. These results are acceptable.

Confidence Limits on P. Pallidum Interference

Since the available clump size is calculated using a poisson distribution it is possible to attach 95% confidence limits to each estimate. For clump sizes larger than 10 a standard table of poisson confidence intervals was used (Ricker 1936) but for estimates less than 10 a corrected table (Stevens 1942) was used. The procedure is exemplified by the data in Figure 27. In this example 1000 P. pallidum spores were used and the available clump size was calculated to be 18 spores. Eighteen spores per clump allows P. pallidum growth at 22.7°C (Fig. 26). The 95% confidence limit around the mean estimate of 18 is 10.7 to 28.4 and the corresponding temperatures are 23° and 22.6°C. Therefore, when 1000 spores are used to establish a culture dish the maximum available clump size might vary from 10.7 to 28.4 spores, and the temperature at which P. pallidum might begin producing fruiting bodies varies from 22.6° to 23.0° (Fig. 27).

Inhibition of D. Discoideum

Experiments in which D. discoideum was grown alone indicated that D. discoideum V12 was capable of growing up to about 26°C and that D. discoideum VC4 was capable of growing up to about 26.5°C. But when D. discoideum V12 was mixed with P. pallidum its growth was always terminated at about 24.3°C (Figs. 24, 25, and 41, 42, 43, 44 - Appendix II).

Apparently, there was no interaction between P. pallidum spore concentration and D. discoideum fruiting ability.

Changes in D. discoideum spore concentration altered D. discoideum fruiting ability in the presence of P. pallidum. As the spore concentration increased from 200 spores per plate to 2000 spores per plate the temperature at which fruiting occurred increased (Fig. 28). This relationship can be expressed by the following equation:

$$T_F = 23.9^\circ - C/1800 \quad ; \quad C \leq 2000 \quad (5)$$

Above 2000 spores per plate (Fig. 28) D. discoideum VC4 was, on the average, unable to fruit above 23.9°C . This relationship can be expressed as:

$$T_F = 23.9^\circ \quad ; \quad C > 2000 \quad (6)$$

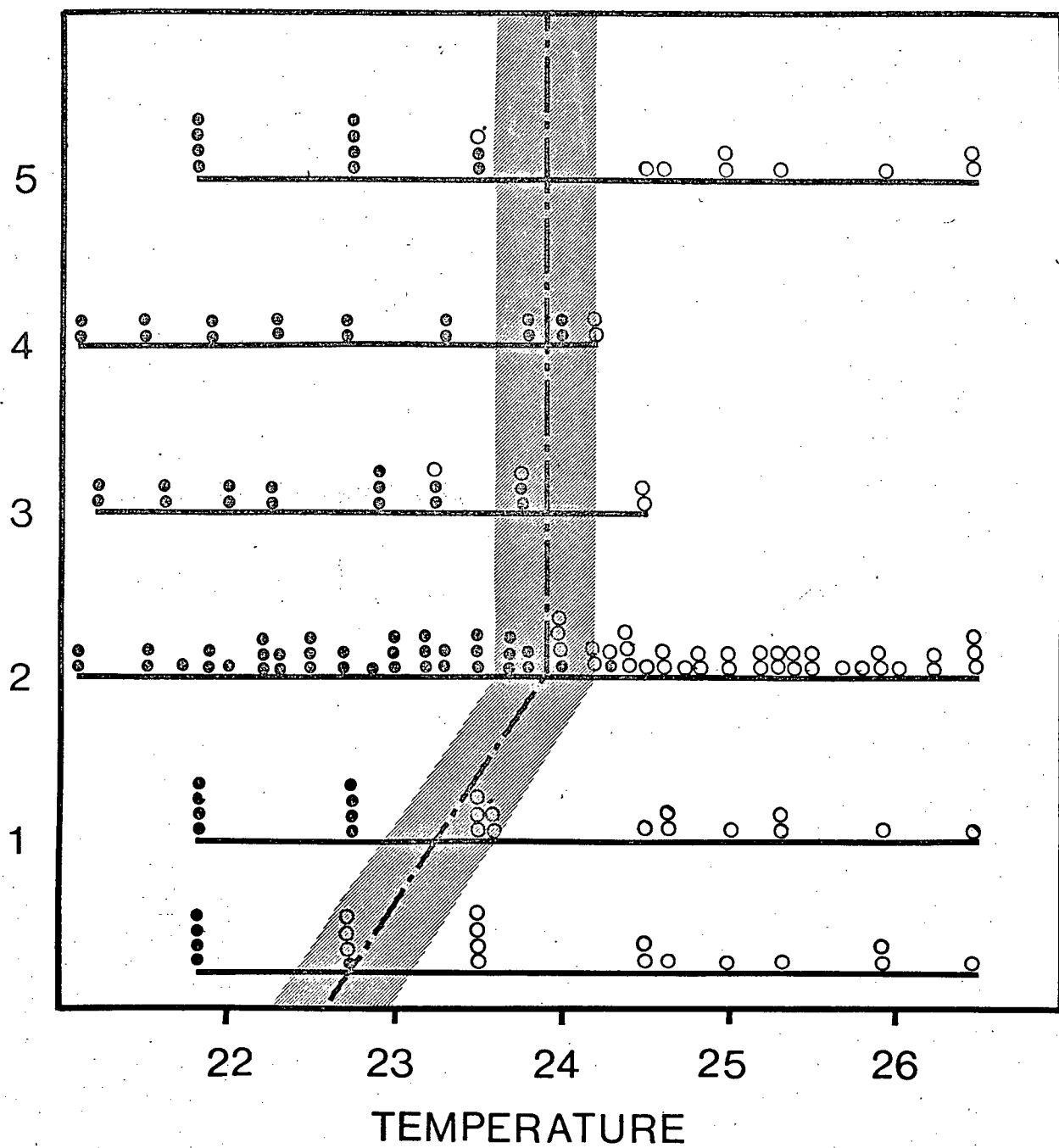
where T_F is the maximum temperature at which D. discoideum can fruit and C is spore concentration.

The foregoing information was incorporated into Program IX (Appendix I) as a subroutine, and was added to the diagrammatic representation (Fig. 29, page 87) as components 10, 11, and 12. The relationships described by the above equations (DATA INPUT), the temperature (TEMP), and the D. discoideum spore concentration (SPORE DD) all feed into a selection function (DD SELECTION) which regulates the fruiting ability of D. discoideum.

Figure 28

The fruiting ability of D. discoideum grown in the presence of 1000 P. pallidum spores is plotted with respect to temperature and D. discoideum spore concentration. A black dot represents the presence of D. discoideum fruiting bodies in a culture dish. An open circle represents a culture dish with no D. discoideum fruiting bodies. The limiting line is fit by eye and 0.3°C temperature intervals have been established on either side of the line.

SPORE CONCENTRATION $\times 10^3$



The lines drawn in Figure 28 and described by equations (5) and (6) do not have 95% confidence limits. However, the positioning of the line is imprecise because there undoubtedly is some data error and there is also error in the measurement of temperature. It was impossible to measure the error in the data but the temperature error was measured and could have been as much as $\pm 0.3^{\circ}\text{C}$. For this reason an interval rather than a line must be used to describe the temperature above which D. discoideum is unable to fruit in the presence of P. pallidum (Fig. 28).

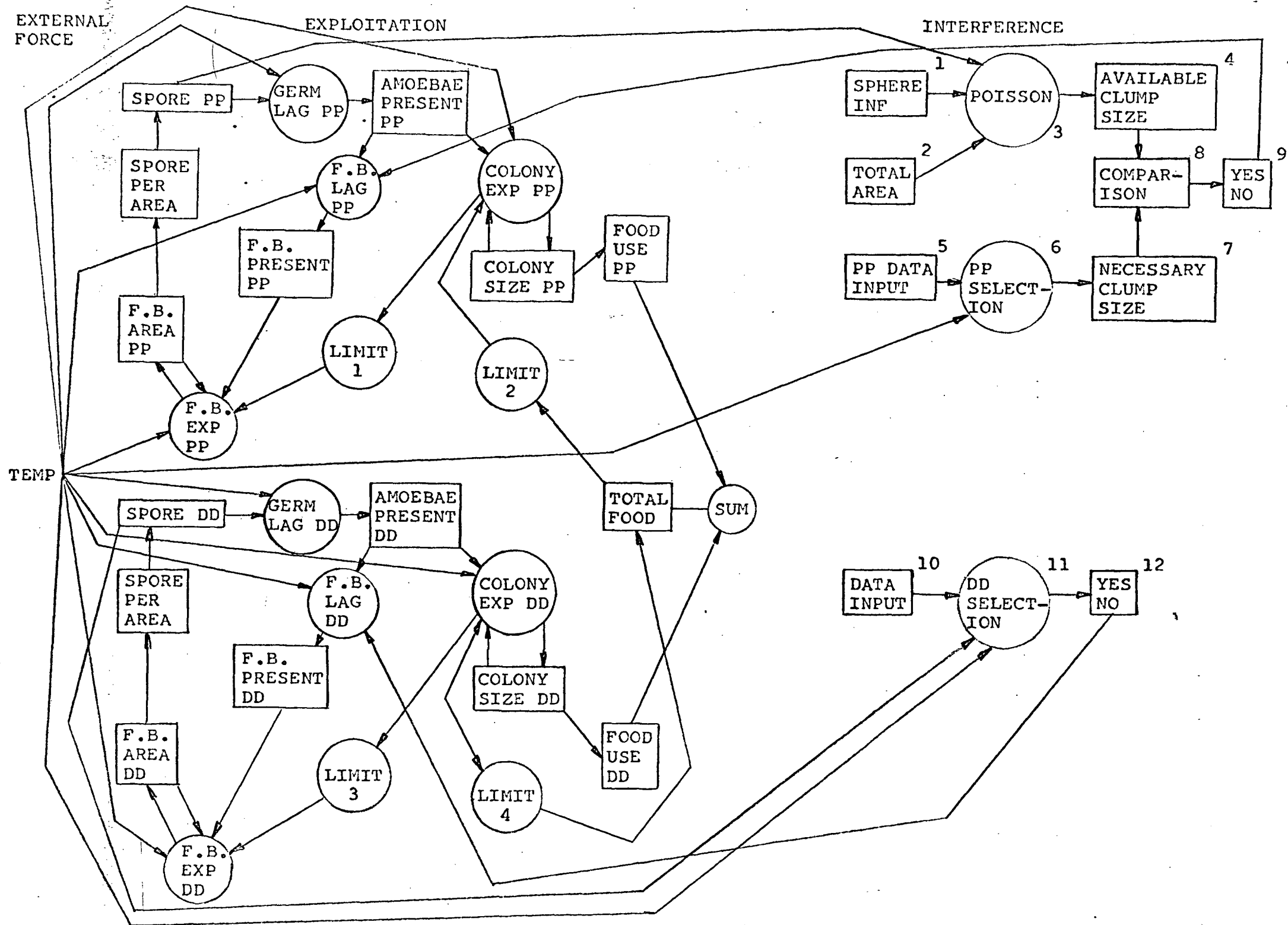
The Completed Model

The model describing competition between D. discoideum and P. pallidum must include an exploitation section, a P. pallidum interference section, a D. discoideum interference section, and an external force section. These four components have been linked together in Program IX (Appendix I - Program IX). A components representation of the model appears in Figure 29.

From the model, several quantities can be predicted. These are: (1) the germination rates, (2) the rate of food use, (3) the rate of fruiting body formation, (4) the number of spores produced, (5) the presence or absence of fruiting bodies, (6) the number of available clumps of the necessary size formed by P. pallidum.

Figure 29

Flow diagram describing the way in which the components describing laboratory competition of D. discoideum and P. pallidum are linked together. The terms are explained in the text. Program IX includes all of the information summarized here.



Estimates of the error attached to each of these outcomes can also be made. In a previous section it was demonstrated that 95% confidence limits could be established around the temperature at which P. pallidum fruited. The error in the estimate of the temperature at which D. discoideum should fruit has also been considered. Finally, it is also possible to estimate the error attached to the model's predictions of food use and area covered by fruiting bodies. These quantities depend upon many sources of error such as: (1) error in temperature measurement, (2) error in germination times, (3) error in colony expansion, (4) error in fruiting body lag time, (5) error in fruiting body expansion rates, and (6) error in spore counts. With the exception of the spore count error, it has been impossible to do any more than assume that the errors attached to each of these components is random. Since all of these components together produce a prediction of the amount of area occupied by fruiting bodies at any time it can be assumed from the theorem for the addition of Poisson distributions (Brownlee 1965) that the error attached to this and similar predictions is also randomly distributed.

Therefore 95% confidence limits from a Poisson distribution may be placed around simulated predictions of spore number, area occupied by fruiting bodies, and food use.

Tests of the Model

Area Occupied by Fruiting Bodies

Several predictions are made by the model, but the one that calls upon the most components is the prediction of the relative areas covered by D. discoideum and P. pallidum fruiting bodies after all of the food and space has been used. For this reason most of the emphasis in testing the model has been in this area. Cultures were established using known spore numbers of both species. Method II (methods section) was employed. Temperature and growth progress were monitored, and when all the food and area were used, the areas occupied by the fruiting bodies of the two species were noted. Experiments were conducted at a range of temperatures and spore concentrations. With each change in P. pallidum concentration the minimum fruiting temperature changed, and with each change in D. discoideum spore concentration the maximum fruiting temperature changed.

The data from these experiments are presented in a series of figures (Fig. 30-A-F), one figure for each pair of P. pallidum and D. discoideum spore concentration values. The results of these tests are summarized as follows:

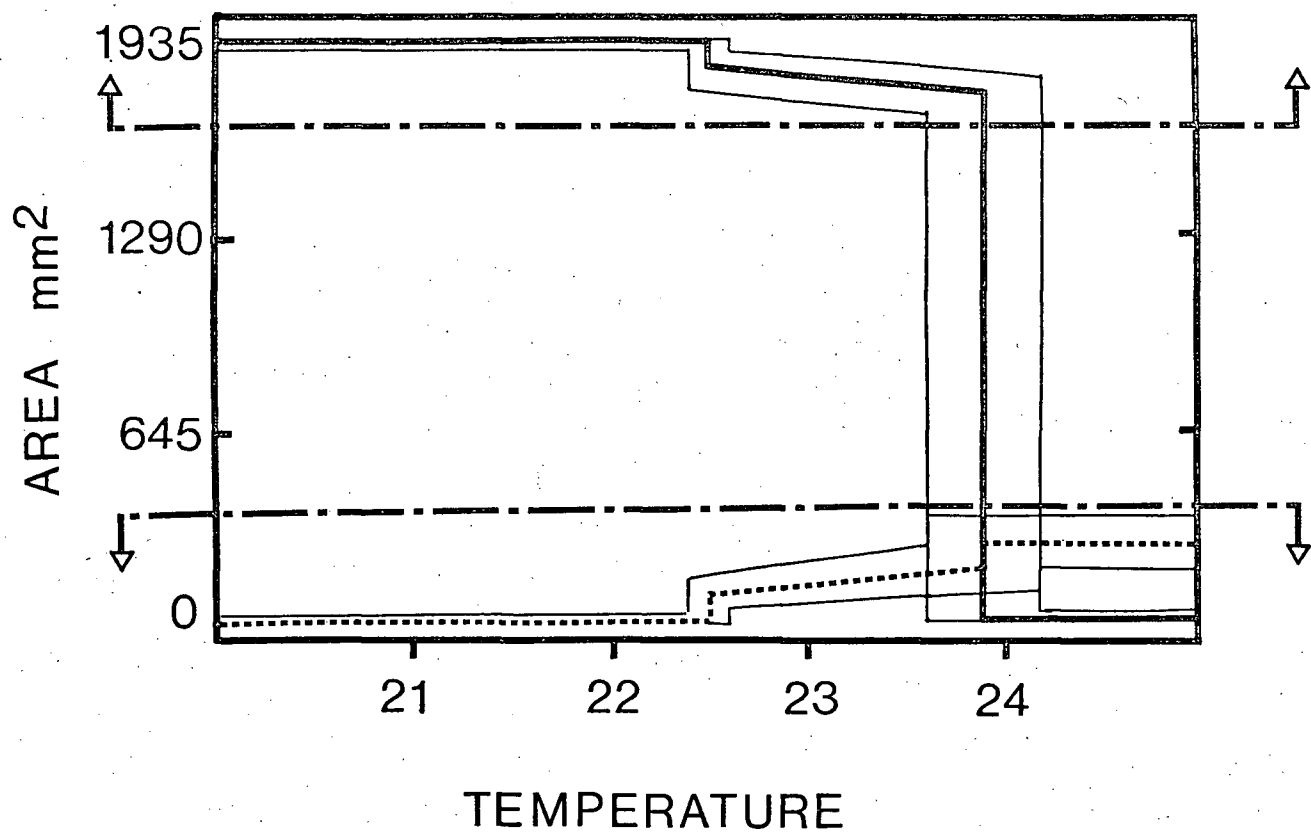
Figure 30

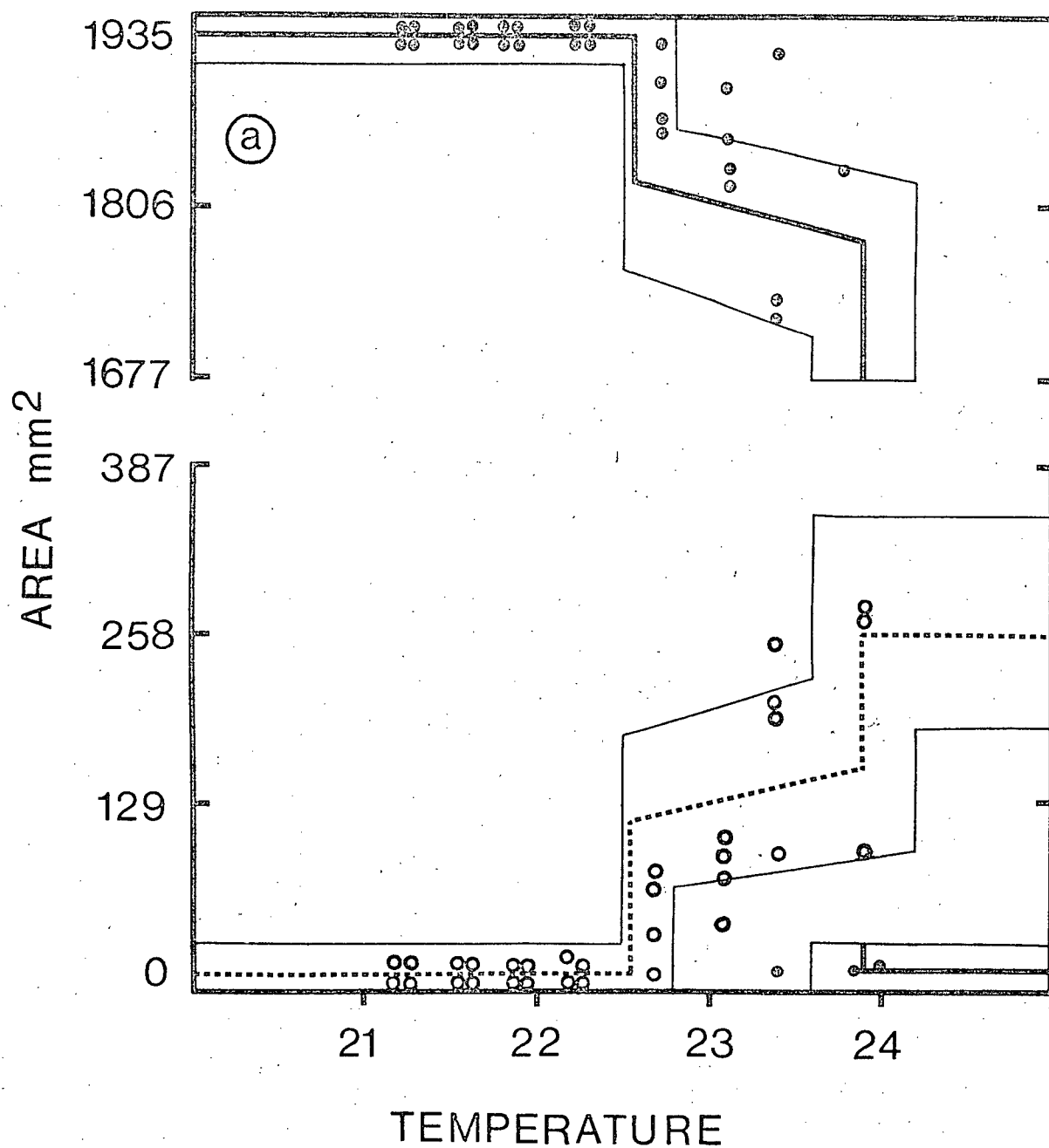
The actual and theoretical areas covered by D. discoideum and P. pallidum fruiting bodies after all the available food and space has been used. Area occupied is measured along the y-axis and temperature on the x-axis. The solid line for D. discoideum and the dotted line for P. pallidum are predicted by the completed model (Fig. 20) (Program IX). Appropriate 95% confidence limits around the predictions are indicated with shaded areas.

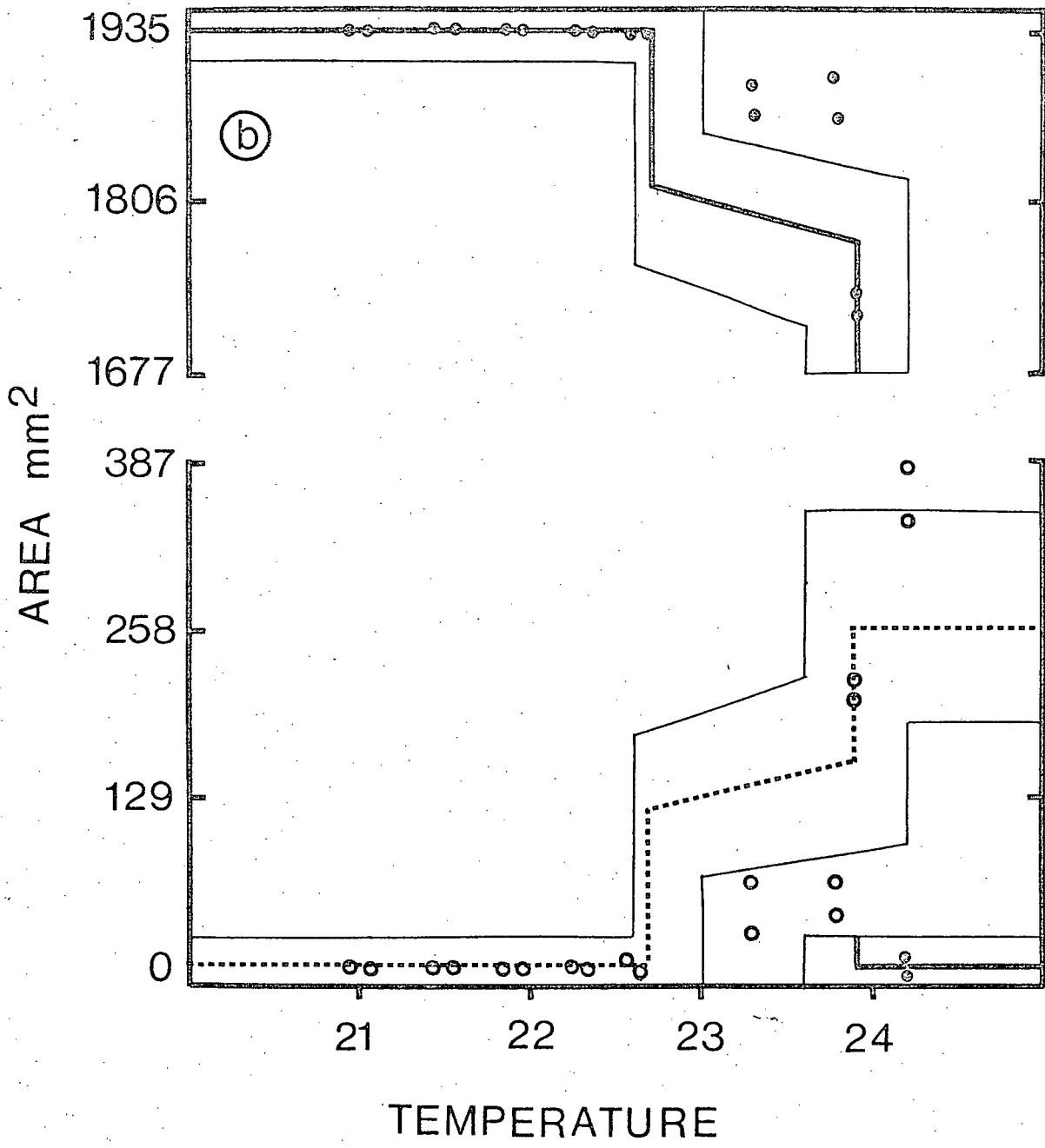
The figure on the first page includes all of the area occupied by the two species. From this the confidence limits can be viewed in proper perspective.

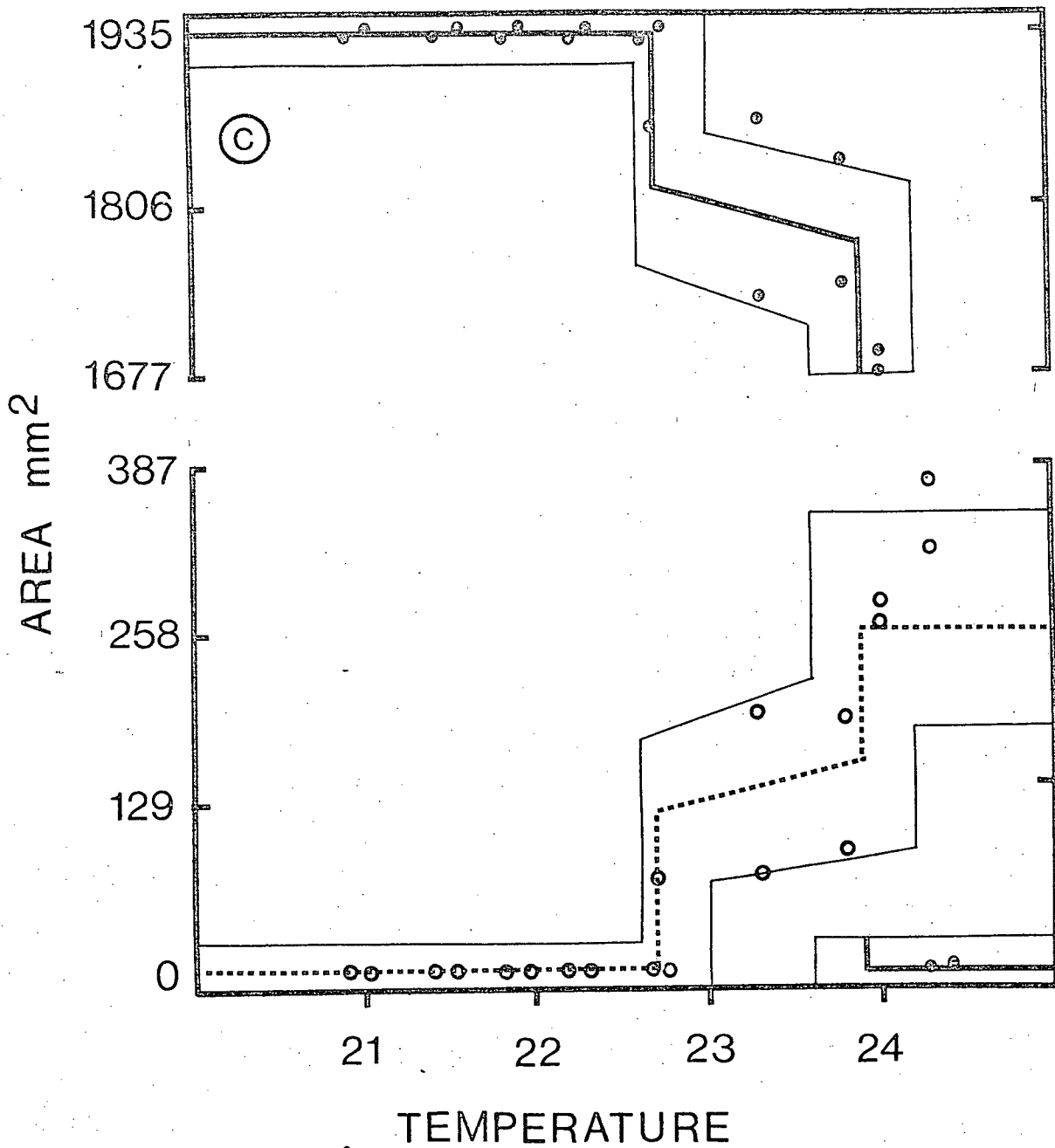
The remaining six pages of figures are blow-ups of the sections which data points appear. The experiments were conducted at several different spore concentrations and one figure has been allotted to each concentration.

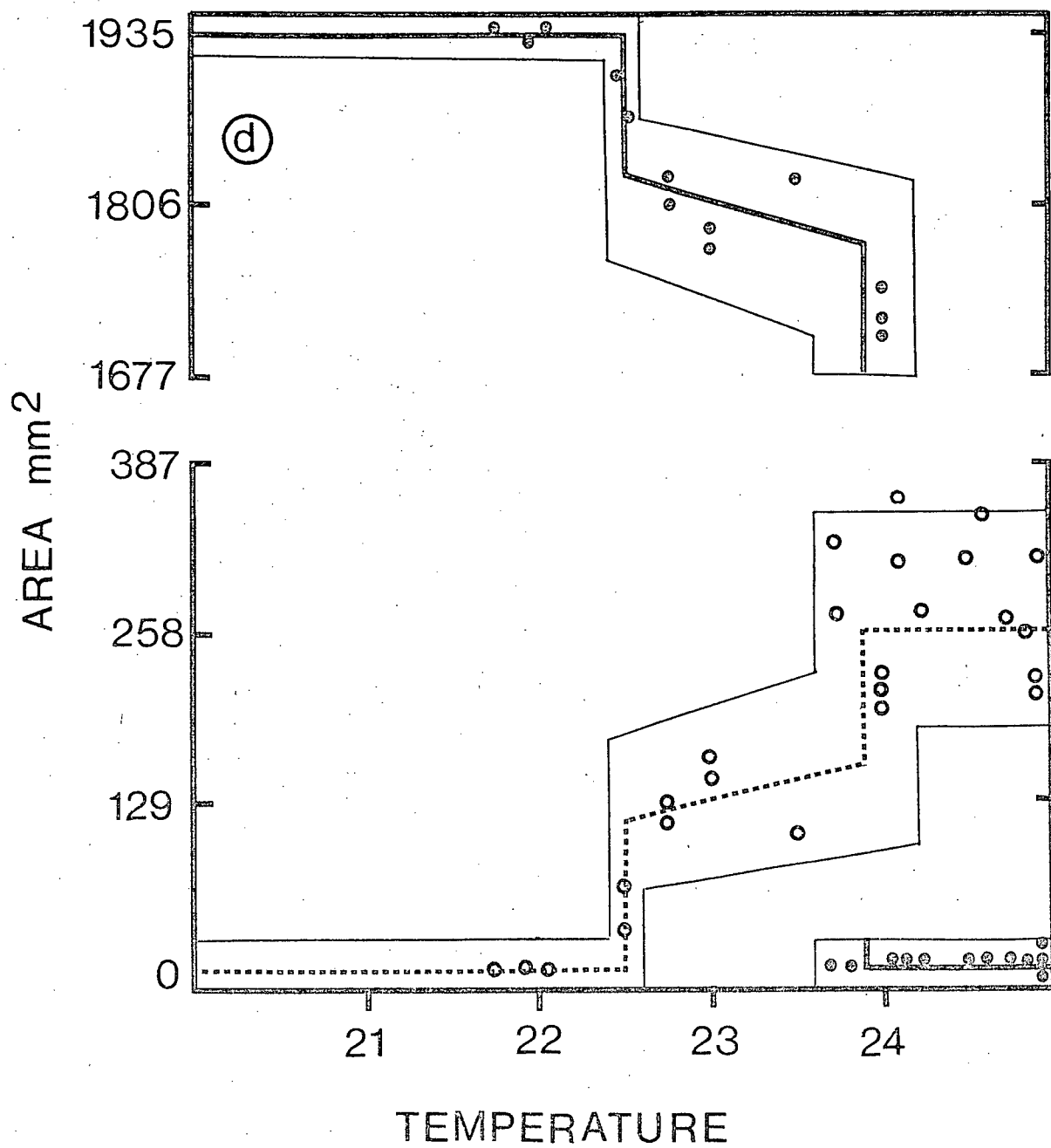
FIGURE NUMBER	SPORE CONCENTRATION	
	D. DISCOIDEUM	P. PALLIDUM
30-A	3000	1500
30-B	4000	1000
30-C	2000	1000
30-D	3000	3000
30-E	4000	4000
30-F	5000	5000

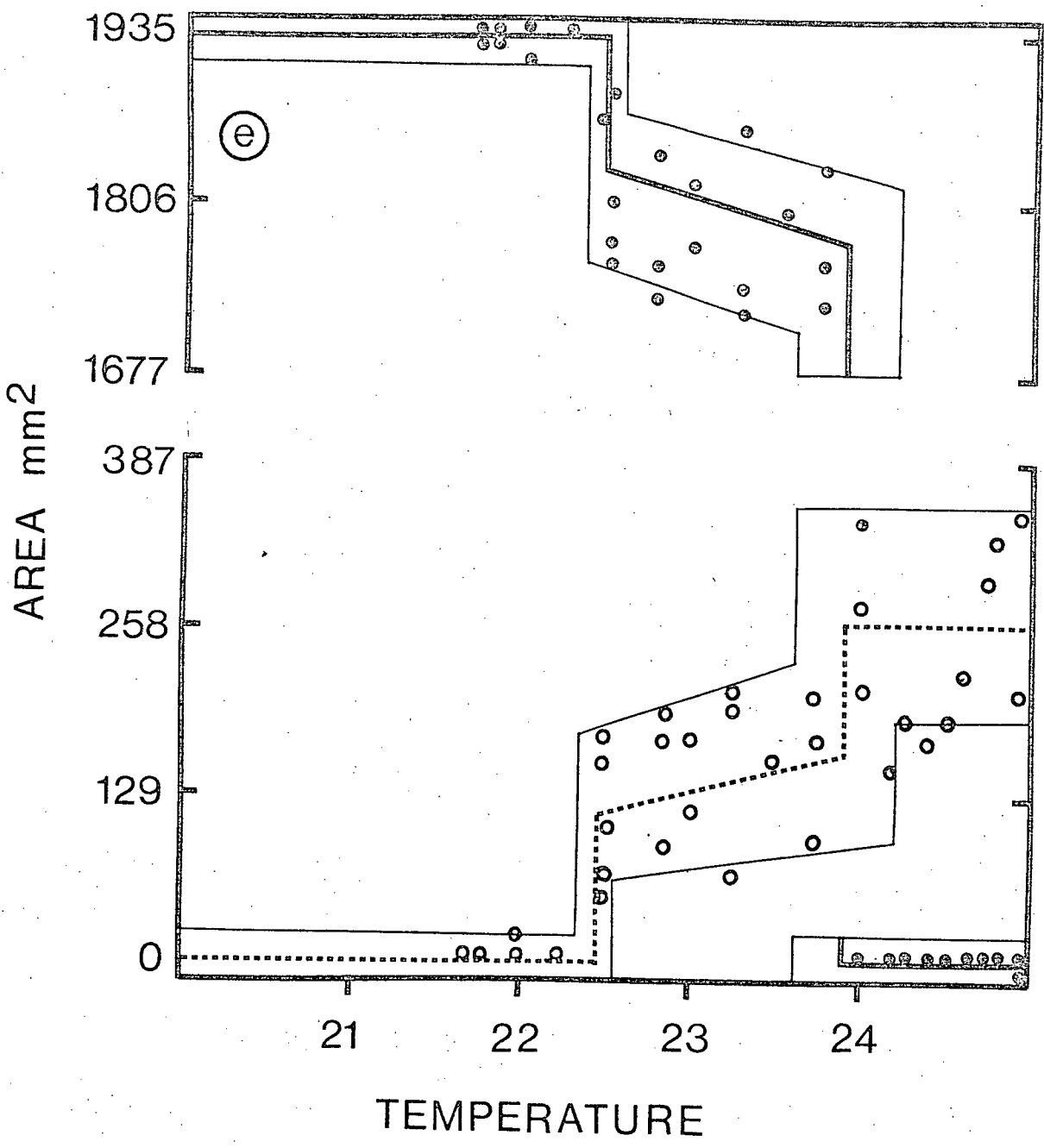












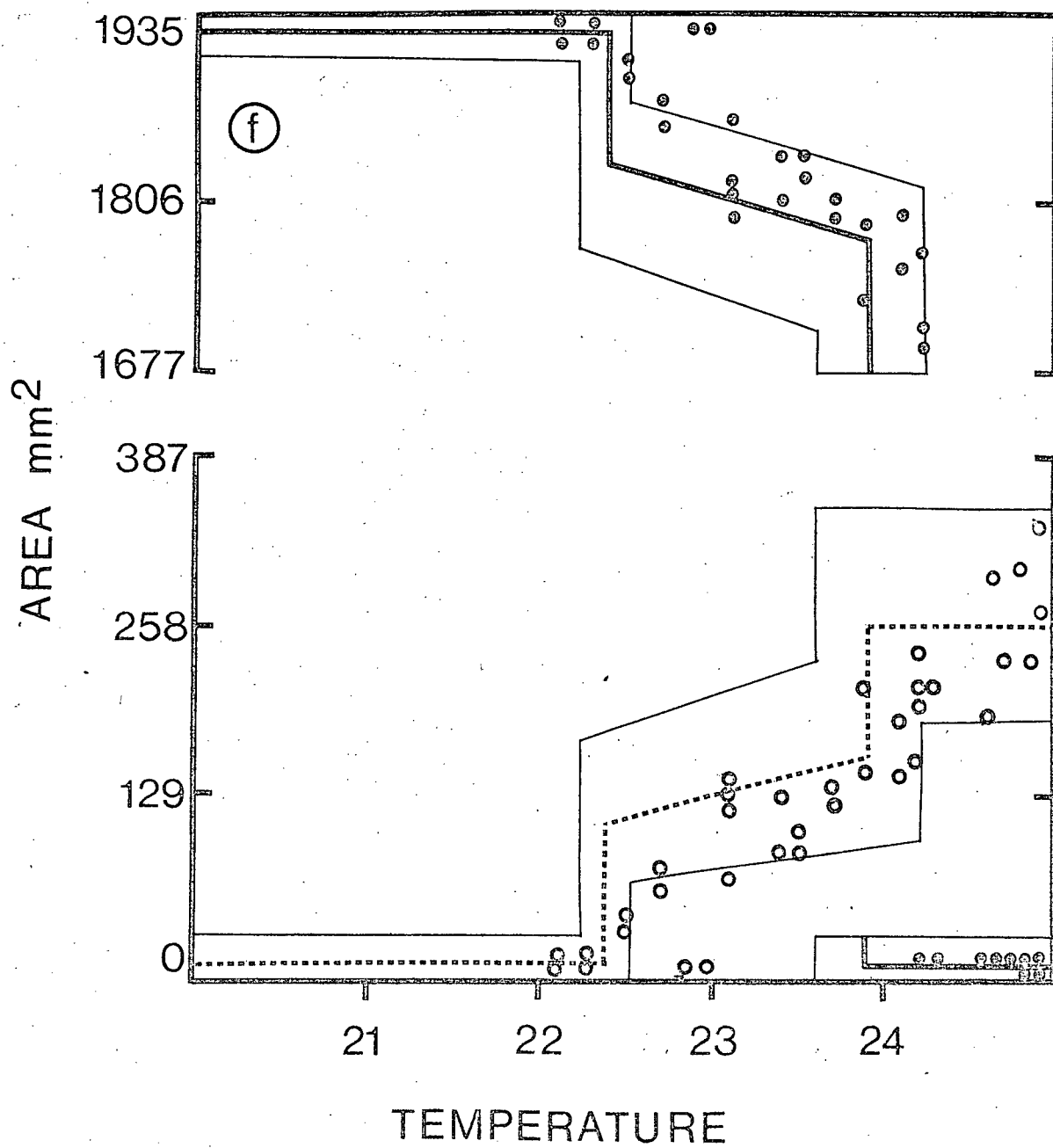


FIGURE	REPLICATE NUMBER	NUMBER OF FAILURES	% FAILURE
30-A	62	5	8.0%
30-B	36	9	25.0%
30-C	38	2	5.2%
30-D	50	1	2.0%
30-E	66	7	10.6%
30-F	72	8	11.1%
TOTAL	324	32	9.9%

Close inspection of the figure indicates that many of the failures are of the type where P. pallidum is too low and D. discoideum is too high. These findings can possibly be explained by the fact that at temperatures close to the minimum fruiting temperature, only one or two available clumps are larger than the necessary clump size. Therefore, all the amoebae from every section of the plate must converge on one or two fruiting centers. It is difficult to believe that the concentration gradient from one center would be great enough to attract all amoebae, and therefore, some amoebae might be unable to find a fruiting center and add to the area occupied by fruiting bodies. This situation results in an over-estimate of P. pallidum fruiting body area. Unfortunately, there is no legitimate way to incorporate this hypothesis into the model until more is known about the attractive area of acrasin in mixed cultures.

Continued Competition

Since the average P. pallidum fruiting body contains from 100 to 10000 spores the model predicts that once such a fruiting body forms anywhere above 22.5°C it should be able to perpetuate itself in a situation where food is constantly being replaced. To test this hypothesis two culture dishes containing 4000 D. discoideum and 4000 P. pallidum spores were grown at 23.5°C. When the food was gone and fruiting bodies had formed the completed dishes were placed face to face with new bacteria covered dishes. The second dishes were allowed to grow and a face to face transfer was made to a third dish. The results of this short experiment are as follows:

<u>TIME PERIOD</u>	<u>AREA P. PALL.</u>	<u>AREA D. DISC.</u>
1	8, 12	292, 288
2	10, 11	290, 289
3	10, 13	290, 287

P. pallidum fruited the first time as expected and continued to fruit throughout the entire time sequence. Also P. pallidum did not gain or lose any area during the sequence.

Summary - Interference

- (1) The predictions made by the exploitation model did not agree with the results of experiments in which the two

species were grown together. Interference was occurring.

- (2) P. pallidum interfered with D. discoideum fruiting above about 24°C.
- (3) D. discoideum interfered with P. pallidum fruiting below about 24°C.
- (4) The fruiting ability of P. pallidum depended upon maximum clump size. The term "clump" was defined as the number of spores occupying 1/100th of the surface area of a 60 mm petri dish.
- (5) The clump size necessary for fruiting was temperature dependent and was assessed experimentally.
- (6) The maximum clump size available for any P. pallidum spore concentration was calculated using a Poisson distribution.
- (7) When "available clump size" exceeded "necessary clump size" fruiting occurred. The model for this process is described in Program VIII (Appendix I).
- (8) D. discoideum fruiting ability changed with respect to temperature and spore concentration (Fig. 28).
- (9) The two interference models were linked to the exploitation model (Fig. 29) (Program IX - Appendix I) and the completed model was tested.

- (10) A total of 324 predicted culture areas were used to test the model which proved accurate in 90.1% of the test cases.

RESULTS SECTION II

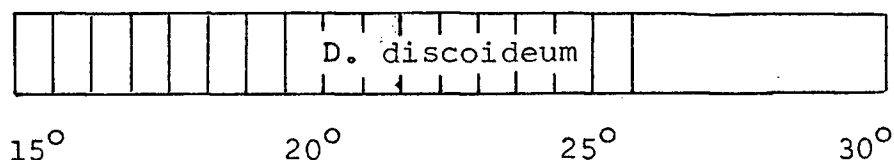
CONSEQUENCES OF COMPETITION

The model constructed in the preceding section deals only with cellular slime mold competition over short time intervals. No attempt was made to predict the results of competition continuing for long periods of time, because such predictions could only be made if the organisms did not change in response to competitive pressure. The results of other studies (Keast 1968, Ficken et al, 1968, Miller 1967) suggest that this is unlikely, particularly with simple organisms like the cellular slime molds.

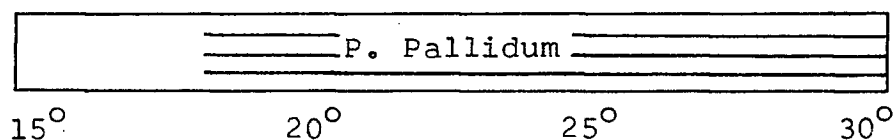
In view of this information, a series of long term competitive situations were contrived in the laboratory. It was hoped that the results of these experiments would describe any changes that D. discoideum and P. pallidum might experience, and that the results might also add to our knowledge of the selective forces involved in the maintenance of exclusion, or the development of coexistence.

The competitive model demonstrated that the two species excluded one another between 18° and 26°C, making it impossible to compete for long time periods. To remedy this situation refuges were provided by growing the two species in long culture dishes which were placed on a temperature gradient extending from about 15°C to 30°C. When grown alone, D. discoideum was able to consume food and fruit from

about 9°C to 26°C.



When grown alone, *P. pallidum* could consume food and fruit between about 18° and 37°C.



Therefore the laboratory design provided refuges for both species.

If divergence, convergence or continued exclusion were to occur, an area of conflict should be provided. The above diagrams indicate that if the two species were grown together they both could compete for the food and space between 18° and 26°C.

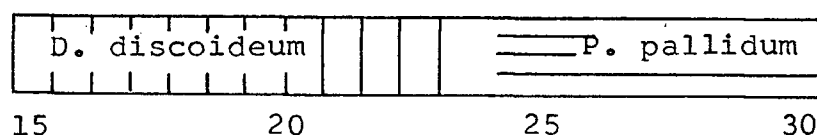
Finally, an attempt was made to produce an environment that was homogeneous in every respect save temperature. The environment was two-dimensional, the agar surface was as flat as possible, and only one bacterial species was used as a food source.

Under these conditions it was hypothesized that the animals might both converge to use the resource at the

same rate, and thereby ensure coexistence; or that one species might increase its rate of resource use making it impossible for the other to compete, thereby ensuring competitive exclusion.

Mixture of Stock Spores

A fifty-fifty mixture of spores coming directly from the stock cultures was used to establish culture gradients periodically throughout the two year period over which this study was conducted. In every case the two species did not fruit together. D. discoideum V12 fruited up to about 24.5°C (Fig. 31-A, B, C). D. discoideum DF fruited up to about 26.5°C (Fig. 31-D, E). In both cases P. pallidum fruited over the remaining portion of the culture gradient. This information is summarized in the following diagram:



Continued Mixtures

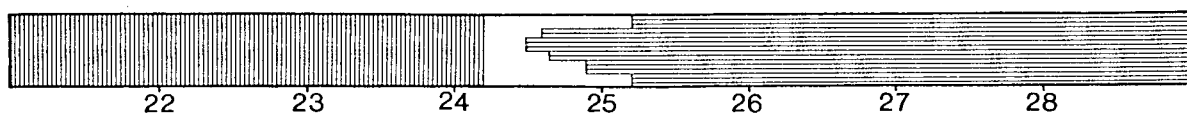
It was hypothesized that if the two competitors were to continue to compete in the homogeneous culture gradients they might converge and coexist.

Since it was impossible to promote continued competition by simply leaving the two species in the same

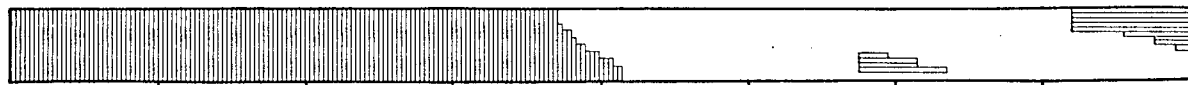
Figure 31

Culture gradient drawings demonstrating the area occupied by fruiting bodies of both species. The spores used came from stock cultures. The vertically shaded areas were covered with D. discoideum fruiting bodies, the horizontally shaded areas with P. pallidum fruiting bodies. The unshaded areas had no fruiting bodies. For gradients A, B, and C D. discoideum V12 was used. For gradients D and E D. discoideum DF was used.

A



B



C



D



E



culture gradient, because the media soon became fouled with waste products and the food was soon used up; competition was continued by a serial transfer technique. The first gradient was left for about two weeks, until the food was gone and fruiting was finished, then then the spore production from this gradient was harvested at random and used to establish a new culture gradient. The spores from the second gradient were used to establish a third, and so on. The changes experienced by the two competitors were observed in four replicate culture experiments. Since minor differences were encountered in each of the series they must be considered separately.

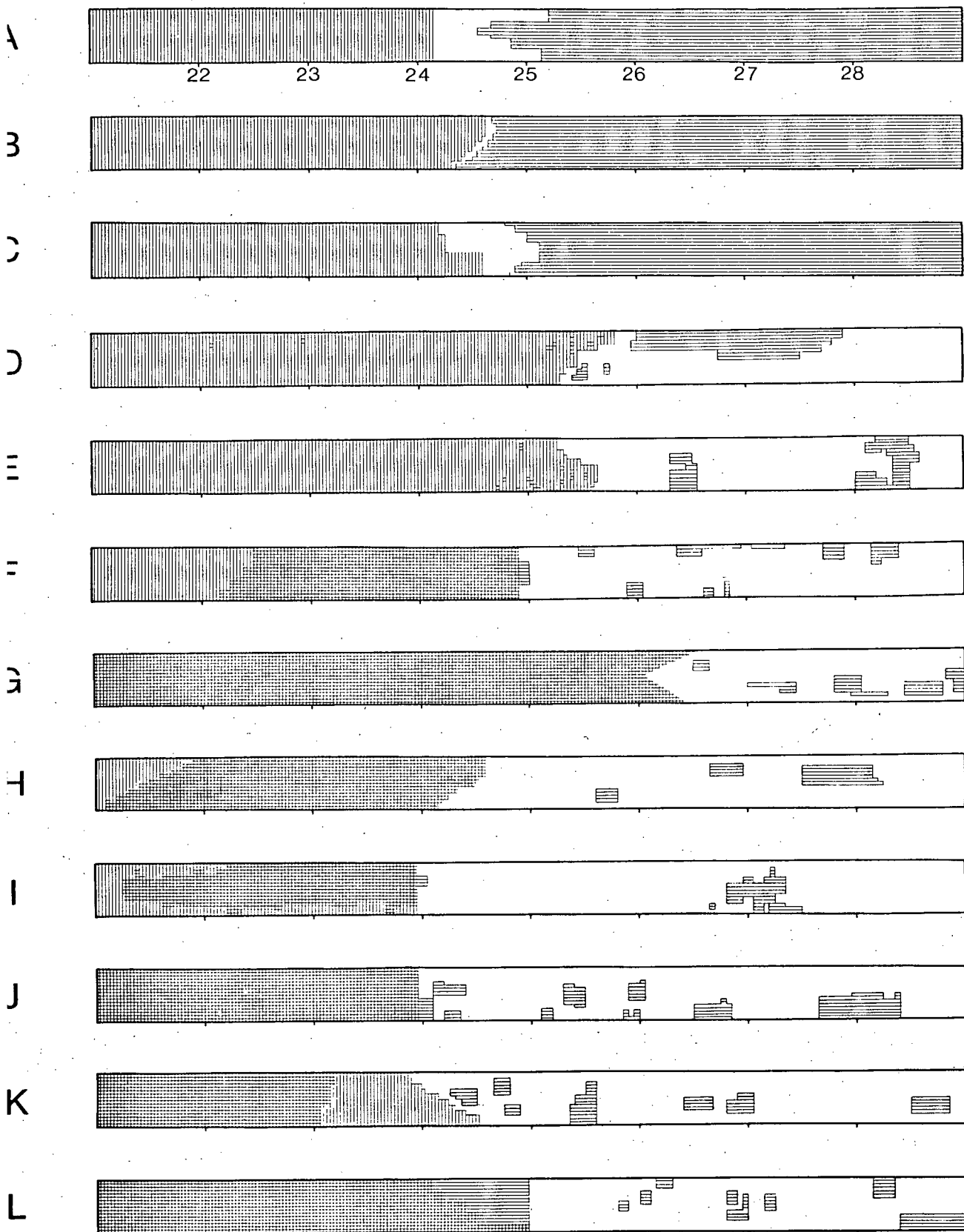
Culture Gradient I

Culture gradient I was established during July 1968 and completed during March 1969. The spore progeny of each gradient was used to establish the next in the series, but in this one case, the spores used to make the serial transfer were not chosen at random. They came from the area of the trough that was closest to the area occupied by the other species. This practice was not followed in the other gradient experiments.

The data indicated that D. discoideum V12 occupied the area from about 15° to 24.5 - 25.5°C and continued to occupy this area throughout the series. P. pallidum, on the other hand, occupied the area extending from 24 - 25°C to

Figure 32

Culture gradient I: gradient drawings demonstrating the changes in fruiting ability exhibited by P. pallidum during continued competition. The horizontally shaded areas were occupied by P. pallidum fruiting bodies, the vertically shaded area by D. discoideum fruiting bodies. The areas shaded with horizontal and vertical lines were occupied by fruiting bodies of both species. The unshaded areas were unoccupied. Temperature is marked at intervals under each diagram.



30°C during the first few cultures but with continued competition P. pallidum extended its range down to about 20°C (Fig. 32).

To summarize: before competition the two species were unable to co-fruit. After continued competition P. pallidum began to fruit between 20° and 24°C, along with D. discoideum V12. It should be noted that P. pallidum was always capable of fruiting in this area when grown alone, it was only the interference by D. discoideum that prevented it from doing so. This interference factor was apparently overcome by some change resulting from continued competition.

Culture Gradient II

Culture gradient II was begun during September 1968 and finished during May 1969. Its establishment and continuance was exactly the same as that of culture gradient I except that the spores used to make the serial transfers came from the entire fruiting area and were chosen at random.

As in culture gradient I the two species began by fruiting in separate areas but after continued competition P. pallidum began to co-fruit with D. discoideum. Co-fruiting in culture gradient II was not as extensive nor as regular as it was in culture gradient I (Fig. 46 - Appendix II). No real explanation can be given for this, except that the spore selection in culture gradient II was not as precise as that

employed in I, and therefore, selection may not have occurred as quickly or as surely. However, co-fruited did occur.

As in culture gradient I the results of II suggest that D. discoideum was unable to fruit between 25° and 26°C through the entire series. On the other hand P. pallidum overcame the inhibitor that prevented it from fruiting.

Finally, gradient II-E (Fig. 46 - Appendix II) and gradient I-G (Fig. 32) both depict D. discoideum fruiting up to 26°C. Both gradients were run at the same time (October 1968) and experienced an equipment failure which caused a shift in the gradient temperature during the first two days of incubation. This short term shift was enough to alter the final competitive outcome, suggesting that the temperatures during the first stages of incubation determine the outcome of the entire competitive situation.

Culture Gradient III

Culture gradient III was established during May 1969 and was completed during September 1969. The general results of this experiment are exactly as they were for the preceding gradients (Fig. 47 - Appendix II). There were two differences however: (1) The culture dishes were only half as wide as those used in other experiments, but the spore number relative to area was identical. (2) The D. discoideum strain was D. discoideum DF which has a higher temperature tolerance than

either VC4 or V12. This higher tolerance allowed D. discoideum DF to grow up to about 26°C as it did in the preliminary cultures (Fig. 30-D, E).

Despite the fact that the D. discoideum strain was different, the results were the same. Before continued competition P. pallidum was unable to co-fruit with D. discoideum, and after continued competition co-fruiting occurred. D. discoideum did not alter its fruiting ability.

Culture Gradient IV

A rather elaborate experimental design was used to generate the spores that were eventually used to establish gradient IV. The data from the three previous gradients has shown that continued competition over a long temperature gradient could result in co-fruiting. But whether the same results could be achieved at any one temperature within the range extending from 18°C to 26°C was unknown. To answer this question D. discoideum V12 cultures were grown at 18°C and 24°C, and P. pallidum cultures were grown at 24°C and 36°C. The spores that came from these four stock cultures were mixed in all combinations and the mixtures were grown at 24°C. The percent of cultures yielding fruiting bodies from both species was then calculated. The stocks were maintained by serial transfers, and mixtures and comparisons were made a second time. This procedure was continued for nine time periods. The following diagram may help to explain the method:

TIME	TEMPERATURE			REMARKS
	18°	24°	36°	
1	Dd stock	Dd stock PP stock	P _p stock	- stocks were set up
2		Dd stock PP stock (1) mix (2) mix	P _p stock	- all stocks continued - (1) mix is Dd stock 24° and PP stock 24° - (2) mix is Dd stock 18° and PP stock 36°
	Dd stock	Dd stock PP stock (1) mix (2) mix (3) mix	P _p stock	- all stocks continued - (1) mix is Dd stock 24°, and PP stock 24° - (2) mix is Dd stock 18° and PP stock 36° - (3) mix is from number (1) mix
	Dd stock		P _p stock	

4 etc...

From the previous work it was apparent that none of the mixtures would produce fruiting bodies of both species if homogeneous mixtures of spores were used as the inoculum. To circumvent this problem the mix cultures were established by making two small holes in the agar, 5 mm in diameter and 1 to 2 mm apart. P. pallidum spores were placed in one hole and D. discoideum spores in the other. The mixtures were incubated at 24°C for 4 to 7 days, and the presence or absence of co-fruited was noted. The following series of tables summarizes the findings. It should be noted that:

- mix (1) = Dd stock 24° + PP stock 24°
 mix (2) = Dd stock 18° + PP stock 18°
 mix (3) = output of mix (3) from time t-1

TIME 2 - TEMPERATURE 24.0° ± 0.5°

MIX NUMBER	REPLICATE	% CO-FRUIT
1	7	100%

TIME 3 - TEMPERATURE 23.6° ± 0.6°

MIX NUMBER	REPLICATE	% CO-FRUIT
1	4	25%
2	4	75%
3	4	100%

TIME 4 - TEMPERATURE $23.0^{\circ} \pm 0.0^{\circ}$

MIX NUMBER	REPLICATE	% CO-FRUIT
1	4	100%
2	4	100%
3	4	100%

TIME 5 - TEMPERATURE $22.5^{\circ} \pm 0.5^{\circ}$

MIX NUMBER	REPLICATE	% CO-FRUIT
1	4	100%
2	3	100%
3	3	100%

By this time it had become clear that very little difference could be found between the three types of mix cultures when they were established using small and separate agar holes. It had been observed, however, that in some instances the fruiting bodies of the two species were actually inter-dispersed with one another. It was really the degree of intermingling that was of interest, and therefore, a mixing index was established. A culture was awarded one point on the mixing index for every pair of P. pallidum and D. discoideum fruiting bodies that were separated by not more than five mm. Therefore, if a D. discoideum fruiting body grew in the middle

of a "forest" of P. pallidum fruiting bodies, it would have a mixing index of four. One point for each of the four sides adjacent to P. pallidum.

From this point on the mixing tables register the percentage of the culture which had a mixing index of one or more. The mean index $\pm$ one standard deviation is also given.

TIME 6 - TEMPERATURE $23.2^{\circ} \pm 0.2^{\circ}$

MIX NUMBER	REPLICATE	% WITH INDEX	INDEX
3	8	6%	1.6 ± 3.0
2	none	-	-
1	none	-	-

TIME 7 - TEMPERATURE $24.5^{\circ} \pm 0.5^{\circ}$

MIX NUMBER	REPLICATE	% WITH INDEX	INDEX
1	none	-	-
2	none	-	-
3	11	23%	2.0 ± 4.4

TIME 8 - TEMPERATURE $22.7^{\circ} \pm 1.2^{\circ}$

MIX NUMBER	REPLICATE	% WITH INDEX	INDEX
1	8	40%	1.8 ± 1.8
2	8	0.0%	0.0 ± 0.0
3	24	40%	5.1 ± 9.1

TIME 9 - TEMPERATURE $23.5^{\circ} \pm 0.5^{\circ}$

MIX NUMBER	REPLICATE	% WITH INDEX	INDEX
1	8	12%	0.9 ± 1.8
2	8	0%	0.0 ± 0.0
3	18	22%	2.0 ± 4.1

Mixture type 3 consistently had the greatest mixing index and usually the highest percentage of mixing, but there was still no way to support or refute the hypothesis that continued mixing increased the chance of co-fruited. The experimental method used was simply not sensitive enough. To rectify this situation the mix cultures were established by suspending the two types of spores together in water and inoculating the cultures with the suspension.

TIME 10 - TEMPERATURE $21.0^{\circ} \pm 1.0^{\circ}$

MIX NUMBER	REPLICATE	% MIXING
3	16	43.6%

TIME 11 - TEMPERATURE $22.0^{\circ} \pm 1.0^{\circ}$

MIX NUMBER	REPLICATE	% MIXING
3	6	100%

TIME 12

MIX NUMBER	REPLICATE	% WITH INDEX	INDEX
TEMPERATURE $21.5^{\circ} \pm 0.7^{\circ}$			
3	1	100%	25
TEMPERATURE $23.6^{\circ} \pm 0.7^{\circ}$			
3	6	83%	23 ± 16.8
TEMPERATURE $24.4^{\circ} \pm 0.6^{\circ}$			
3	6	83%	9.8 ± 6.3

The mix cultures 3 that came from previous mix cultures appeared to be able to co-fruit in almost every instance, particularly after the selection during time period 10. To prove this point the spores from time period 12 were used to establish culture gradient IV. In gradient IV co-fruiting occurred immediately and continued throughout the experiment (Fig. 48 - Appendix II).

To summarize; this experiment demonstrated that the ability of P. pallidum to overcome D. discoideum inhibition does not depend upon growth over the wide range of temperatures provided in the gradient but can occur at one temperature in the overlap range of 18° to 25°C .

It might be hypothesized that competition had nothing to do with the change in fruiting ability undergone by P. pallidum. One or both species might have acclimated to temperatures in the middle range (20°C to 24°C), and this acclimation might have been enough to cause co-fruited. This hypothesis was tested in the following manner. The cultures that were used to establish gradients I and II were grown at 18°C (D. discoideum) and 34°C (P. pallidum) for about four months before the gradients were established. In both cases no co-fruited occurred in the first culture gradient. The cultures that were used to establish the preliminary gradients (Fig. 31A, B, C) were grown at room temperature ($22.0^{\circ} \pm 2.0^{\circ}$) for a minimum of four months before being used to establish these gradients. Again no overlap occurred. Therefore, temperature alone was not enough to cause co-fruited.

At this point in the study the data strongly suggests that competition alone is both necessary and sufficient to cause co-fruited. But three questions remain unanswered: (1) which species changed? (2) what are the mechanics of the change? (3) what kind of change occurred?

Changes Between 18° And 24°

Which Competitor Changed?

With the data available it was known only that competition caused something to happen which resulted in

co-fruiting. These results could have been obtained in one of three ways: (1) D. discoideum could have stopped inhibiting P. pallidum, (2) P. pallidum could have overcome the inhibition from D. discoideum, or (3) both competitors could have changed.

To find which of these hypotheses was the most reasonable the following experiment was designed:

<u>D. discoideum</u> (stock)	}	gradient A
was grown with		
<u>P. pallidum</u> (changed)		

<u>D. discoideum</u> (changed)	}	gradient B
was grown with		
<u>P. pallidum</u> (stock)		

where "stock" denotes stock cultures that had not experienced competition and "changed" denotes cultures which came from co-fruiting gradients. If gradient A resulted in co-fruiting, then P. pallidum must have changed. If gradient B resulted in co-fruiting then D. discoideum must have changed. If both gradients A and B resulted in co-fruiting then both must have changed.

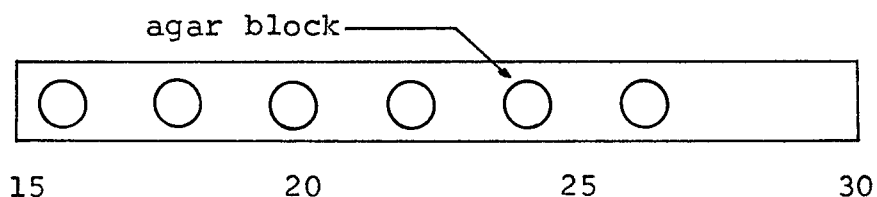
The experiment outlined above was replicated four times and in every case only gradient A resulted in co-fruiting (Fig. 49 - Appendix II). It is safe to assume that only

P. pallidum changed. In all probability this species was, in some way, able to overcome D. discoideum inhibitory ability.

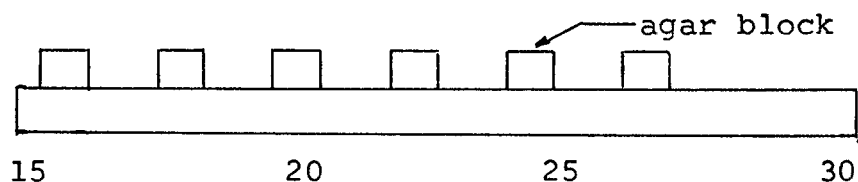
Mechanics of the Change

Having found that P. pallidum reacted to competitive pressure by overcoming its inability to grow in the presence of D. discoideum it would be desirable to know something about the nature of the inhibitory effect. All of the work up to this point indicated only that P. pallidum fruiting bodies were not found in the presence of D. discoideum before competition and that they were found after an extended period of competition. P. pallidum could have been stopped at any one of three stages and the above description would still be applicable. The three stages are: (1) P. pallidum spores might have been unable to germinate, (2) the spores might have germinated to produce amoebae but the amoebae may have been unable to reproduce, or (3) both the spores and amoebae may have acted normally but the amoebae may have been unable to aggregate to produce fruiting bodies.

The following experiment was designed to find which of the three hypotheses was correct. A culture gradient was set up in the usual way with a bacteria lawn and a random dispersal of spores of both species. Small blocks of agar, also covered with a bacteria lawn, were placed at intervals down the length of the gradient. In plan view the gradient appears:



and in side view:



The agar block gradient was then incubated on the temperature gradient. If P. pallidum spores were germinating in the presence of D. discoideum and if the amoebae were moving around and dividing it should have been possible to find the amoebae on top of the agar blocks. Therefore, after incubation a smear was taken from the top of each agar block and incubated at 36.0°C. P. pallidum was found down the length of the gradient (Fig. 33-A, B, C) (stars denote blocks on which P. pallidum was found). These data strongly suggest that D. discoideum was able to inhibit P. pallidum at the fruiting body formation stage.

As a check against the hypothesis that the amoebae found on top of the agar blocks migrated down from the area above 25°C, the culture gradients were run in pairs, one undivided, and one with aluminum foil dividers between each agar block. The dividers were sealed in place with silicone

Figure 33

Culture gradient drawings demonstrating the ability of P. pallidum to fruit below about 24°C. Horizontally shaded areas were occupied by P. pallidum fruiting bodies. Vertically shaded areas by D. discoideum. Vertically and horizontally shaded areas by both species. Unshaded areas by neither. The circles represent the agar blocks in plan view. The dividers between blocks are shown in A DIV, B DIV AND C DIV. The stars over the blocks denote blocks on which P. pallidum was found. Temperature is measured in degrees centigrade along the x-axis.

A



A'



B



B'



C



C'



21

22

23

24

25

26

27

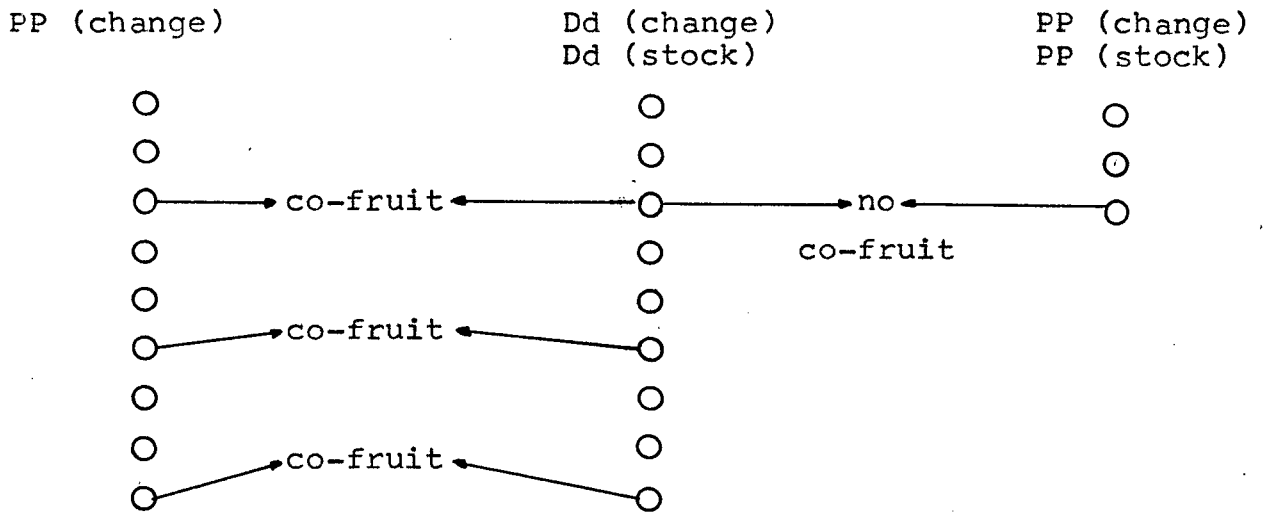
28

grease. In the divided gradients P. pallidum was still found on top of the agar blocks (Fig. 33-A DIV, B DIV, C DIV).

To summarize: D. discoideum was, in some way, able to stop P. pallidum vegetative amoebae from aggregating and fruiting, but the two species of amoebae compete for food at all non-lethal temperatures. The type on inhibitor used by D. discoideum remains unknown.

The Type of Change

Since the species that changed, and the point at which the change occurred were identified, the kind of change that took place was considered. P. pallidum could have changed genetically, or adaptively, or in some other un-hypothesized manner. In an attempt to narrow the field two experiments were conducted. In the first experimental set P. pallidum (changed) (from gradient I) was mixed 50:50 with P. pallidum (stock) and the culture dish was grown at 27°C. At the same time D. discoideum V12 from gradient I was mixed 50:50 with D. discoideum V12 (stock) (it was unnecessary to make the D. discoideum mixture but when this experiment was done it was not known that only P. pallidum had changed). At the same time P. pallidum (changed) was grown by itself. The following diagram explains the design.



During time period 1 the first plate of each line was allowed to grow until all fruiting stopped (a period of one week). The spore progeny were then used to set up plates for time period 2, and so on. At the end of time period 3 a culture gradient was established with the output of line 1 (PP change) and line 2 (Dd change + Dd stock). Co-fruiting occurred. The lines were continued and co-fruiting continued to occur (Fig. 50-A, B, C - Appendix II). When line 2 was mixed with line 3 (PP change + PP stock) no co-fruiting occurred in time period 3 (Fig. 50-D - Appendix II).

Two kinds of information are yielded by this set of experiments. First, the cultures containing only *P. pallidum* (changed) were able to maintain their co-fruiting ability even in the absence of interspecific competitive pressure. Second, the cultures containing both (changed) and (stock) strains lost their ability to produce co-fruiting spores,

suggesting that the (changed) stock was at an intraspecific competitive disadvantage.

These data suggest, but do not prove, that P. pallidum did not experience an adaptive change. If the change were adaptive one might expect that the cultures containing only P. pallidum (changed) would lose some, or all, of their co-fruiting ability in the absence of interspecific selective pressure. The stability of the change suggests, but does not prove, that the change may have been genetic.

Cloning Experiments

If selection really was occurring there are several possible pathways which might be followed. For example:

(1) A co-fruiting mutant might have occurred and been favoured during competition, or (2) the population's gene pool might have contained one or more co-fruiting genotypes which were favoured during competition.

One of the most straightforward methods of solving this type of problem is to clone out individual spores and test their ability to co-fruit. In the case of cellular slime molds the cloning procedure presents no problems, but the testing procedure involves the establishment of a separate mixed culture gradient for each clone. Despite this difficulty it was decided that a maximum of ten tests would be conducted using ten separate P. pallidum clones and a standard stock

D. discoideum culture.

The fifth test resulted in co-fruiting by P. pallidum and D. discoideum (Fig. 51 - Appendix II) suggesting that the stock population of P. pallidum contained spores with co-fruiting ability. This experiment does not allow any statistical significance to be attached to the evidence. Apparently, however, the ability to co-fruit was contained within the stock gene pool.

Changes Between 24.0° And 26.5°C

When grown alone D. discoideum was capable of using food and space, and fruiting between 24° and 26.5°C. But, when grown with P. pallidum, fruiting bodies were not observed above 24° to 25°C. After continued periods of competition D. discoideum V12 was unable to improve its fruiting ability over this short temperature range.

The lack of improvement in fruiting ability is unexplained, but it was observed that beyond 24.5°C the expansion rate of D. discoideum V12 dropped rapidly to become zero at 26.4°C (Fig. 12). This put D. discoideum at a very severe competitive disadvantage in this area, so that the changes that would have to occur would undoubtedly involve changes in growth rate, as well as changes in fruiting ability.

Despite the fact that no change was observed it was still important to ascertain the point at which

D. discoideum was inhibited. Just as in the case of P. pallidum the inhibition could have occurred at the spore stage, the vegetative amoebae stage, or the aggregation stage. To distinguish between these possibilities, tests like those used for P. pallidum were employed. A culture gradient was inoculated with equal numbers of both D. discoideum and P. pallidum spores. Bacteria covered agar blocks were set on top of the agar surface and the gradient was incubated. After incubation smears were taken from the tops of the agar blocks and incubated at 20°C. D. discoideum was found on all of the agar blocks (Fig. 52-A, B, C - Appendix II) suggesting that the spores germinated and the amoebae divided and consumed food, but that aggregation did not occur.

To test the hypothesis that amoebae were moving from the gradient area below 24°C, a divided culture gradient was used (Fig. 52-C-DIV - Appendix II) and D. discoideum amoebae were still found growing above 25°C. While the inhibition of D. discoideum fruiting is probably relatively unimportant due to the small temperature span over which it occurs, the mechanism involved appears to be the same as the mechanism involved in P. pallidum inhibition.

Similarity of Resource Use

The foregoing data have indicated that competitive pressure selected for P. pallidum individuals that were able

to co-fruit with D. discoideum between 20° and 24.5°C. This occurrence makes continued coexistence possible. When the species were unable to co-fruit it was impossible for P. pallidum to produce more than one generation since the lack of spores precluded the production of further generations. But with the acquisition of co-fruited ability the chances that P. pallidum could successfully coexist between 18° and 24°C was increased.

Logically, however, continued coexistence could only come about in situations where the competitors were divergent with respect to resource use or where they used the resource at exactly the same rate. Therefore, during continued competition it might be expected that one of three types of selection with respect to resource use might occur. These are: (1) the competitors might diverge and use alternate resources, (2) they might converge using the resource at exactly the same rate, or (3) one competitor might increase its rate of resource use to exclude the other.

In an attempt to find which of these paths were followed in the cellular slime mold situation, the components determining rates of resource use were assessed after a period of continued competition. The components involved are the germination lags and the colony expansion rates.

Germination Lags

For P. pallidum salvador, germination lag data was collected after a period of continued competition, from culture gradients I and II, by regressing culture expansion data and noting the points at which the regression lines intercepted the x-axis (Fig. 10). These data were compared with measurements of spore germination lag collected before competition began (Fig. 7). There was no significant difference before and after competition (Table X). For D. discoideum V12 germination lag data was collected both before and after continued competition by the regression method. Again data from culture gradients I and II were considered together. No change before and after continued competition was detected in D. discoideum at the 95% level of confidence (Table XI).

Colony Expansion Rates

The second and most important component capable of directly altering rates of resource use are the colony expansion rates. For P. pallidum the rates of colony expansion were measured separately for culture gradient I and II after a period of continued competition (measurements were made on the progeny of gradients J, K, L in both cases). Two points should be noted: (1) the post-competitive data from gradient I (Fig. 35) and gradient II (Fig. 37) are very

TABLE X

P. pallidum salvador (from gradients I and II) spore germination lags before (stocks) and after (change) continued competition. Replication is shown from the changed cultures and 95% confidence intervals around the change mean are given. Stock lags come from Figure 7.

TEMPERATURE	REPLICATE	GERM LAG CHANGE	95% CON CHANGE	GERM LAG STOCK
22.5	4	1.17	0.60-1.74	1.05
23.5	12	1.02	0.79-1.25	0.94
24.5	11	1.05	0.88-1.22	0.88
25.5	10	0.95	0.68-1.22	0.84
26.5	12	0.84	0.69-0.99	0.78
27.5	13	0.73	0.61-0.85	0.75
28.5	12	0.67	0.46-0.88	0.71
29.5	8	0.60	0.36-0.83	0.70

TABLE XI

D. discoideum V12 (from gradients one and two) spore germination lags before (stock) and after (change) continued competition. Replication is listed for the change cultures and confidence interval around the change mean are given.

TEMP	REP	GERM LAG CHANGE	95% CON CHANGE	REP	GERM LAG STOCK	95% CON STOCK
18.5	7	0.75	0.37-1.14	4	0.72	0.20-1.24
19.5	7	0.57	0.32-0.82	5	0.70	0.18-1.21
20.5	9	0.56	0.36-0.77			
21.5	8	0.51	0.32-0.69	5	0.58	0.19-0.96
22.5	10	0.51	0.37-0.65			
23.5	5	0.58	0.34-0.81			
24.5	3	0.73	0.44-1.02	7	0.58	0.28-0.88

similar. This was expected because the two gradients are replicates, (2) the post-competitive data is not significantly different from the pre-competitive data (Fig. 13).

For D. discoideum the rates of colony expansion were also measured separately for gradient I and II, and measurements were also made on gradients J, K, L. Here too there are two points which should be noticed: (1) the post-competitive data for gradients I (Fig. 34) and II (Fig. 36) are not different, and (2) the post-competitive data and the pre-competitive data (Fig. 12) are very different. Both culture gradients yielded organisms which were able to expand the colony at a much greater rate after competition than they were before.

The hypothesis that rates of resource use changed in response to competition cannot be tested on the basis of these data alone. A search must be made for changes in germination rates and rates of colony expansion of stock cultures which never experienced competition but which were grown for the same length of time that the competition experiments were run. It is entirely possible that changes in rates of food use experienced in the competitive cultures were the result of media conditioning or some other cause not related to competition at all.

Figure 34

D. discoideum culture gradient I expansion rates with respect to temperature. Growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from the family described by equation (2c). The points are means $\pm$ 95% confidence limits on the means. $T_H = 27.5$, $T_L = 13.0$, $T_O = 23.25$, $K = 1.16758$, $C = 4.79121$, $G_{max} = 6.2$.

Figure 35

P. pallidum culture gradient I expansion rates with respect to temperature. Growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from the family described by equation (2c). The points are means $\pm$ 95% confidence limits on the means. $T_H = 37.5$, $T_L = 18.0$, $T_O = 30.0$, $K = 1.73781$, $C = -4.82781$, $G_{max} = 8.4$.

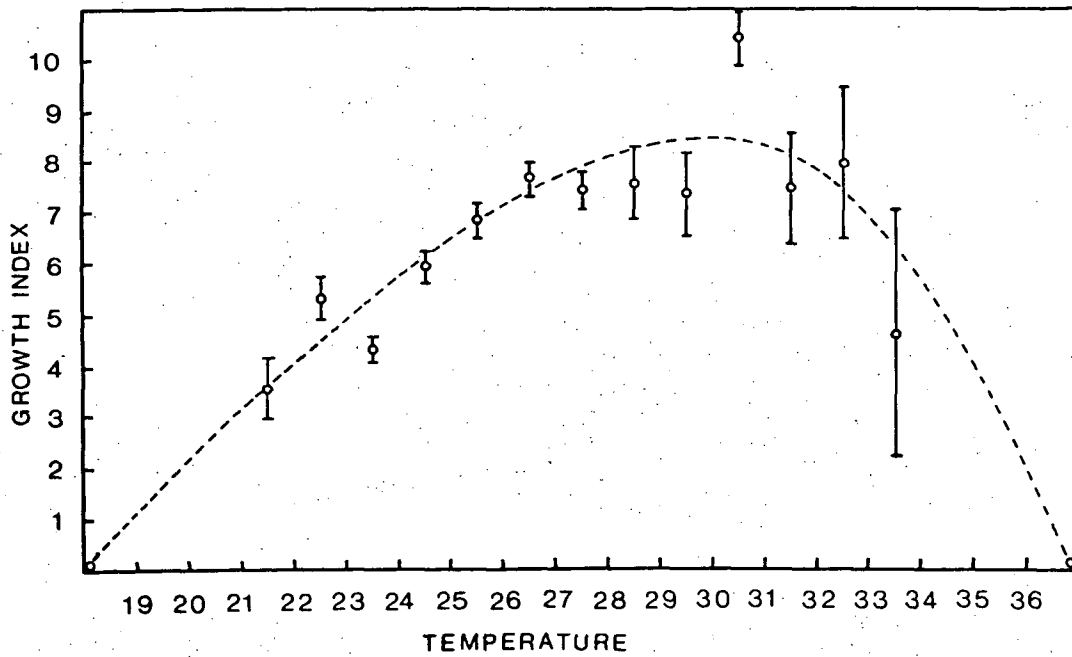
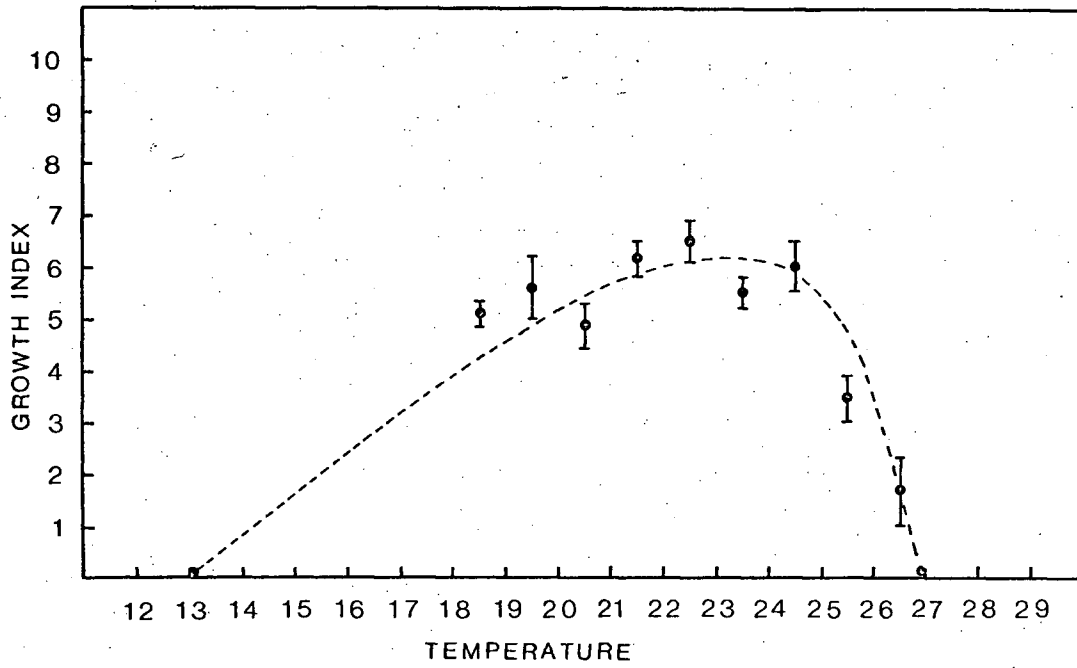
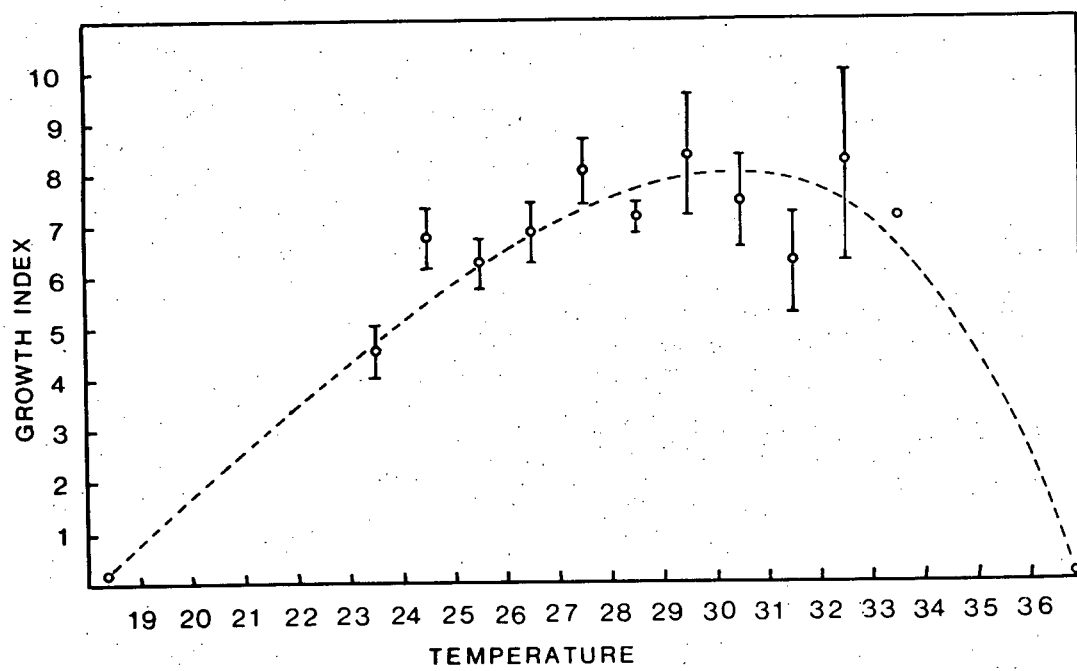
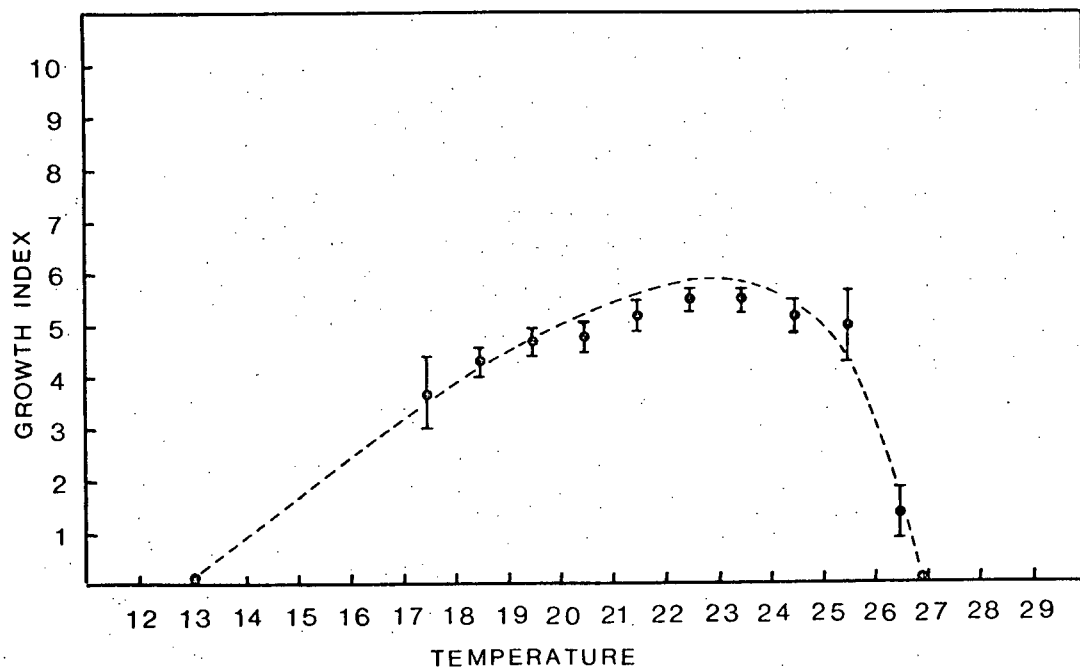


Figure 36

D. discoideum culture gradient II expansion rates with respect to temperature. Growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from the family described by equation (2c). The points are means $\pm$ 95% confidence limits on the means. $T_H = 27.5$, $T_L = 13.0$, $T_O = 23.0$, $K = 1.22500$, $C = 3.02126$, $G_{max} = 5.8$.

Figure 37

P. pallidum culture gradient II expansion rates with respect to temperature. Growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from the family described by equation (2c). The points are means $\pm$ 95% confidence limits on the means. $T_H = 37.5$, $T_L = 18.0$, $T_O = 30.4$, $K = 1.53059$, $C = -2.4335$, $G_{max} = 8.0$.



Stock Spore Germination Rates

For *P. pallidum* the spore germination rates were measured during October and November of 1968. The cultures were then maintained by a series of bi-weekly serial transfers and the spore germination times were again calculated using the regression method. The data indicates that no significant change in spore germination time was experienced (Table XII).

D. discoideum presented problems. Due to an equipment failure in the spring of 1969 the V12 stock that was used to establish gradients I and II was lost. This meant that it was impossible to test for changes that might have occurred in the stock culture in the absence of competitive pressure. As a partial remedy to this problem *D. discoideum* VC4 stock was established in August 1969 and run through 12 bi-weekly transfers which ended in February 1970. Despite the fact that the strain was different the changes that occurred during this period of time probably do parallel the changes experienced by the V12 stock. The data from these experiments (Table XIII) indicates that no change in spore germination time was experienced by the stock cultures.

Stock Colony Expansion Rates

For *P. pallidum* the colony expansion rates were measured in October of 1968 (Fig. 13) and again during June 1969 (Fig. 38). A visual comparison of the two figures

TABLE XII

P. pallidum salvador: comparison of stock germination lags before and after an extended period of intraspecific growth. Replication is listed for cultures tested after a period of growth and 95% confidence limits around the "after" means are given.

TEMPERATURE	REPLICATE	GERM LAG AFTER	95% CON AFTER	MEAN BEFORE
18.5	7	2.08	1.89-2.28	2.30
19.5	6	1.75	1.30-2.19	1.62
20.5	5	1.72	0.98-2.45	1.30
21.5	8	1.17	0.90-1.44	1.15
22.5	8	0.98	0.71-1.25	1.05
23.5	7	1.01	0.83-1.19	0.94
24.5	6	1.03	0.72-1.34	0.88
25.5	9	0.84	0.67-1.01	0.84
27.5	7	0.91	0.65-1.17	0.75
28.5	11	0.69	0.53-0.85	0.71
29.5	7	0.57	0.32-0.85	0.70
30.5	13	0.60	0.36-0.77	0.70

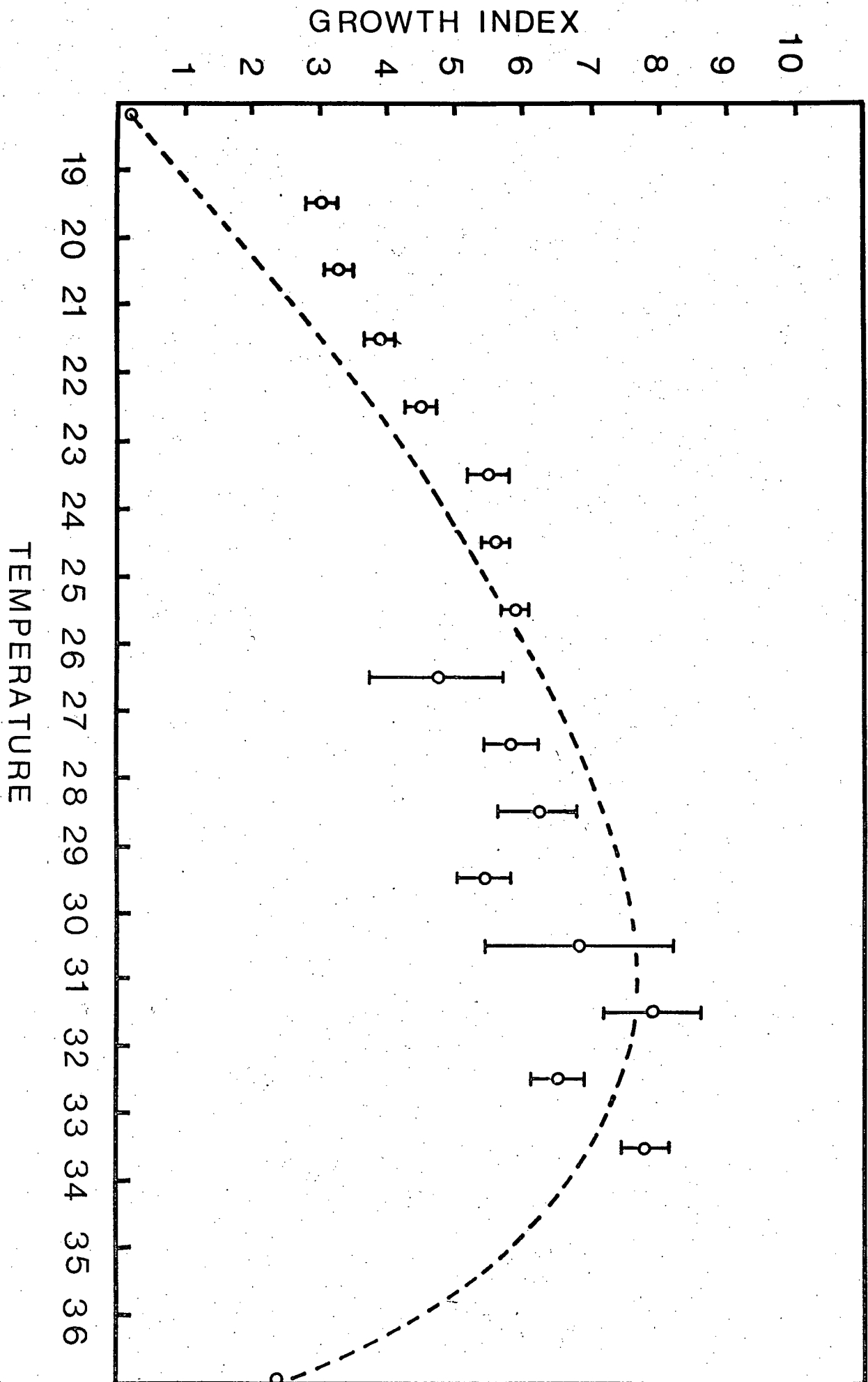
TABLE XIII

D. discoideum VC4: comparison of stock germination lags after extended periods of intraspecific growth. Replication is listed for cultures tested after growth and 95% confidence intervals around the "after" means are given.

TEMPERATURE	REPLICATE	GERM LAG AFTER	95% CON AFTER	MEAN BEFORE
15.9	3	1.60	0.86-2.33	1.43
17.9	9	1.40	1.18-1.61	1.18
18.8	5	0.98	0.62-1.33	1.10
19.4	4	1.00	0.77-1.22	1.04
20.1	4	1.20	0.67-1.72	1.02
21.0	4	1.10	0.97-1.22	1.00
22.5	8	0.98	0.90-1.07	0.98
23.5	3	0.90	0.40-1.39	0.96
24.1	3	1.20	0.95-1.44	0.98
25.0	3	1.20	0.95-1.44	1.03

Figure 38

P. pallidum amoebae expansion rates with respect to temperature. The spores used had experienced media conditioning. Growth index is measured without units, temperature is measured in degrees centigrade. The points are mean data points $\pm$ 95% confidence limits on the means. The dotted line is the line of best fit from equation (2c) with $T_H = 41.0$, $T_L = 18.0$, $T_O = 31.0$, $G_{max} = 7.6$, $K = 1.62709$, $C = -13.59423$.



suggests that the rates of colony expansion may have decreased slightly but the 95% confidence intervals around the data points used to establish these curves overlap, making it impossible to find any significant statistical difference in rates of colony expansion before and after a long period of growth in stock cultures.

Since the D. discoideum V12 stock was lost, the VC4 stock was used as an indicator of changes that might have occurred in the absence of competitive pressure. Colony expansion rates were measured during August 1968 (Fig. 39) and again during February 1970 (Fig. 40) after a series of 12 bi-weekly transfers. The rates of colony expansion changed significantly (95% level) over the six month period. The rate of colony expansion "after" (Fig. 40) was greater than "before" (Fig. 39). Evidently media conditioning or some other unknown factor allowed D. discoideum to increase its rate of resource use.

The changes that did occur due to competitive pressure alone are summarized in the following table.

P. PALLIDUM	BEFORE	AFTER
Co-fruiting 19° to 24.5°	No	Yes
Spore germination	Same	Same
Colony expansion	Same	Same

Figure 39

D. discoideum VC4 expansion rates with respect to temperature. The spores used had not experienced media conditioning. Growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from the family described by equation (2c). The points are means $\pm$ 95% confidence limits on the means.
 $T_H = 27.5$, $T_L = 9.0$, $T_0 = 22.25$, $K = 0.63277$,
 $C = 2.01331$, $G_{max} = 4.2$.

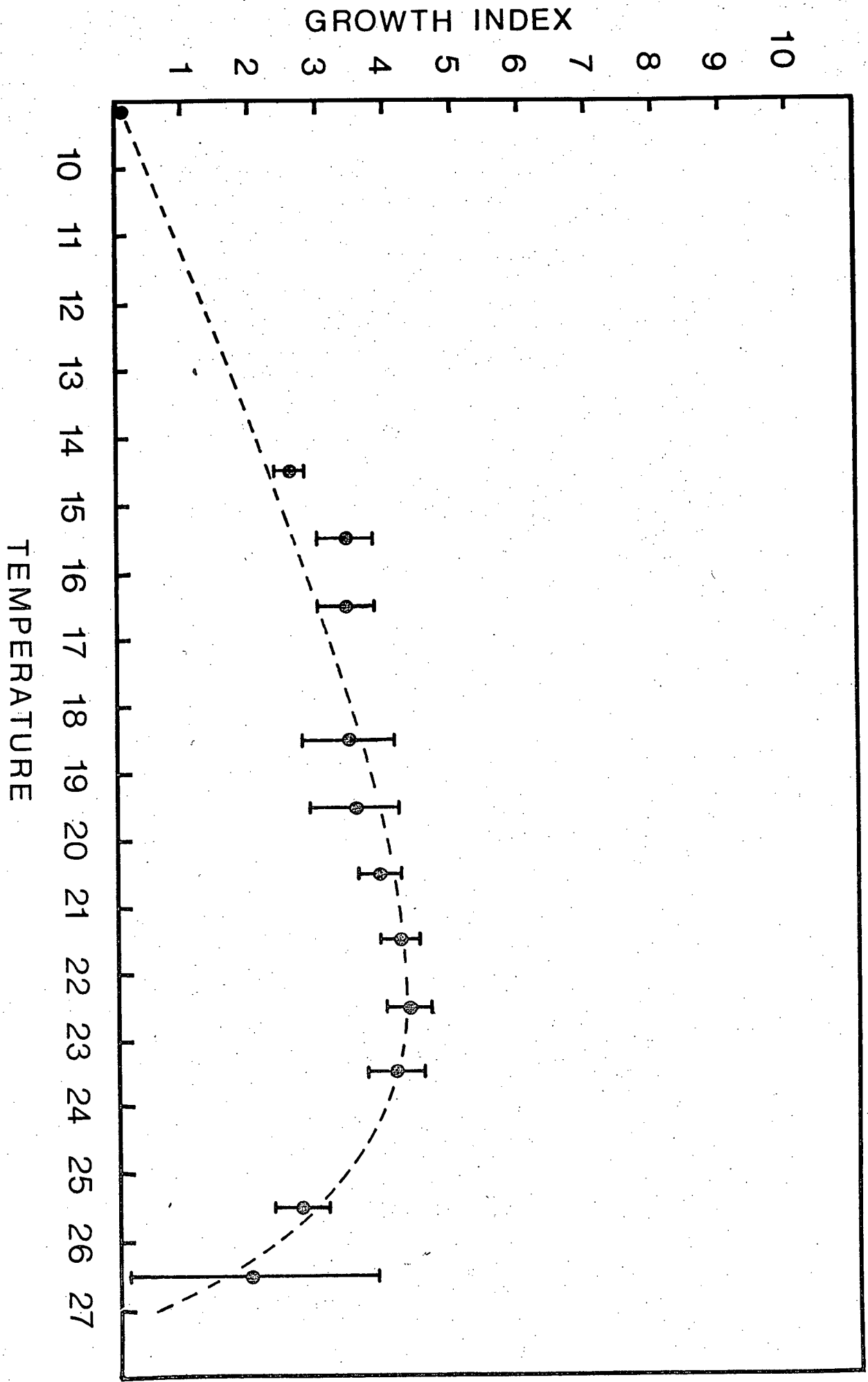
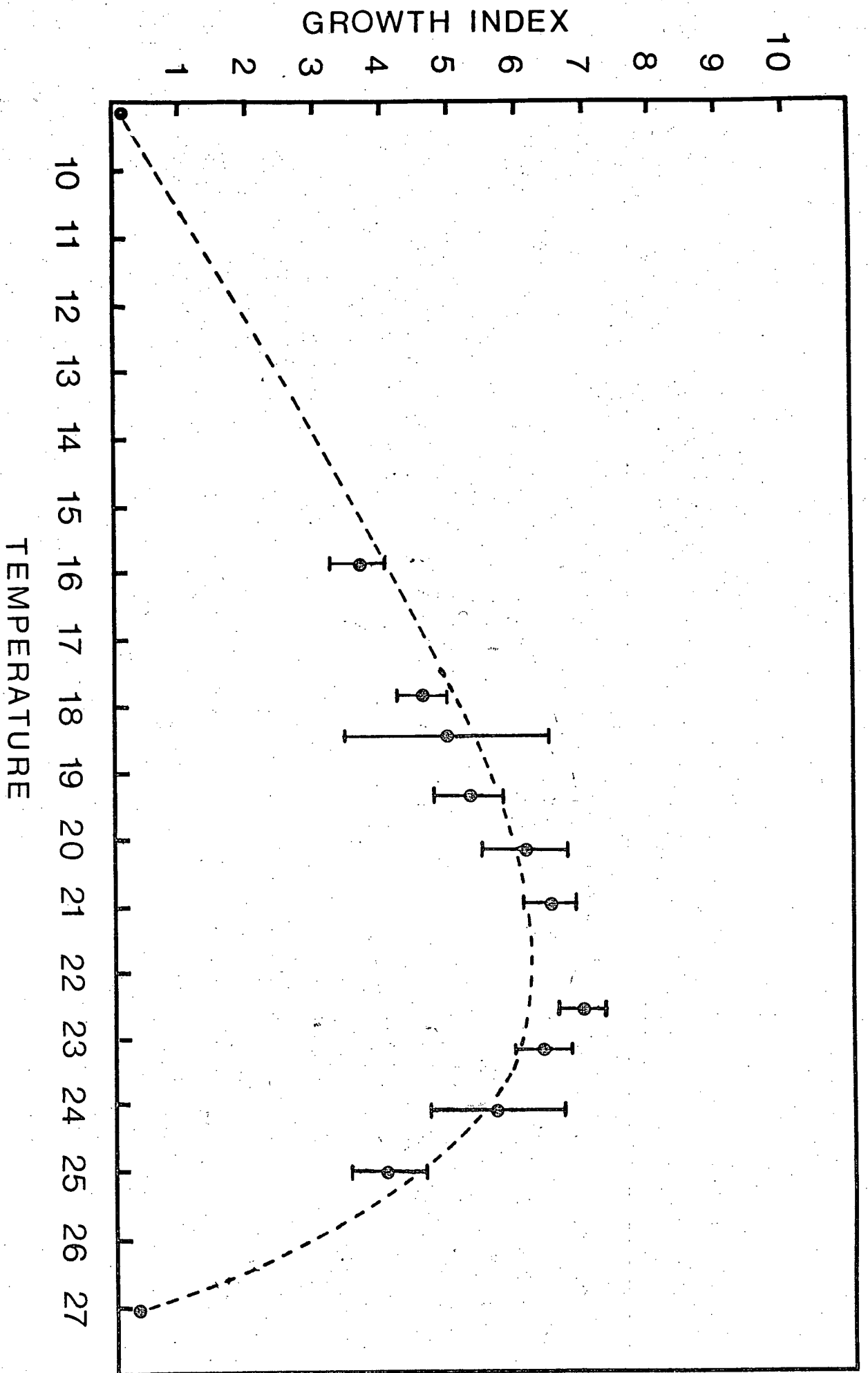


Figure 40

D. discoideum VC4 expansion rates with respect to temperature. The spores used had experienced media conditioning. Growth index has no units, temperature is measured in degrees centigrade. The dotted line is the line of best fit from the family described by equation (2c). The points are means $\pm$ 95% confidence limits on the means. $T_H = 27.0$, $T_L = 9.0$, $T_O = 22.0$, $K = 0.90973$, $C = 3.22786$, $G_{max} = 6.0$.



D. DISCOIDEUM	BEFORE	AFTER
Co-fruiting 24.5° to 26.5°	No	No
Spore germination	Same	Same
Colony expansion	Lower	Possibly* Higher

* Since the V12 stock could not be tested it is impossible to be exactly sure that the colony expansion rate did not change in response to competitive pressure. The VC4 data does suggest that the change that was observed was not caused by competition.

In summary, competitive pressure changed the co-fruiting ability of P. pallidum and possibly altered the rate of food use in D. discoideum. All the other parameters tested remained unaltered. But these two changes alone are enough to greatly influence the outcome of competition between the two species.

This can be demonstrated using Program VII with parameter values derived before competition and after competition. The output indicates that in time period one the rates of resource use were more similar before continued competition (Fig. 41-A) than after (Fig. 41-B). But, if the spore progeny of time period one were used to establish a new competitive situation in time period two, only D. discoideum would be available to use the resource before competition (Fig. 41-C), while both species would use food after competition,

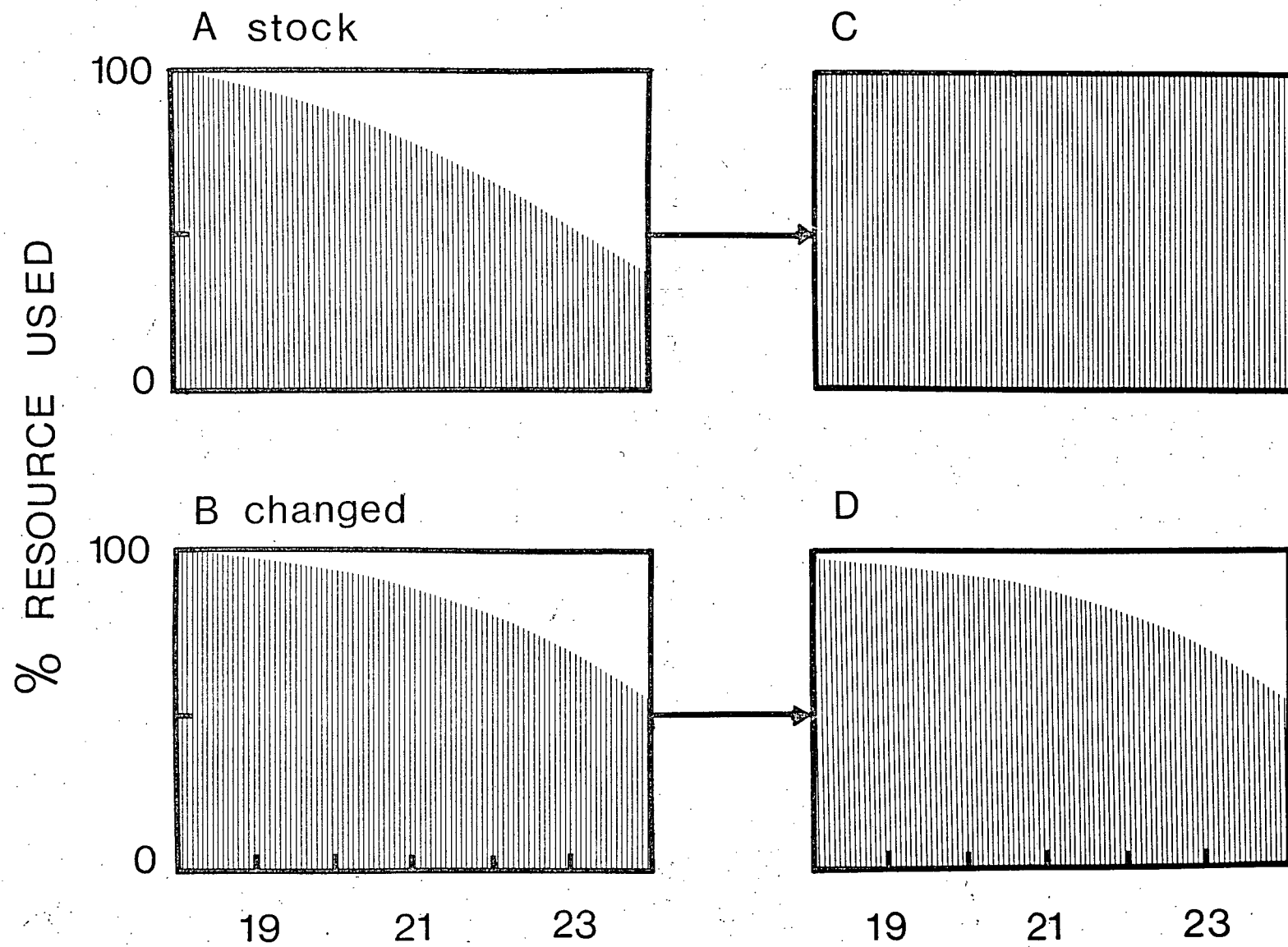
because the co-fruiting P. pallidum could produce spores (Fig. 41-D). Logically it would seem that P. pallidum would eventually be excluded because of its inability to use food as quickly as D. discoideum, but the interference experiments suggested that once P. pallidum becomes established it could inhibit D. discoideum in areas of high spore concentration and persist even in the face of considerable exploitation pressure.

These findings suggest that the original hypothesis of exclusion on the basis of unequal resource use or co-existence due to convergence and/or divergence, are too simple. In the case of the cellular slime mold species used in this work, P. pallidum converged towards D. discoideum when it became able to co-fruit, and D. discoideum diverged from P. pallidum in response to either competitive or media conditioning pressure. Both of these changes worked against one another.

In time period one it appeared that the rates of resource use had diverged during the period of extended competition and that P. pallidum would soon be excluded. However, in time period two the rates of resource use after competition were more convergent than they were before, because P. pallidum was able to produce progeny which could use the resource.

Figure 41

A comparison of the amount of resource used by competitors before and after competition. Percent of the total resource available is found on the y-axis, temperature on the x-axis. The shaded area represents the amount of resource used by D. discoideum, the unshaded area the amount of resource used by P. pallidum. The progeny of time period one were used to establish the competitive situation of time period two.



Summary - Continued Competition

- (1) Stock spores grown in culture gradients which extended from about 15° to 30°C produced D. discoideum fruiting bodies up to about 24°C and P. pallidum fruiting bodies beyond.
- (2) After periods of continued competition P. pallidum changed its fruiting ability but D. discoideum experienced no change. D. discoideum fruited up to about 24°C and P. pallidum fruited down to about 20°C.
- (3) P. pallidum overcame D. discoideum fruiting body inhibition between about 20° and 24°C.
- (4) D. discoideum did not overcome P. pallidum inhibition between about 24° and 26°C.
- (5) Competition alone was both a necessary and sufficient force to cause P. pallidum to change.
- (6) P. pallidum amoebae always reproduced and competed for food from about 20° to 24°C, but before the competition induced change, P. pallidum fruiting body production was stopped in this area.
- (7) Apparently the P. pallidum gene pool contained genotypes that could co-fruit and these were selected for by competition.

- (8) P. pallidum spore germination lags and amoebae colony expansion rates did not change in response to continued competition.
- (9) D. discoideum V12 spore germination lags did not change, but the colony expansion rates increased during continued competition. (Competition alone may not have been responsible for the change).
- (10) The simulation model suggested that P. pallidum could co-exist with D. discoideum between 20° and 24°C more readily after continued competition than before.

DISCUSSION

The experiments reported here were conducted in an attempt to describe the way in which two species of cellular slime mold compete in the laboratory; to test some of the hypotheses relating competition, convergence or divergence, and coexistence; and to devise a preliminary general method for approaching competitive problems in the laboratory and in the field. The results have indicated that it was overly optimistic to hope for an immediate solution in any of these areas, but it has been possible to add a little information to each. The work also touched on some aspects of cellular slime mold biology which are relevant to work being conducted in other laboratories. These findings are considered in section one of the discussion, while the more ecologically oriented work is discussed in the second section.

Section I: Cellular Slime Mold Biology

Inhibition

During the study it was observed that cellular slime mold competition was composed of both an exploitation and an interference component. Since the environment was simple it was possible to describe the major components involved in the exploitation of food and space accurately enough to simulate the amount of food and space used at any time. But, when the two competitors were grown together, they interfered with one

another's ability to produce fruiting bodies. The interference components that were identified were difficult to assess because the actual inhibitory substance could not be identified.

The results suggested that two separate inhibitory actions took place. D. discoideum inhibited P. pallidum fruiting body formation below some temperature, which was determined by P. pallidum spore concentration, and P. pallidum inhibited D. discoideum above some temperature, which was dependent upon D. discoideum spore concentration. Similar observations have been made by Raper and Thom (1941) who found that P. violaceum inhibited D. discoideum fruiting, and Cohen and Ceccarini (1967) reported that D. purpureum prevented P. violaceum from aggregating. The nature of the inhibitor remains unknown.

Any one of at least three classes of chemicals produced during fruiting body formation might be responsible. Bonner and Dodd (1962) and Bonner and Hoffman (1963) studied ammonia and carbon dioxide production and found that in some species, fruiting body spacing was linked to the production of these gases. Bonner and Hoffman (1963) also found that when D. mucoroides was confronted with D. purpureum or D. discoideum in petri dishes which had agar on the top and bottom surfaces, total inhibition resulted in four of the seven cultures run. In view of these findings it is possible that these chemicals are responsible for the interspecific inhibition observed in this study.

A second class of inhibitory chemical was first observed by Russell and Bonner (1960) and has subsequently been studied by Snyder and Ceccarini (1966), Ceccarini and Cohen (1967) and Cohen and Ceccarini (1967). They observed that a chemical intraspecifically inhibited spore germination. That is, Polysphondylium species were not inhibited at the spore stage by Dictyostelium species. They also observed that P. violaceum was unable to fruit in the presence of D. purpureum. This fruiting inhibition appears to be very much like the phenomena observed in the present study, but again there was no direct link between the intraspecific inhibitory chemical which Ceccarini and Cohen (1967) isolated and interspecific fruiting body inhibition. At the present time, cellular slime mold biologists (Bonner pers. comm.) tend to believe that the chemical of Ceccarini and Cohen is only effective in the fruiting body, where it prevents spore germination within the fruiting body head.

A third general class of chemical products produced by cellular slime mold species might be responsible. Raper and Thom (1941) observed that Dictyostelium species mixed during aggregation but separated by the time fruiting bodies formed. Dictyostelium and Polysphondylium species, however, did not approach one another, even in mixed cultures. Bonner (1947) showed that the aggregations were forming along gradients of a chemical which he called acrasin. Konijn et al (1968) have shown that cyclic AMP attracts D. discoideum and

P. pallidum. Konijn (1969) has shown that both of the above species produce cyclic AMP and that D. discoideum also produces phosphodiesterase which converts cyclic AMP to 5'AMP (Chang 1968). Cyclic AMP acts as an attractant like acrasin, while 5'AMP is inert with respect to attractive ability. More recent work (Bonner pers. comm.) suggests that other chemicals also act like acrasin and that cyclic AMP is produced by many organisms such as E. coli the bacterial food used in most cellular slime mold work.

From all of this work several points regarding the production and effects of acrasin can be made: (1) cyclic AMP acts like acrasin, (2) cyclic AMP is produced by D. discoideum and P. pallidum, (3) cyclic AMP is produced by many other organisms, (4) D. discoideum produces a small protein which breaks down cyclic AMP and (5) other chemicals also have the attributes of acrasin. Some facts are also unknown and these might hold the key to interspecific inhibition of fruiting bodies. It is possible, for example, that the phosphodiesterase produced by D. discoideum might break down the acrasin produced by P. pallidum. It is also possible that the acrasin produced by D. discoideum might interfere with the production or the reception of the acrasin produced by P. pallidum.

Since the interspecific inhibition observed here, and in the other studies, occurs at the aggregation stage, the most likely hypothesis is that the substances produced during

aggregation act as interspecific aggregation inhibitors. Acrasin and chemicals which break it down are most abundant at this period in the life cycle of cellular slime molds, and therefore, must be the most likely candidates. However, until P. pallidum acrasin is isolated, and until its response to phosphodiesterase is characterized, it is impossible to make any definite statements.

Genetics

The results of this study also suggested that D. discoideum was able to inhibit P. pallidum fruiting before competition, but after a period of continued competition P. pallidum overcame the inhibition and began to fruit in the presence of D. discoideum. Apparently the change was genetic rather than acclimative and the gene pool of stock P. pallidum Salvador contained spores with co-fruiting abilities.

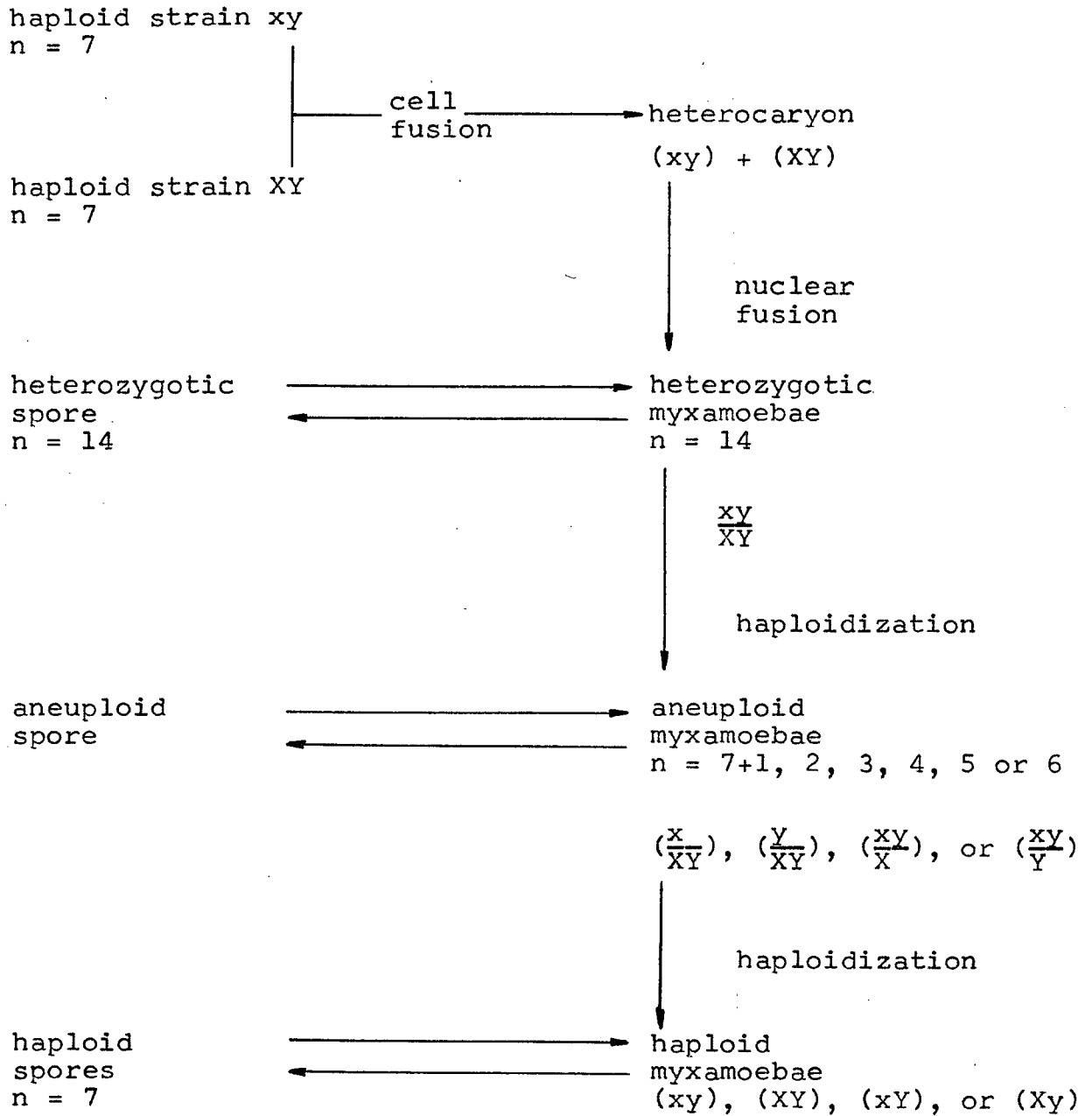
These conclusions are difficult to accept when considered along with two other pieces of information. (1) Olive (1963), Sussman and Sussman (1963), Huffman and Olive (1964) and Huffman (1967) all concluded that there is no meiosis in cellular slime molds. (2) The data from this study indicates that when stock P. pallidum and co-fruiting P. pallidum are mixed and grown for approximately 120 generations at 27°C co-fruiting P. pallidum can no longer be detected.

The data makes it appear that the co-fruiting strain

is less fit than the stock strain, and that during intra-specific competition the co-fruiting strain completely or almost completely disappears. Yet a co-fruiting spore was found in a stock culture. Without meiosis it is difficult to see how the co-fruiting strains are protected.

The best answer to this dilemma comes from some recent genetical work on cellular slime molds. Sussman and Sussman (1963) report that haploid, diploid and unstable diploid strains exist. Wilson and Ross (1957), Huffman and Olive (1964), Huffman (1967), and Sinha and Ashworth (1969) have observed cell fusions as the vegetative amoebae aggregate to form fruiting bodies. Loomis and Ashworth (1968), and Sinha and Ashworth (1969) state that these fusions result in the formation of one diploid cell from two haploid cells. Loomis (pers. comm. 1970) reports that fusion occurs about once in a thousand times. These observations plus the results of genetic marker experiments had led to the conclusion that para-sexuality (Pontecorvo 1958) takes place.

The general mechanism can be summarized by a modified version of a diagram presented by Sinha and Ashworth (1969).



where x/X and y/Y are two unlinked genes.

The diagram demonstrates that recessive characteristics can be hidden by dominants and that less fit alleles can be protected. Since spores considerably larger (diploid) than the average (haploid) have been observed in the P. pallidum stock culture used for the work presented in this paper, it appears that para-sexuality might be involved. It seems possible that the ability of P. pallidum Salvador to co-fruit in the presence of D. discoideum is determined by a number of alleles, some of which may be recessive and less fit in the intraspecific cultures but more fit in the interspecific cultures. Because of para-sexuality the necessary alleles could be protected in the stock cultures and be selected for during competition.

In general, the periodic fusion of cells, recombination, and chromosome loss, results in an extremely variable population despite the fact that meiosis does not occur. This variability makes it possible to find individual co-fruiting spores in the stock culture, and at the same time, to mask this attribute at the population level, provided that a significant number of co-fruiting cells are necessary for fruiting to take place in mixed cultures.

The supposition that para-sexuality and its attendant genetic variability occurs in P. pallidum leads to the prediction that in situations where competition is continuous, P. pallidum and D. discoideum should co-fruit almost all the time. These two species have been found together in their

natural habitat by Cavender (1963), Cavender and Raper (1965), and Horn (1969). And Horn demonstrated that the two species could fruit together in the laboratory. The P. pallidum strain used here came from Salvador and the information available indicates that it never experienced competition from D. discoideum. In the laboratory D. discoideum inhibited P. pallidum fruiting.

Section II: Ecological Relevance

Convergence and Coexistence

The effects of continued competition have been the center of some controversy for a considerable period of time. Grinnell (quoted in Udvardy 1959 and Ross 1958), and later Gause (1934), advanced the theory of competitive exclusion which basically states that animals coexist only when their resource use is divergent. This axiom has prompted a considerable number of studies which have linked continued competition to morphological and ecological divergence (Johannes and Larkin 1961, Ficken et al 1967, Keast 1967), which in turn resulted in coexistence. On the other hand, recent work suggests that continued competition might also result in convergence in the form of mimicry Moynihan (1968), or morphological and behavioral interference related to territoriality Cody (1969). Miller (1964) has also shown

that ecological convergence in fruit flies can result in co-existence and Park and Lloyd (1955) list instances of flour beetle competition lasting for several years in small homogeneous laboratory environments.

In all probability the axiom of competitive exclusion and the hypothesis of convergent coexistence are both correct in many situations. But the work presented here suggests that both may be too simple to have any real predictive value.

Following the hypothesis of convergent coexistence, the cellular slime mold experiments were designed so that the environment was homogeneous offering no alternate source of food, and the two species each had a refuge to guard against immediate extinction. It was assumed that under these conditions only convergence could occur and that this would result in coexistence. Two changes did occur; P. pallidum gained co-fruiting ability and D. discoideum used food faster.

The two species did end up co-fruiting and co-existing but for the wrong reasons. They didn't converge and therefore coexist, nor did they completely diverge with respect to resource use. They used the same resource at different rates and coexisted only because P. pallidum was always able to produce at least one fruiting body in an area where it was at a real disadvantage from an exploitative point of view. Coexistence occurred because the advantage

gained from interference was greater than the disadvantage from exploitation. Because Grinnell's axiom (Udvardy's term) and its opposite (Miller 1964) are only concerned with rates of resource use, this result could not be predicted by either.

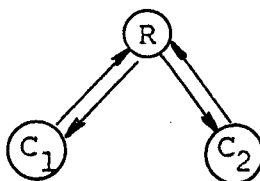
Park (1954) has stated that "no prediction about the outcome of sustained competition is inherent in the definition - this being a matter for empirical investigation or abstract deduction." To this I would add that empirical knowledge, rather than abstract deduction, is necessary, before any conclusions can be reached with respect to the consequences of continued competition.

Components of Competition

A second objective of this study was to analyze the mechanics of competition and to identify the basic components and feedbacks which might be applicable to most competitive situations. The cellular slime mold study has suggested that there are five basic components involved.

The primary consideration is exploitation (Park 1954). Since it is generally accepted that organisms must compete for something other than life itself, competition cannot occur until some resource is exploited. The cellular slime mold species demonstrated this point by competing for both food and space. Their interaction was mediated through the resource, because resource used by one competitor could not

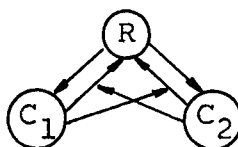
be used by the other. Symbolically the process can be represented as:



where C_1 and C_2 are competitors 1 and 2 and where R is the limited resource.

The cellular slime mold situation was complicated by other components, but there have been literature reports of situations in which exploitation was the only competitive component involved. For example, Ulliyett (1950) observed that when Chrysomya chloropyga and Lucilia sericata were placed on 140 grams of meat they interacted only because the food eaten by one could not be eaten by the other.

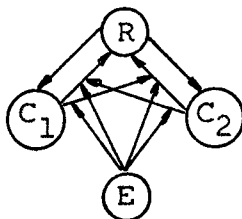
The other important competitive component observed in this study is interference. It was found that the two species interfered with one another's fruiting ability at certain temperatures, and it was hypothesized that the chemicals produced during aggregation and fruiting were responsible. Since interference alters the competitive outcome, changing the exploitation rates, it may be symbolically expressed as:



where the two competitors directly alter exploitation rates.

The cellular slime mold work suggested that animals could interfere by producing toxic chemicals. Similar observations have been made by Grummer and Beyer (1960) who report that flax is inhibited by chemicals washed out of the leaves of Camelina. And toxic interference has been invoked to explain the spacing in Larrea. Grummer (1961) provides numerous other examples of chemically induced inhibition. Two other classes of interference appear in the literature but were not observed in the cellular slime mold study. Orians and Horn (1969), Lack (1954), and numerous other authors have reported that behavioral interference, particularly with respect to territoriality, alters exploitation for both food and space. And cannibalistic or predatory interference also alters exploitation rates. Park (1965) reported that Tribolium castaneum ate both its own pupae and the pupae of T. confusum at 20°C and 70% RH.

External forces, defined as any forces outside the control of the competitors, were very important in the cellular slime mold situation. In this case temperature was the external force, and it was found that below about 22°C D. discoideum could always exclude P. pallidum while above about 24°C the reverse was true. Between 22° and 24°C interference became important but temperature controlled that also. External force can be symbolically represented as:



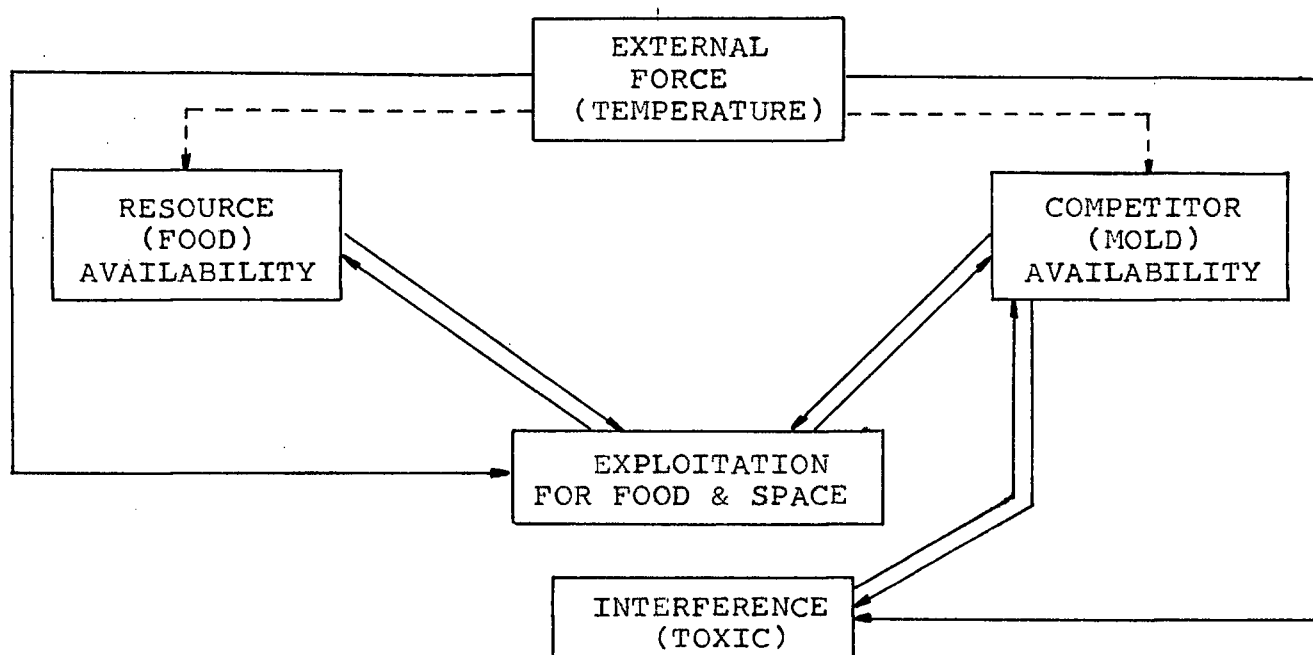
where E represents an external force. External forces are usually abiotic and can be exemplified by temperature, pH, humidity, hours of sunlight, etc. The literature suggests that they may also be biotic. Paine (1966) noted that starfish remove several foreshore species which ordinarily compete for nutrients and space, and Connell (1967) has observed that three species of Thais curtail or prevent Balanus glandula and B. cariosus competition for space at Friday Harbor.

A fourth consideration and one that did not play an important role in this study is resource availability. In the laboratory situation constant amounts of food were used at all times, but Horn (pers. comm.) reports that the rate of colony expansion is proportional to food concentration, thereby altering the rate of exploitation. In field situations this component can be very important. Filter feeding zooplankton alter their rates of exploitation with changes in food availability (Mullin 1963), and Griffiths and Holling (1969) report that the degree of contagion of hosts alter parasite attack rates.

Finally, competitor availability greatly altered the effect of the interference component in this study. Field evidence also suggests that competitor availability over a long time span is important. Orians and Horn (1969) found that three species of blackbirds compete for food and space in the Potholes of central Washington. But they only

compete at certain times of the day and during one period of the year. In this situation it is possible that exclusion does not occur because the period of competition is too short, and regulating mortality occurs at other places and during different periods of time.

Because a laboratory situation was used in which external forces and availabilities could be controlled it was possible to assess in some detail the factors involved in a competitive situation. It was also possible to determine the ways in which the components interact.



Exploitation was the center of competition and altered interference by altering the number of competitors available. It, in turn, was altered by the amount of bacterial

food available and the number of competitors available. Interference altered exploitation by inhibiting feeding in areas of high density for short periods of time and by altering the number of competitors available in time period two. Temperature affected exploitation and interference but was not altered by competition. In the laboratory the temperature did not directly change food availability or competitor availability but in field situations these links (shown by the dotted lines) would almost certainly be made.

The real value of the general system which has emerged from this study can only be assessed by future field studies. But if it has any generality, the component parts of field studies reported in the literature should fit the above outline.

One of the best studies in the literature was conducted by Ulliyett (1950), and concerned competition between four species of blow flies; Lucilia sericata, Chrysomya chloropyga, C. albiceps, and C. marginalis. Both field and laboratory data were included. Ulliyett found that all four species exploited carrion in both the laboratory and the field. He did not measure rates of food use, but when he considered interspecific competitive situations, all larval numbers were converted to Lucilia units. In most interspecific situations only exploitation was important but when C. albiceps was involved he found that this species interfered with the others by eating larvae. The extent of predatory

interference depended upon resource quality and larval numbers. One biotic and two abiotic external forces were also important. Temperature and humidity altered the rates of oviposition and adult dispersal, and a parasite Mormoniella vitripennis Walk increased C. albiceps pupal mortality. Food availability in the field was very important with respect to quantity, quality and dispersion. Unfortunately this component was very difficult to assess and for this reason little information was included. Finally, competitor availability restricted field competition. Because two of the competitors breed in the summer and two in the winter a potentially complex four competitor situation was reduced to two separate two competitor situations.

In general, all of the components of competition found in the cellular slime mold situation were also found in Ulliyett's study and all of Ulliyett's data could be included in the system. It would have been better if the system could have been fit to cellular slime mold field studies. Unfortunately, however, the only field work known to this author was concerned only with the distribution of species in various habitats (Cavender 1963). However, Horn (1969) has pointed out that food quality was very important to the four species he studied, and he hypothesized that since most species had a series of foods on which they could out-compete the others, competition could be resolved and co-existence could be attained on the basis of food quality alone. The present study suggests that while food quality

is very important, response to environmental factors, and in some cases interference, also determine the outcome of competition on any patch of bacteria. Beyond this, little can be said due to the lack of information about the natural ecology of cellular slime mold species.

It is hoped that the general model developed from the laboratory studies includes most of the major components and feedbacks which are involved in competitive systems. The kinds of information included within each component should also be general in nature. During the course of this work each component was studied in some detail and the resulting model simulated competition between two species of cellular slime mold with a reasonable amount of accuracy. It is unlikely, however, that the minute mechanics involved in this situation would be useful in other instances of competition. The ways in which information is assessed and incorporated into each component must depend upon the system under study.

Studies of the short term mechanics and the long term results of continued competition suggests that to date there are no general laws which will allow ecologists to make predictions about competition. However, there do seem to be a finite number of strategies available and a finite number of selective forces acting on competitors. For the moment, it appears that competitive problems can only be solved by on-site study of both of these factors.

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APPENDIX I
COMPUTER PROGRAMS

// JOB

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

// FOR

*LIST SOURCE PROGRAM

*ONE WORD INTEGERS

*IOCS (CARD,1132 PRINTER)

REAL KON

REAL LMIN(20)

REAL LAG(40)

DIMENSION T(30),Q(30),TT(30),TO(30),SQ(30),ACT(30)

TH =

TL =

II =

JJ =

KK =

LL =

READ(2,1000) (T(I),Q(I), I=1,II)

1000 FORMAT(2F10.5)

READ(2,2000) (TO(J),J=1,JJ)

2000 FORMAT(F10.5)

READ(2,3000) (LMIN(K),K=1,KK)

3000 FORMAT(F10.5)

READ(2,4000) (TT(L),ACT(L),L=1,LL)

4000 FORMAT(2F10.5)

DO 1 I = 1,II

A = (-.5*ALOG((-1.0)*T(I)**2.0 + T(I)*(TH+TL) - TH*TL))-

1 (((TH+TL)/2.0)*(1.0/(TH-TL)) *

1 (ALOG(ABS(T(I) - TH)/(T(I)-TL))))

B = (((1.0)/(TH-TL))*ALOG(ABS(T(I)-TH)/(T(I)-TL)))

DO 2 J = 1,JJ

D = (-.5*ALOG((-1.0)*TO(J)**2.0 + TO(J)*(TH+TL) - TH*TL))-

1 (((TH+TL)/2.0)*((1.0)/(TH-TL))*

1 (ALOG(ABS(TO(J)-TH)/(TO(J)-TL))))

E = ((1.0)/(TH-TL))*(ALOG(ABS(TO(J)-TH)/(TO(J)-TL)))

DO 3 K=1,KK

KON = (Q(I) -LMIN(K))/(A+B*TO(J)-D-E*TO(J))

CON = (LMIN(K)-((Q(I)-LMIN(K))*(D+E*TO(J))) /

1 (A+B*TO(J)-D-E*TO(J)))

WRITE(3,200)T(I),Q(I)

```

200  FORMAT( ' TEMP OF POINT USED IS ' F10.5 ' AND POINT IS ' F10.5)
      WRITE(3,300) TO(J)
300  FORMAT( ' TEMP OPTIMUM IS ' F10.5)
      WRITE(3,400) LMIN(K)
400  FORMAT( ' LAG MIN IS ' F10.5)
      WRITE(3,500) KON
500  FORMAT( ' K IS ' F10.5)
      WRITE(3,600) CON
600  FORMAT( ' C IS ' F10.5)
      WRITE (3,700)
700  FORMAT( ' TEMP          LAG PRE    LAG ACT    SUMSQ ' )

```

```

      SUMSQ = 0.0
      DO 4 L = 1,LL
        LAG(L) = KON*((-.5)*ALOG((-1.0)*TT(L)**2.0 + (TT(L)*(TH+TL))-
1  (TH*TL))) -
1  KON*(((TH+TL)/2.0)*((1.0)/(TH-TL))*
1  ALOG(ABS((TT(L)-TH)/(TT(L)-TL))))+
1  KON*TO(J)*((1.0)/(TH-TL))*
1  ALOG(ABS((TT(L)-TH)/(TT(L)-TL))) + CON
        SQ(L) = (LAG(L) - ACT(L))**2.0
        SUMSQ = SUMSQ + SQ(L)
        WRITE(3,100)TT(L),LAG(L),ACT(L),SUMSQ
100  FORMAT(4F10.5)
4    CONTINUE
3    CONTINUE
2    CONTINUE
1    CONTINUE
      CALL EXIT
      END

```

PROGRAM I - CURVE FITTING (Equation 2b)

Program I is designed to fit curves described by equation (2b) to spore germination lag data. This problem is complicated by the fact that in equation (2b) there are two unknowns; Lag minimum, and T_0 , which in turn determine K and C. In view of this, equation (2b) has been fitted to the lag data by trying many possible Lag minimum and T_0 values, comparing the calculated Lag values with those observed, calculating the sum of the squared deviation between observed and calculated, and choosing the curve that best fits the data.

In mathematical terms the following steps were taken. Equation 2b is broken up so that:

$$a = [(-.5 \log |(-1)T^2 + T(T_H + T_L) - T_H T_L|) -$$

$$\left[\left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] - \log \left| \frac{T - T_H}{T - T_L} \right| \right]$$

$$b = \left[\left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right]$$

then equation 2b can be written as:

$$L = K \cdot a + K \cdot T_0 \cdot b + C \quad (2c)$$

If $T = T_0$ then equation 2b can be broken up again to yield:

$$d = \left[(-5 \log | (-1) T_O^2 + T_O (T_H + T_L) - T_H \cdot T_L |) - \left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right]$$

$$e = \left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T_O - T_H}{T_O - T_L} \right|$$

and equation (2b) with $T = T_O$ could be written as:

$$L_{\min} = K \cdot d + K \cdot T_O \cdot e + C \quad (2d)$$

where $L_{\min}$ is the minimum lag value, which, by definition, must occur at $T = T_O$.

To solve (2c) and (2d) simultaneously some data value for L and its corresponding T value is substituted into (2c), and $L_{\min}$ and T_O are estimated and substituted into (2d). Having made these substitutions it is possible to solve for K and C . These K and C and T_O values may then be substituted back into (2b), which can then be solved. There is no way of knowing which data point should be substituted into (2d) and there is no way of knowing the best values of $L_{\min}$ and T_O . For this reason Program I has been designed so that all of the available data points may be tried in combination with all of the reasonable $L_{\min}$ and T_O values.

In general about 200 combinations of T_O , $L_{\min}$, and data values, are examined before a curve is chosen by the least of squares method. The curve chosen must have a sum of

squared deviations which is in a "sink" (ie. the sum of squared deviations for every other combination of $L_{\min}$, T_o , and data is larger).

// JOB

MCQUEEN

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

// FOR

*LIST SOURCE PROGRAM

*IOCS (TYPEWRITER,1132 PRINTER,KEYBOARD)

DIMENSION A(25)

Z=1.0

16 WRITE(3,5)Z

5 FORMAT(3H1Z=,F10.5)

WRITE(3,12)

12 FORMAT(20X,20H J A)

DO 1 J = 1,25

IF(J-1)2,2,3

3 A(J)=(((Z*3.54490 * J) + 1)/2)**2

GO TO 4

2 A(1) = 1.0

4 CONTINUE

WRITE(3,13) J,A(J)

13 FORMAT (20X,13,7X,F10.5)

1 CONTINUE

READ(6,14)Z

14 FORMAT(F6.0)

IF (Z)15,15,16

15 CALL EXIT

END

PROGRAM II - FORM OF COLONY EXPANSION (Equation 1c)

Program II is designed to calculate the area occupied by a colony at any time t . In the program notation $A(J)$ is area, J is time, Z is g in equation (1d), and C has been set to 1.0 area units. The output indicates that as Z (or g) increases; the slope of the line, which may be interpreted as the growth rate of the colony, also increases. Values of Z are input, using the keyboard and $A(J)$ and J are output via the printer.

// JOB

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

// FOR

*LIST SOURCE PROGRAM

*ONE WORD INTEGERS

*IOCS(CARD, 1132 PRINTER, PLOTTER)

DIMENSION A(14), X(50), Y(50), ANS(16), STORE(8,65), IUSED(65),

1 TABLE(30), TTEST(50)

READ(2,900)(TTEST(JJ),JJ=1,50)

900 FORMAT(8F10.5)

READ(2,901)(TABLE(NN),NN=1,30)

901 FORMAT(8F10.5)

DO 501 I = 1,75

501 IUSED(I) = 0

L=0

C CARD COUNTER TO GET DATA FOR EACH REGRESSION

K=0

3 READ(2,100)TEMP,TIM,A

100 FORMAT(15F5.0,F4.0)

IF(TEMP)2,1,2

2 IF(K)16,6,16

6 SAVE = TEMP

L=L+1

N=0

K=1

GO TO 3

1 DO 4 I=1,14

IF (A(I))3,3,5

5 N=N+1

X(N) =TIM

Y(N) = SQRT(A(I))

C DATA IS WRITTEN OUT HERE

WRITE (3,200) X(N),Y(N)

200 FORMAT(2F10.5)

4 CONTINUE

GO TO 3

16 WRITE (3,101) SAVE

101 FORMAT (// ' L= ', F6.2)

C REGRESSION CARRIED OUT AND ANSWERS ARE WRITTEN

CALL LREG(X,Y,N,ANS)

CALL LREGO(ANS)

WRITE (3,400)

```

400  FORMAT (1H0)
      WRITE (3,401) ANS(4)
401  FORMAT ( ' SUM OF XY = ', F10.5)
      WRITE (3,402) ANS(5)
402  FORMAT ( ' SUM OF X SQUARED = ', F10.5)
      WRITE (3,403) ANS(9)
403  FORMAT ( ' SUM OF Y SQUARED = ', F10.5)
      SUMDS = ANS(9) - (ANS(4)**2)/ANS(5)
      WRITE (3,404) SUMDS
404  FORMAT ( ' SUM OF DEVIATION SQUARED OF XY = ', F10.5)
      WRITE(3,832)
      WRITE(3,832)
      WRITE(3,832)
832  FORMAT(1H0)

```

```

C    ALL THE BASIC PLOTTING INFORMATION IS COLLECTED HERE
      STORE(1,L) = ANS(1)
      STORE(2,L) = ANS(2)
      STORE(3,L) = ANS(3)
      STORE(4,L) = ANS(4)
      STORE(5,L) = ANS(5)
      STORE(6,L) = ANS(9)
      STORE(7,L) = SUMDS
      STORE(8,L) = SAVE
      IF (TEMP)11,11,6

```

```

C    THE DATA IS SORTED ACCORDING TO TEMPERATURE
11   DO 600 NN = 1,30

```

```

      LC = 0
      SUMN = 0.
      YMEAN = 0.
      XMEAN = 0.
      SUMXY = 0.
      SUMXS = 0.
      SUMYS = 0.
      SSUMD = 0.
      DO 601 LL = 1,L
      IF (IUSED(LL)) 601,602,601
602  IF (STORE(8,LL) - TABLE(NN)) 603,601,601
603  IUSED(LL)=1
      T = STORE(8,LL)
      WRITE (3,525) STORE(8,LL)
525  FORMAT ( ' STORE(8,LL) IS TEMP = ', F10.5)
      WRITE (3,888)
888  FORMAT ( '      N      YBAR      XBAR      SUMXY      SUMXSQ
1  SUMYSQ      SUMDXY ' )
      WRITE (3,526) (STORE(KK,LL),KK = 1,7)
526  FORMAT (7F10.5)
      SLOP = (STORE(4,LL))/(STORE(5,LL))
      WRITE (3,560) SLOP
560  FORMAT ( ' SLOP OF SINGLE LINE = ',F10.5)
      WRITE(3,832)

```

```

        LC = LC +1
        IF(LC - 1) 61,61,62
61      CALL SCALF (7.0/7.0,10.0/20.0, 0.0,0.0)
        CALL FGRID(0.0,0.0,0.0,1.0,7)
        CALL FGRID(1.0,0.0,0.0,1.0,20)
        CALL FCHAR(0.0,0.0,0.07,0.07,1.570796)
62      YE = STORE(2,LL) + SLOP*(7.0 - STORE(3,LL))
        IF ( YE - 20. ) 88,88,89
88      XE = 7.0
        GO TO 90
89      XE = (20. - STORE(2,LL) + SLOP*(STORE(3,LL)))/SLOP
        YE = 20.
90      CONTINUE
        XS = (-STORE(2,LL)/SLOP) + STORE(3,LL)
        IF (XS) 75,76,76
75      YS = STORE(2,LL) - ( SLOP*(STORE(3,LL)) )
        GO TO 77
76      YS = 0.
        GO TO 78
77      XS = 0.
78      CONTINUE
        CALL FPLOT (-2,XS,YS )
        CALL FPLOT (-1,XE,YE)
        WRITE(7,628) LC
628     FORMAT (I2)
        CALL FPLOT ( 0,0.0,0.0)
        SUMN = STORE(1,LL) + SUMN
        YMEAN = STORE(2,LL) + YMEAN
        XMEAN = STORE(3,LL) + XMEAN
        SUMXY = STORE(4,LL) + SUMXY
        SUMXS = STORE(5,LL) + SUMXS
        SUMYS = STORE(6,LL) + SUMYS
        SSUMD = STORE(7,LL) + SSUMD
601     CONTINUE
        IF (LC) 600,600,22
22      WRITE(3,527) T
527     FORMAT ( ' T           IS TEMP = ' F10.5)
        WRITE (3,889)
889     FORMAT( ' SUMN      YMEAN      XMEAN      SUMXY      SUMXS
1      SUMYS      SSUMD ' )
        WRITE(3,528) SUMN, YMEAN, XMEAN, SUMXY, SUMXS, SUMYS, SSUMD
528     FORMAT(7F10.5)
        SLOPE = (SUMXY) / (SUMXS)
        DFREE = (SUMN) - (LC) - (1.)
        SSYX = (SSUMD) / (DFREE)
        SSB = (SSYX) / (SUMXS)
        SB = SQRT(SSB)
        JJ = DFREE
        DEVIA = TTEST(JJ)* SB
        XPLOT = XMEAN/LC
        YPLOT = YMEAN / LC
        WRITE (3,999)
999     FORMAT ( ' SLOPE      DFREE      SSYX      SSB      SB      DEVIA
1      XPLOT      YPLOT ' )
        WRITE (3,529) SLOPE, DFREE, SSYX, SSB, SB, DEVIA,XPLOT, YPLOT
529     FORMAT(8F9.5)

```

```
WRITE(3,832)
WRITE(3,832)
WRITE(3,832)
WRITE(3,832)
YEM = YPLOT + SLOPE*(7.0 - XPLOT)
IF (YEM - 20.) 30,30,31
30 XEM = 7.0
GO TO 32
31 XEM = (20.0 - YPLOT + SLOPE*XPLOT)/SLOPE
YEM = 20.
32 CONTINUE
XSM = XPLOT - (YPLOT/SLOPE)
IF (XSM)33,34,34
33 YSM = YMEAN - (SLOPE*XMEAN)
GO TO 35
34 YSM = 0.0
GO TO 36
35 XSM = 0.0
36 CONTINUE
CALL FPLOT (-2,XSM,YSM)
CALL FPLOT (-1,XEM,YEM)
WRITE(7,629)
629 FORMAT( ' MEAN LINE ' )
CALL FCHAR (1.0,19.0,0.14,0.14,0.0)
WRITE (7,223) T
223 FORMAT( ' TEMP = ' F10.5)
CALL FCHAR (1.0,18.0,0.14,0.14,0.0)
WRITE(7,224) SLOPE
224 FORMAT( ' SLOPE = ' F10.5)
CALL FCHAR (1.0,17.0,0.14,0.14,0.0)
WRITE(7,225) DEVIA
225 FORMAT( ' DEVIA = ' F10.5)
CALL FCHAR ( 1.0,16.0,0.14,0.14,0.0)
WRITE(7,226)SUMN
226 FORMAT( ' SUMN = ' F10.5)
CALL FPLOT ( 0,10.0,0.0)
600 CONTINUE
CALL EXIT
END
```

FEATURES SUPPORTED
ONE WORD INTEGERS
IOCS

CORE REQUIREMENTS FOR
COMMON 0 VARIABLES 1610 PROGRAM 1452

END OF COMPILATION

// XEQ

PROGRAM III - CALCULATION OF MEAN SLOPE

Program III calculates the mean slopes of the lines which result from plotting area occupied by a colony against time (Fig. 11).

In the first section of the program the data from each culture are considered separately. The culture temperature is read and then the time-area data for that culture are read. A regression line (time and square root of area) is then calculated using the standard IBM 1130 LREG and LREGO subroutines. After all the cultures have been processed and a regression line obtained for each, the regression lines are grouped with respect to temperature (ie. all the lines resulting from cultures grown at 20.5° are grouped, all those grown at 21.5° are grouped etc.). During this grouping process eight pieces of information are printed out from each regression. These are:

- (1) n = number of data points
- (2) $\bar{y}$ = mean time
- (3) $\bar{x}$ = mean square root of area
- (4) $\sum_{xy} = \sum XY - [(\sum X)(\sum Y)]/n$
- (5) $\sum x^2 = \sum X^2 - (\sum X)^2/n$
- (6) $\sum y^2 = \sum Y^2 - (\sum Y)^2/n$
- (7) $\sum d_{xy}^2 = \sum Y^2 - (\sum xy)^2/x^2$
- (8) T = temperature

After all of the regressions for any particular

temperature have been calculated a mean regression line is calculated. This is accomplished in the following manner:

Four quantities are summed:

$$\sum \sum x^2 = \sum x_1^2 + \sum x_2^2 \dots \sum x_k^2$$

$$\sum \sum y^2 = \sum y_1^2 + \sum y_2^2 \dots \sum y_k^2$$

$$\sum \sum xy = (\sum xy)_1 + (\sum xy)_2 \dots (\sum xy)_k$$

$$\sum \sum d_{yx}^2 = (\sum d_{yx}^2)_1 + (\sum d_{yx}^2)_2 \dots (\sum d_{yx}^2)_k$$

where k is the total number of regression lines for the temperature being considered. With this information a mean slope can be calculated.

$$b = \frac{\sum \sum xy}{\sum \sum x^2}$$

where b is the mean slope. A 5% confidence limit can also be established around b.

$$s_{yx}^2 = \sum \sum d_{yx}^2 / \sum n - k - 1$$

$$s_b^2 = s_{yx}^2 / \sum \sum x^2$$

so that:

$$b \pm s_b \cdot t_{(\sum n - k - 1), \alpha = .05}$$

where n is the total number of regression points and k is the total number of regression lines incorporated to produce the

mean regression line. This mean regression line is placed in space by calculating the mean x and y values so that

$$\text{mean } x = \sum_{i=1}^k \bar{x}_i / k$$

$$\text{mean } y = \sum_{i=1}^k \bar{y}_i / k$$

When all of the regression lines have been grouped and their mean regression lines calculated the plotter routine is called and each set of lines along with their mean line is plotted. The plotter also writes the mean slope and the 5% confidence limit around the mean slope for every temperature. This slope and 5% confidence interval can then be used as the growth index.

// JOB

MCQUEEN

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

*IOCS (CARD, 1132 PRINTER)

REAL K

DIMENSION G(20),GD(20),T(20),SSQ(20),Q(20),TO(20)

TL =

TH =

KK =

JJ =

II =

READ (2,51)(GD(I),T(I),I=1,II)

51 FORMAT (2F10.5)

READ(2,200)(TO(J),J=1,JJ)

200 FORMAT (F10.5)

READ(2,201)(Q(L),L=1,LL)

201 FORMAT(F10.5)

DO 203 J=1,JJ

A = (ALOG(TH-TL))*(TH-TO(J)) - (TH) + (TL)

B = (ALOG(TH-TO(J)))*(TH-TO(J)) - (TH) + (TO(J))

DO 204 L=1,LL

K=Q(L)/(B-A)

C= -A*(Q(L)/(B-A))

DO 205 I=1,II

G(I) = (ALOG(TH-T(I)))*K*(TH-TO(J))-K*TH+K*T(I)+C

205 CONTINUE

SSQ(1) = (GD(1) - G(1))*2.

DO 206 I =2,II

SSQ(I) =SSQ (I-1)+(GD(I)-G(I))*2.

206 CONTINUE

1 WRITE (3,5) TO(J)

5 FORMAT (4HOTO=,F10.5)

WRITE (3,209) Q(L)

209 FORMAT (1X,5HQ(I)=,F10.5)

WRITE (3,100)K

100 FORMAT(3H K=,F10.5)

WRITE (3,101)C

101 FORMAT(3H C=,F10.5)

WRITE (3,102)

102 FORMAT(36H T GD G SSQ)

WRITE (3,103)(T(I), GD(I), G(I), SSQ(I) ,I=1,II)

103 FORMAT(4F10.5)

204 CONTINUE

203 CONTINUE

30 CALL EXIT

END

PROGRAM IV - CURVE FITTING (Equation 3c)

Program IV fits equation (3c) to the growth index data. This problem is complicated by the fact that two unknowns must be found before the equation can be fit. These are the maximum growth of the colony (Gmax) and the optimum temperature (T_o) at which Gmax occurs. T_o and Gmax determine K and C.

One solution to the equation can be found by setting $T = T_L$ and $G = 0.0$ in equation (3c) so that:

$$0.0 = \log |T_H - T_L| \cdot K (T_H - T_o) - K \cdot T_H + K \cdot T_L + C$$

where T_H and T_L are known. It is also known that $g = g_{max}$ when $T = T_o$ so that (2c) may also be written:

$$G_{max} = \log |T_H - T_o| \cdot K (T_H - T_o) - K \cdot T_H + K \cdot T_o + C$$

There are now two equations and two unknowns (K, C) so a solution may be found.

However, the best values for T_o and Gmax are unknown. To overcome this problem the curve was fitted iteratively to the data. All the reasonable Gmax and T_o values were tried, equation (3c) was solved for each pair, values of G at every T were calculated, and the sum of the squared deviations between the observed and calculated values of G was calculated. The pair of T_o and Gmax values which yielded the curve of best fit (smallest sum of squared deviations) was used.

// JOB

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

// FOR

*LIST SOURCE PROGRAM

*ONE WORD INTEGERS

*IOCS (CARD,1132 PRINTER)

REAL KON

DIMENSION T(30),Q(30),TT(30),TO(30),SQ(30),ACT(30),GMAX(40),

1 GRO(40)

TL =

TH =

LL =

KK =

JJ =

II =

READ(2,1000) (T(I),Q(I), I=1,II)

1000 FORMAT(2F10.5)

READ(2,2000) (TO(J),J=1,JJ)

2000 FORMAT(F10.5)

READ(2,3000) (GMAX(K),K=1,KK)

3000 FORMAT(F10.5)

READ(2,4000) (TT(L),ACT(L),L=1,LL)

4000 FORMAT(2F10.5)

DO 1 I = 1,II

A = (-.5*ALOG((-1.0)*T(I)**2.0 + T(I)*(TH+TL) - TH*TL))-

1 (((TH+TL)/2.0)*(1.0/(TH-TL)) *

1 (ALOG(ABS(T(I) - TH)/(T(I)-TL))))

B = (((1.0)/(TH-TL))*ALOG(ABS(T(I)-TH)/(T(I)-TL)))

DO 2 J = 1,JJ

D = (-.5*ALOG((-1.0)*TO(J)**2.0 + TO(J)*(TH+TL) - TH*TL))-

1 (((TH+TL)/2.0)*((1.0)/(TH-TL))*

1 (ALOG(ABS(TO(J)-TH)/(TO(J)-TL))))

E = ((1.0)/(TH-TL))*(ALOG(ABS(TO(J)-TH)/(TO(J)-TL)))

DO 3 K=1,KK

KON = (Q(I) -GMAX(K))/(-A-B*TO(J)+D+E*TO(J))

CON = (GMAX(K)+((Q(I)-GMAX(K))*(D+E*TO(J))) /

1 (-A-B*TO(J)+D+E*TO(J)))

WRITE(3,200)T(I),Q(I)

200 FORMAT(' TEMP OF POINT USED IS ' F10.5 ' AND POINT IS ' F10.5)

WRITE(3,300) TO(J)

300 FORMAT(' TEMP OPTIMUM IS ' F10.5)

WRITE(3,400) GMAX(K)

400 FORMAT(' GRO MAX IS ' F10.5)

WRITE(3,500) KON

500 FORMAT(' K IS ' F10.5)

WRITE(3,600) CON

600 FORMAT(' C IS ' F10.5)

WRITE (3,700)

700 FORMAT(' TEMP GRO PRE GRO ACT SUMSQ ')

SUMSQ = 0.0

DO 4 L = 1,LL

```
GRO(L) = -KON*((-.5)*ALOG((-1.0)*TT(L)**2.0 + (TT(L)*(TH+TL))-  
1 (TH*TL))) +  
1 KON*(((TH+TL)/2.0)*((1.0)/(TH-TL))*  
1 ALOG(ABS((TT(L)-TH)/(TT(L)-TL))))-  
1 KON*TO(J)*((1.0)/(TH-TL))*  
1 ALOG(ABS((TT(L)-TH)/(TT(L)-TL))) + CON  
SQ(L) = (GRO(L) - ACT(L))**2.0  
SUMSQ = SUMSQ + SQ(L)  
WRITE(3,100)TT(L),GRO(L),ACT(L),SUMSQ  
100 FORMAT(4F10.5)  
4 CONTINUE  
3 CONTINUE  
2 CONTINUE  
1 CONTINUE  
CALL EXIT  
END
```

PROGRAM V - CURVE FITTING (Equation 4b)

Program V was used to fit curves of the type described by equation (4b) to fruiting body expansion data from P. pallidum. In equation (4b) where:

$$G = -K \left[(-.5) \log \left| (-1) T^2 + T (T_H + T_L) - T_H \cdot T_L \right| \right] -$$

$$\left[\left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right] -$$

$$K \cdot T_O \left[\left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right] + C$$

The fitting procedure is complicated by the fact that there are two unknowns; growth maximum and T_O which in turn determine K and C . There is also only one equation. These factors have made it necessary to use an iterative fitting method using all the reasonable combinations of growth max and T_O , comparing the calculated expansion rate values with those observed, calculating the sum of the squared deviations between observed and calculated, and choosing the curve that best fits the data.

This fitting procedure was mathematically conducted in the following manner: In equation (4b) let:

$$a = \left[(-.5 \log \left| (-1) T^2 + T (T_H + T_L) - T_H \cdot T_L \right|) - \right.$$

$$\left. \left[\left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] - \log \left| \frac{T - T_H}{T - T_L} \right| \right] \right]$$

$$b = \left[\left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right]$$

so that (4b) may be written as:

$$G = -K \cdot a - K \cdot T_O \cdot b + C \quad (4c)$$

Again in equation (4b) if $T = T_O$ then:

$$d = \left[\left(-5 \log \left| (-1) T^2 + T_O (T_H + T_L) - T_H T_L \right| \right) - \left[\left[\frac{T_H + T_L}{2} \right] \left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T - T_H}{T - T_L} \right| \right] \right]$$

$$e = \left[\left[\frac{1}{T_H - T_L} \right] \log \left| \frac{T_O - T_H}{T_O - T_L} \right| \right]$$

and therefore equation (4b) with $T = T_O$ could be written as:

$$G_{\max} = -K \cdot d - K \cdot T_O \cdot e + C \quad (4d)$$

where $G_{\max}$ is the maximum growth value which must by definition occur at $T = T_O$.

From this point on the actual mechanics of the fitting procedure are identical to those employed in Program I. Briefly, G in equation (4c) is set equal to zero and T is set equal to T_L . G in equation (4d) is set equal to $G_{\max}$ and T is set equal to T_O . Equations (4c) and (4d) are solved simultaneously, K and C are found and substituted into equation (4b), and G is calculated for every temperature between T_L and T_H .

// JOB T

MCQUEEN 1

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

*EQUAT(PRNTZ,PRNTY)

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

SUBROUTINE DLAG(T,TO,TL,TH,K,C,DL)

REAL K

IF(T-9.0) 20,20,21

20 DL = 9999.00

GO TO 24

21 IF(T-27.) 22,22,23

23 DL = 9999.00

GO TO 24

22 DL = K*((-.5)*ALOG((-1.0)*T**2.0 + (T*(TH+TL))-(TH*TL)))-

1 K*(((TH+TL)/2.0)*((1.0)/(TH-TL)))*

1 ALOG(ABS((T-TH)/(T-TL)))) +

1 K*TO*(((1.0)/(TH-TL)))*

1 ALOG(ABS((T-TH)/(T-TL)))+C

24 RETURN

END

FEATURES SUPPORTED

ONE WORD INTEGERS

CORE REQUIREMENTS FOR DLAG

COMMON 0 VARIABLES 34 PROGRAM 220

END OF COMPILATION

// DUP

*STORE WS UA DLAG

CART ID 0001 DB ADDR 5361 DB CNT 0012

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

SUBROUTINE PLAG (T,TO,TL,TH,K,C,PL)

REAL K

IF(T-18.) 30,30,31

30 PL = 9999.00

GO TO 34

31 IF(T-37.) 32,32,33

33 PL = 9999.00

GO TO 34

32 PL = K*((-.5)*ALOG((-1.0)*T**2.0 + (T*(TH+TL))-(TH*TL)))-

1 K*(((TH+TL)/2.0)*((1.0)/(TH-TL)))*

1 ALOG(ABS((T-TH)/(T-TL)))) +

1 K*TO*(((1.0)/(TH-TL)))*

```

1      ALOG(ABS((T-TH)/(T-TL)))+C
34     RETURN
      END

```

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR PLAG

COMMON 0 VARIABLES 34 PROGRAM 220

END OF COMPILATION

// DUP

```

*STORE      WS  UA  PLAG
CART ID 0001  DB ADDR 5373  DB CNT 0012

```

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

```

      SUBROUTINE PFGRO(T,TO,TL,TH,K,C,PG)
      REAL K
      IF(T-18.)40,40,41
40     PG = 0.0
      GO TO 44
41     IF(T-37.)42,42,43
43     PG = 0.0
      GO TO 44
42     PG = -K*((-.5)*ALOG((-1.0)*T**2.0 + (T*(TH+TL))-(TH*TL)))+
1       K*(((TH+TL)/2.0)*((1.0)/(TH-TL)))*
1       ALOG(ABS((T-TH)/(T-TL)))
1       -K*TO*(((1.0)/(TH-TL)))*
1       ALOG(ABS((T-TH)/(T-TL)))+C
44     RETURN
      END

```

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR PFGRO

COMMON 0 VARIABLES 36 PROGRAM 226

END OF COMPILATION

// DUP

```

*STORE      WS  UA  PFGRO
CART ID 0001  DB ADDR 5385  DB CNT 0012

```

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

```

      SUBROUTINE DGROW(T,TO,TL,TH,K,C,DG)
      REAL K
      IF(T-9.0) 1,1,2
1     DG = 0.0

```

```

GO TO 5
2   IF(T-26.5)3,3,4
4   DG = 0.0
    GO TO 5
3   DG = (ALOG(TH-T))*K*(TH-TO)-K*TH+K*T+C
5   RETURN
    END

```

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR DGROW
COMMON 0 VARIABLES 8 PROGRAM 98

END OF COMPILATION

// DUP

```

*STORE      WS  UA  DGROW
CART ID 0001  DB ADDR 5397  DB CNT 0008

```

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

```

SUBROUTINE PGROW (T,TO,TL,TH,K,C,PG)
REAL K
    IF(T-18.) 10,10,11
10   PG = 0.0
    GO TO 14
11   IF(T-37.) 12,12,13
13   PG = 0.0
    GO TO 14
12   PG = (ALOG(TH-T))*K*(TH-TO)-K*TH+K*T+C
14   RETURN
    END

```

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR PGROW
COMMON 0 VARIABLES 8 PROGRAM 98

END OF COMPILATION

// DUP

```

*STORE      WS  UA  PGROW
CART ID 0001  DB ADDR 539F  DB CNT 0008

```

// FOR

*IOCS (CARD,1132 PRINTER,TYPEWRITER)

*LIST SOURCE PROGRAM

*ONE WORD INTEGERS

```

C   THE FOLLOWING COMMENT CARDS IDENTIFY THE SYMBOLS USED
C   T = TEMP.
C   TL = TEMP. LOW
C   TO = TEMP. OPTIMUM

```

```

C      TH = TEMP HIGH
C      K = CONSTANT
C      C = CONSTANT
C      LDA = LAG FOR DD AMOEBAE
C      GDA = GROWTH INDEX FOR DD AMOEBAE
C      LDF = LAG FOR DD FRUITING BODIES
C      GDF = GROWTH INDEX FOR DD FRUITING BODIES
C      LPA = LAG FOR PP AMOEBAE
C      LPF = LAG FOR PP FRUITING BODIES
C      GPA = GROWTH FOR PP AMOEBAE
C      GPF = GROWTH FOR PP FRUITING BODIES
C      THE DATA THAT IS INPUT SO THAT THE SUBROUTINES CAN FUNCTION
C      IS CODED IN FOUR PARTS .....THE FIRST LETTER IS L FOR LAG
C      OR G FOR GROWTH .....THE SECOND ONE OR TWO LETTERS ARE TO,TH,
C      TL,K,C ALL OF WHICH HAVE BEEN IDENTIFIED BEFORE.....THE SECOND TO
C      THE LAST LETTER IS P OR D STANDING FOR PP OR DD.....THE LAST
C      LETTER IS A OR F STANDING FOR AMOEBAE OR FRUITING BODY.....AN
C      EXAMPLE..LTHPA..STANDS FOR LAG TEMPERATURE HIGH PP AMOEBAE..
C      THIS IS THE TEMP. HIGH FOR THE PP AMOEBAE LAG SUBROUTINE
      REAL LTODA,LTLDA,LTHDA,LKDA,LCDA,LDA,
1     LTODF,LTLDF,LTHDF,LKDF,LCDF,LDF,
1     LTOPA,LTLPA,LTHPA,LKPA,LCPA,LPA,
1     LTOPF,LTLPF,LTHPF,LKPF,LCPF,LPF
99    CONTINUE
      READ(2,101)T
101   FORMAT(F10.5)
C      ALL OF THE FOLLOWING INFORMATION IS READ INTO THE SUBROUTINES
C      D.DISCOIDEUM AMOEBAE
      LTODA = 23.0
      LTLDA = 9.
      LTHDA = 27.5
      LKDA = 1.60137
      LCDA = 4.74403
      CALL DLAG (T,LTODA,LTLDA,LTHDA,LKDA,LCDA,LDA)
      GTODA = 21.5
      GTLDA = 9.
      GTHDA = 27.5
      GKDA = 0.80084
      GCDA = 0.79554
      CALL DGROW (T,GTODA,GTLDA,GTHDA,GKDA,GCDA,GDA)
C      D.DISCOIDEUM FRUITING BODY
      LTODF = 24.
      LTLDF = 9.
      LTHDF = 27.5
      LKDF = 2.47681
      LCDF = 7.62542
      CALL DLAG (T,LTODF,LTLDF,LTHDF,LKDF,LCDF,LDF)
      GTODF = 21.
      GTLDF = 9.
      GTHDF = 27.5
      GKDF = 0.86318
      GCDF = -0.65368
      CALL DGROW(T,GTODF,GTLDF,GTHDF,GKDF,GCDF,GDF)
C      P.PALLIDUM AMOEBAE
      LTOPA = 31.
      LTLPA = 18.

```

```

LTHPA = 37.
LKPA = 0.81132
LCPA = 2.59356
CALL PLAG (T,LTOPA,LTLPA,LTHPA,LKPA,LCPA,LPA)
GTOPA = 31.
GTLPA = 18.
GTHPA = 41.
GKPA = 1.62709
GCPA = -13.59423
CALL PGROW (T,GTOPA,GTLPA,GTHPA,GKPA,GCPA,GPA)
C P.PALLIDUM FRUITING BODY
LTOPF = 30.
LTLPF = 18.
LTHPF = 37.
LKPF = 1.28527
LCPF = 3.76145
CALL PLAG (T,LTOPF,LTLPF,LTHPF,LKPF,LCPF,LPF)
GTOPF = 31.
GTLPF = 18.
GTHPF = 37.5
GKPF = 0.78172
GCPF = 0.97552
CALL PFGRO(T,GTOPF,GTLPF,GTHPF,GKPF,GCPF,GPF)
WRITE(3,300)T
300 FORMAT( ' TEMPERATURE = 'F10.5)
WRITE(3,309)
309 FORMAT( '          LDA          GDA          LDF          GDF          LPA
1GPA          LPF          GPF ' )
WRITE(3,308) LDA,GDA,LDF,GDF,LPA,GPA,LPF,GPF
308 FORMAT(8F10.2)
WRITE (3,310)
310 FORMAT(1H0)
WRITE(3,301)
301 FORMAT( ' TIME      DDA          DDF          PPA          PPF ' )
C IN THE FOLLOWING AREA CALCULATIONS ARE MADE.....AREAS ARE
C CALCULATED FOR DD AMOEBAE, PP AMOEBAE, DD FRUITING BODIES,
C AND PP FRUITING BODIES.
DO 1000 I = 1,50
C D.DISCOIDEUM AMOEBAE
400 DATIM = I*.1 -LDA
IF(DATIM ) 120,120,121
120 DATIM = 0.0
121 ADA = ( GDA*DATIM +2.0)**2.
ADA = ADA*6.4516
C P.PALLIDUM AMOEBAE
PATIM = I*.1 - LPA
IF(PATIM ) 140,140,141
140 PATIM = 0.0
141 APA = ( GPA*PATIM + 2.0)**2.
APA = APA*6.4516
C D.DISCOIDEUM FRUITING BODY
DFTIM = I*.1 - LDF
IF(DFTIM ) 130,130,131
130 DFTIM = 0.0
131 ADF = ( GDF*DFTIM )**2.
ADF = ADF*6.4516

```

```
C      P.PALLIDUM FRUITING BODY
      PFTIM      = I*.1 - LPF
      IF(PFTIM    ) 150,150,151
150    PFTIM      = 0.0
151    APF        = (      GPF*PFTIM      )**2.
      APF = APF*6.4516
      TIME= I*.1
      WRITE(3,302)TIME,ADA,ADF,APA,APF
302    FORMAT(F5.2,4F10.2)
1000   CONTINUE
      WRITE(3,303)
303    FORMAT(1H1)
      IF(T-38.) 99,99,98
98     CALL EXIT
      END
```

PROGRAM VI - EXPLOITATION OF SINGLE SPECIES

Program VI was used to calculate the area occupied by amoebae and fruiting bodies at any temperature and at any time. Both D. discoideum and P. pallidum were run together but did not interact in any way.

The program is broken into four portions: (1) subroutines, (2) data input, (3) calculations and (4) output.

SUBROUTINES:

There are five subroutines: (1) DLAG: calculates the spore germination lag and the fruiting body lag for D. discoideum. Equation (2b) is used. Below 9.0°C and above 27.0°C the lag is set equal to infinity. (2) PLAG: calculates the spore germination lag and the fruiting body lag for P. pallidum. Equation (2b) is used. Below 18.0°C and above 37.5°C the lag is set equal to infinity. (3) PFGRO: calculates the rate of fruiting body colony expansion for P. pallidum. Equation (4b) is used. Below 18.0°C and above 37.5°C the expansion rate is set equal to 0.0. (4) DGROW: calculates the rate of colony expansion for D. discoideum amoebae and fruiting bodies. Equation (3b) is used. Below 9.0°C and above 27.0°C the rate of expansion is set equal to 0.0. (5) PGROW: calculates the rate of colony expansion for P. pallidum amoebae. Equation (4b) is used. Below 18.0°C and above 37.5°C the expansion rate is set equal to 0.0.

DATA INPUT:

Data is input for eight quantities: (1) D. discoideum amoebae lag, (2) D. discoideum fruiting body lag, (3) D. discoideum amoebae expansion, (4) D. discoideum fruiting body expansion, (5) P. pallidum amoebae lag, (6) P. pallidum fruiting body lag, (7) P. pallidum amoebae expansion, (8) P. pallidum fruiting body expansion. Five pieces of information are needed for the calculation of these eight parameters. These are: temperature low, temperature high, temperature optimum, K, and C. The coding of these data is explained in the program.

CALCULATIONS:

The area occupied by the fruiting bodies and the amoebae of both species is calculated here. Equation (1f) is used in all cases. The constant C in equation (1f) is set equal to 13 mm^2 for D. discoideum and P. pallidum amoebae because C equals the initial area occupied by the spores. For D. discoideum and P. pallidum fruiting bodies the constant C is set equal to 0.0 mm^2 because no area is occupied by the fruiting bodies until they begin to form.

OUTPUT:

Six quantities are output. These are (1) temperature, (2) time, measured in days, (3) DDA = D. discoideum amoebae area, (4) PPA = P. pallidum amoebae area, (5) DDF =

D. discoideum fruiting body area, (6) PPF = P. pallidum
fruiting body area.

// JOB T

MCQUEEN 1

M 21 ABOVE RECORD NOT A SUPERVISOR CONTROL RECORD

// FOR

*IOCS (CARD,1132 PRINTER,TYPEWRITER)

*LIST SOURCE PROGRAM

*ONE WORD INTEGERS

REAL LTODA,LTLDLDA,LTHDA,LKDA,LCDA,LDA,

1 LTODF,LTLDLDF,LTHDF,LKDF,LCDF,LDF,

1 LTOPA,LTLPALPA,LTHPA,LKPA,LCPA,LPA,

1 LTOPF,LTLPFLPF,LTHPF,LKPF,LCPF,LPF

C THE FOLLOWING COMMENT CARDS IDENTIFY THE SYMBOLS USED

C T = TEMP.

C TL = TEMP. LOW

C TO = TEMP. OPTIMUM

C TH = TEMP HIGH

C K = CONSTANT

C C = CONSTANT

C LDA = LAG FOR DD AMOEBAE

C GDA = GROWTH INDEX FOR DD AMOEBAE

C LDF = LAG FOR DD FRUITING BODIES

C GDF = GROWTH INDEX FOR DD FRUITING BODIES

C LPA = LAG FOR PP AMOEBAE

C LPF = LAG FOR PP FRUITING BODIES

C GPA = GROWTH FOR PP AMOEBAE

C GPF = GROWTH FOR PP FRUITING BODIES

C THE DATA THAT IS INPUT SO THAT THE SUBROUTINES CAN FUNCTION

C IS CODED IN FOUR PARTSTHE FIRST LETTER IS L FOR LAG

C OR G FOR GROWTHTHE SECOND ONE OR TWO LETTERS ARE TO,TH,

C TL,K,C ALL OF WHICH HAVE BEEN IDENTIFIED BEFORE.....THE SECOND TO

C THE LAST LETTER IS P OR D STANDING FOR PP OR DD.....THE LAST

C LETTER IS A OR F STANDING FOR AMOEBAE OR FRUITING BODY.....AN

C EXAMPLE..LTHPA..STANDS FOR LAG TEMPERATURE HIGH PP AMOEBAE..

C THIS IS THE TEMP. HIGH FOR THE PP AMOEBAE LAG SUBROUTINE

99 CONTINUE

READ(2,101)T

101 FORMAT(F10.5)

C ALL OF THE FOLLOWING INFORMATION IS READ INTO THE SUBROUTINES

C D.DISCOIDEUM AMOEBAE

LTODA = 23.0

LTLDLDA = 9.

LTHDA = 27.5

LKDA = 1.60137

LCDA = 4.74403

CALL DLAG (T,LTODA,LTLDLDA,LTHDA,LKDA,LCDA,LDA)

GTODA = 21.5

GTLDA = 9.

GTHDA = 27.5

GKDA = 0.80084

GCDA = 0.79554

CALL DGROW (T,GTODA,GTLDA,GTHDA,GKDA,GCDA,GDA)

C D.DISCOIDEUM FRUITING BODY

LTODF = 24.

LTLDLDF = 9.

LTHDF = 27.5

LKDF = 2.47681

```

LCDF = 7.62542
CALL DLAG (T,LTODF,LTLDF,LTHDF,LKDF,LCDF,LDF)
GTODF = 21.
GTLDF = 9.
GTHDF = 27.5
GKDF = 0.86318
GCDF = -0.65368
CALL DGROW(T,GTODF,GTLDF,GTHDF,GKDF,GCDF,GDF)
C P.PALLIDUM AMOEBAE
LTOPA = 31.
LTLPA = 18.
LTHPA = 37.
LKPA = 0.81132
LCPA = 2.59356
CALL PLAG (T,LTOPA,LTLPA,LTHPA,LKPA,LCPA,LPA)
GTOPA = 31.
GTLPA = 18.
GTHPA = 41.
GKPA = 1.62709
GCPA = -13.59423
CALL PGROW (T,GTOPA,GTLPA,GTHPA,GKPA,GCPA,GPA)
C P.PALLIDUM FRUITING BODY
LTOPF = 30.
LTLPF = 18.
LTHPF = 37.
LKPF = 1.28527
LCPF = 3.76145
CALL PLAG (T,LTOPF,LTLPF,LTHPF,LKPF,LCPF,LPF)
GTOPF = 31.
GTLPF = 18.
GTHPF = 37.5
GKPF = 0.78172
GCPF = 0.97552
CALL PFGRO(T,GTOPF,GTLPF,GTHPF,GKPF,GCPF,GPF)
WRITE(3,300)T
300 FORMAT( ' TEMPERATURE = 'F10.5)
WRITE(3,309)
309 FORMAT( ' LDA GDA LDF GDF LPA
1GPA LPF GPF ')
WRITE(3,308) LDA,GDA,LDF,GDF,LPA,GPA,LPF,GPF
308 FORMAT(8F10.2)
WRITE (3,310)
310 FORMAT(1H0)
WRITE(3,301)
301 FORMAT( ' TIME DDA DDF PPA PPF SUMA
1 SUMF ')
C IN THE FOLLOWING AREA CALCULATIONS ARE MADE.....AREAS ARE
C CALCULATED FOR DD AMOEBAE, PP AMOEBAE, DD FRUITING BODIES,
C AND PP FRUITING BODIES.
SUMA = 0.0
SUMF = 0.0
APF = 0.0
ADF = 0.0
DO 1000 I = 1,50
IF(SUMF-1935.) 400,400,401
C D.DISCOIDEUM AMOEBAE

```

```
400 DATIM      = I*.1 -LDA
    IF(DATIM      ) 120,120,121
120 DATIM      = 0.0
121 ADA        = (      GDA*DATIM      +2.0)**2.
    ADA = ADA*6.4516
C    P.PALLIDUM AMOEBAE
    PATIM      = I*.1 - LPA
    IF(PATIM      ) 140,140,141
140 PATIM      = 0.0
141 APA        = (      GPA*PATIM      + 2.0)**2.
    APA = APA*6.4516
401 SUMA = ADA+APA
    IF(SUMF-1935.) 500,500,501
500 CONTINUE
C    P.PALLIDUM FRUITING BODY
    IF(APF-40.) 503,503,504
503 CONTINUE
    PFTIM      = I*.1 - LPF
    IF(PFTIM      ) 150,150,151
150 PFTIM      = 0.0
151 APF        = (      GPF*PFTIM      )**2.
    APF = APF*6.4516
C    D.DISCOIDEUM FRUITING BODY
504 CONTINUE
    IF (ADF - ADA) 510,510,501
510 CONTINUE
    DFTIM      = I*.1 - LDF
    IF(DFTIM      ) 130,130,131
130 DFTIM      = 0.0
131 ADF        = (      GDF*DFTIM      )**2.
    ADF = ADF*6.4516
501 SUMF = ADF + APF
    TIME= I*.1
    WRITE(3,302)TIME,ADA,ADF,APA,APF,SUMA,SUMF
302 FORMAT(F5.1,6F10.2)
1000 CONTINUE
    WRITE(3,303)
303 FORMAT(1H1)
    IF(T-38.) 99,99,98
98  CALL EXIT
    END
```

PROGRAM VII - EXPLOITATION OF MIXED SPECIES

Program VII simulates the exploitation interaction between D. discoideum and P. pallidum grown under laboratory conditions and any temperature. The program is generally the same as Program VI with respect to the subroutines used and the data input. The calculations and data output have been modified to account for exploitation.

The environment (60 mm petri dish) contained 1935 mm^2 of space or 1935 mm^2 of bacterial lawn. The area occupied by the two species growing simultaneously is constantly monitored by the SUM function. When SUM equals 1935 mm^2 amoebae expansion stops. The area occupied by P. pallidum fruiting bodies is limited to 1/7th of the area occupied by P. pallidum amoebae. D. discoideum fruiting bodies are allowed to cover the remaining area.

// JOB

MCQUEEN

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

// FOR

*LIST SOURCE PROGRAM

*IOCS (CARD,TYPEWRITER,1132 PRINTER,KEYBOARD)

REAL NEED(33)

REAL KNEED

DIMENSION PROB(100),CUM(100),TQ(100)

READ(2,3051)(NEED(I),I=1,33)

3051 FORMAT(F5.1)

4053 WRITE(1,4000)

4000 FORMAT(' ONWARDS IS ONE ')

READ(6,4001) ON

4001 FORMAT(F10.5)

IF(ON)4002,4002,4003

4003 WRITE(1,4020)

4020 FORMAT(' TEMP = ')

READ(6,4021) T

4021 FORMAT(F5.1)

WRITE(1,4004)

4004 FORMAT(' SPORE NUMBER IS ')

READ(6,4005) SNUM

4005 FORMAT(F10.5)

AREA = 100.

X = SNUM/AREA

WRITE(3,4022) T

4022 FORMAT(' TEMPERATURE IS 'F5.1)

WRITE(3,4006) X

4006 FORMAT(' MEAN SPORE NUMBER IS ' F10.5)

WRITE(3,4007) SNUM

4007 FORMAT(' TOTAL NUMBER PER PLATE IS ' F10.5)

WRITE(3,4008) AREA

4008 FORMAT(' NUMBER OF AREA UNITS IS ' F10.5)

PZERO = EXP((-X))

DO 4018 K = 1,100

IF (K-1) 4010,4010,4011

4010 TQ (1) = X

GO TO 4012

4011 TQ(K) = (X/K)*TQ(K-1)

4012 CONTINUE

PROB(K) = EXP(-X)*TQ(K)

IF(K-1) 4013,4013,4014

4013 CUM(1) = PZERO + PROB(1)

GO TO 4015

4014 CUM(K) = CUM(K-1) + PROB(K)

4015 CONTINUE

SQNUM = CUM(K) * AREA

SQLEF = AREA - SQNUM

IF(1.0-SQLEF) 4016,4016,4017

4016 CONTINUE

4018 CONTINUE

```
4017 AVAIL = K
      WRITE(3,4030) AVAIL
4030 FORMAT( ' NUMBER OF PP SPORES AVAILABLE = ' F5.1)
      IF(T-21.7) 3052,3052,3053
3052 KNEED = 1000.
      GO TO 3081
3053 IF(T-25.) 3054,3054,3055
3055 KNEED = 1.
      GO TO 3081
3054 Z=(T-21.7)*10.
      I = Z
      KNEED = NEED(I)
3081 CONTINUE
      WRITE(3,3056) KNEED
3056 FORMAT( ' KNEED = ' F7.1)
      IF(KNEED - AVAIL) 4050,4051,4051
4050 CONTINUE
      WRITE(3,4052)
4052 FORMAT( ' PP WILL GROW ' )
      GO TO 4053
4051 CONTINUE
      WRITE(3,4054)
4054 FORMAT( ' PP WILL NOOOOOOOOOOOT GROW ' )
      WRITE (3,4019)
4019 FORMAT(1H1)
      GO TO 4053
4002 CALL EXIT
      END
```


PROGRAM VIII - INHIBITION OF P. PALLIDUM

Program VIII simulates D. discoideum inhibition of P. pallidum fruiting body formation. The program is composed of two sections; available clump size, which calculates the maximum clump size available at any given spore concentration; and necessary clump size, which calculates the clump size necessary for fruiting at any temperature.

AVAILABLE CLUMP SIZE

The calculations of available clump size are based on two assumptions. During the experimental work it was observed that a small number of P. pallidum spores placed on an agar surface containing a known number of randomly distributed D. discoideum spores, were able to control the use of food, for at least three days, in the 19 mm^2 area surrounding their point of inoculation. This sphere of influence is about 1/100th of the total surface area of a 60 mm petri dish. Therefore, it was assumed that a petri dish could be divided into 100 spheres of influence. It was also assumed that when spores were spread on the surface of a petri dish using Method II (methods section) that they were distributed at random.

A poisson distribution was used to calculate the number of spores in each of the 100 spheres of influence. The program continues to calculate the number of spheres of influence containing various numbers of spores until all of

the spheres of influence have been used up. In this way the maximum number of spores per sphere of influence is found. This quantity is called the maximum clump size.

NEEDED CLUMP SIZE

The necessary clump size is calculated by reading data input from Figure 26, on which the clump size necessary for fruiting is plotted against temperature.

COMPARISON

When the temperature and the number of P. pallidum spores have been read into the computer the available and necessary clump size can be calculated. The two quantities are compared and if available is larger than necessary then P. pallidum fruiting takes place.

// JOB T

MCQUEEN 1

LOG DRIVE	CART SPEC	CART AVAIL	PHY DRIVE
0000	0001	0001	0000

V2 M06 ACTUAL 8K CONFIG 8K

*EQUAT(PRNTZ,PRNTY)

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

SUBROUTINE DLAG(T,TO,TL,TH,K,C,DL)

REAL K

IF(T-9.0) 20,20,21

20 DL = 9999.00

GO TO 24

21 IF(T-27.0) 22,22,23

23 DL = 9999.00

GO TO 24

22 DL = K*((-.5)*ALOG((-1.0)*T**2.0 + (T*(TH+TL))-(TH*TL)))-

1 K*(((TH+TL)/2.0)*((1.0)/(TH-TL))*

1 ALOG(ABS((T-TH)/(T-TL)))) +

1 K*TO*(((1.0)/(TH-TL))*

1 ALOG(ABS((T-TH)/(T-TL))))+C

24 RETURN

END

FEATURES SUPPORTED

ONE WORD INTEGERS

CORE REQUIREMENTS FOR DLAG

COMMON 0 VARIABLES 34 PROGRAM 220

END OF COMPILATION

// DUP

*STORE WS UA DLAG
 CART ID 0001 DB ADDR 5361 DB CNT 0012

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

 SUBROUTINE PLAG (T,TO,TL,TH,K,C,PL)

 REAL K

 IF(T-18.) 30,30,31

30 PL = 9999.00

 GO TO 34

31 IF(T-37.) 32,32,33

33 PL = 9999.00

 GO TO 34

32 PL = K*((-.5)*ALOG((-1.0)*T**2.0 + (T*(TH+TL))-(TH*TL)))-

1 K*(((TH+TL)/2.0)*((1.0)/(TH-TL))*

1 ALOG(ABS((T-TH)/(T-TL)))) +

1 K*TO*(((1.0)/(TH-TL))*

1 ALOG(ABS((T-TH)/(T-TL))))+C

34 RETURN

 END

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR PLAG

COMMON 0 VARIABLES 34 PROGRAM 220

END OF COMPILATION

// DUP

*STORE WS UA PLAG

CART ID 0001 DB ADDR 5373 DB CNT 0012

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

SUBROUTINE PFGRO(T,TO,TL,TH,K,C,PG)

REAL K

IF(T-18.)40,40,41

40 PG = 0.0

GO TO 44

41 IF(T-37.)42,42,43

43 PG = 0.0

GO TO 44

42 PG = -K*((-.5)*ALOG((-1.0)*T**2.0 + (T*(TH+TL))-(TH*TL)))+

1 K*(((TH+TL)/2.0)*((1.0)/(TH-TL))*

1 ALOG(ABS((T-TH)/(T-TL))))

1 -K*TO*(((1.0)/(TH-TL))*

1 ALOG(ABS((T-TH)/(T-TL))))+C

44 RETURN

END

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR PFGRO
COMMON 0 VARIABLES 36 PROGRAM 226

END OF COMPILATION

// DUP

*STORE WS UA PFGRO

CART ID 0001 DB ADDR 5385 DB CNT 0012

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

 SUBROUTINE DGROW(T,TO,TL,TH,K,C,DG)

 REAL K

 IF(T-9.0) 1,1,2

1 DG = 0.0

 GO TO 5

2 IF(T-26.0) 3,3,4

4 DG = 0.0

 GO TO 5

3 DG = (ALOG(TH-T))*K*(TH-TO)-K*TH+K*T+C

5 RETURN

 END

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR DGROW
COMMON 0 VARIABLES 8 PROGRAM 98

END OF COMPILATION

// DUP

*STORE WS UA DGROW

CART ID 0001 DB ADDR 5397 DB CNT 0008

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

SUBROUTINE PGROW (T,TO,TL,TH,K,C,PG)

REAL K

IF(T-18.) 10,10,11

10 PG = 0.0

GO TO 14

11 IF(T-37.) 12,12,13

13 PG = 0.0

GO TO 14

12 PG = (ALOG(TH-T))*K*(TH-TO)-K*TH+K*T+C

14 RETURN

END

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR PGROW
COMMON 0 VARIABLES 8 PROGRAM 98

END OF COMPILATION

// DUP

*STORE WS UA PGROW

CART ID 0001 DB ADDR 539F DB CNT 0008

// FOR

*ONE WORD INTEGERS

*LIST SOURCE PROGRAM

SUBROUTINE DDFRU(DDCON,T,QUIT,DFRU,TQUIT)

DIMENSION DFRU(1)

IF(DDCON - 2000.) 1,2,2

1 R = (DDCON - 100.)/100.

I = R

TQUIT = DFRU(I)

GO TO 6

2 TQUIT = 23.9

6 IF(T - TQUIT) 3,3,4

3 QUIT = 1.

GO TO 5

4 QUIT = -1.

5 CONTINUE

RETURN

END

FEATURES SUPPORTED
ONE WORD INTEGERS

CORE REQUIREMENTS FOR DDFRU
COMMON 0 VARIABLES 4 PROGRAM 80

// FOR

*IOCS (CARD,TYPEWRITER,1132 PRINTER,KEYBOARD)

*LIST SOURCE PROGRAM

*ONE WORD INTEGERS

REAL LTODA,LTLDA,LTHDA,LKDA,LCDA,LDA,

1 LTODF,LTLDF,LTHDF,LKDF,LCDF,LDF,

1 LTOPA,LTLPA,LTHPA,LKPA,LCPA,LPA,

1 LTOPF,LTLPF,LTHPF,LKPF,LCPF,LPF

REAL NEED(33)

REAL KNEED

DIMENSION TIME(50),ADA(50),ADF(50),APA(50),APF(50),SUMA(50),

1 SUMF(50),PROB(50),CUM(50),TQ(50),DFRU(18)

READ(2,3051)(NEED(I),I=1,33)

3051 FORMAT(F5.1)

READ(2,8040)(DFRU(I),I=1,18)

8040 FORMAT(F10.5)

99 CONTINUE

WRITE(1,4000)

4000 FORMAT(' ONWARDS IS ONE ')

READ(6,4001) ON

4001 FORMAT(F10.5)

IF(ON) 98,98,4003

4003 WRITE(1,4020)

4020 FORMAT(' TEMP = ')

READ(6,4021) T

4021 FORMAT(F5.1)

WRITE(1,4004)

4004 FORMAT(' SPORE NUMBER IS ')

READ(6,4005) SNUM

4005 FORMAT(F10.5)

WRITE(1,4874)

4874 FORMAT(' DD SPORE CONCENTRATION ')

READ(6,4884) DDCON

4884 FORMAT(F10.5)

AREA = 100.

X = SNUM/AREA

WRITE(3,4022) T

4022 FORMAT(' TEMPERATURE IS ' F5.1)

WRITE(3,4006) X

4006 FORMAT(' MEAN P.PALLIDUM SPORE NUMBER IS ' F10.5)

WRITE(3,4007) SNUM

4007 FORMAT(' P.PALL SPORE NUMBER PER PLATE IS ' F10.5)

WRITE(3,4008) AREA

4008 FORMAT(' NUMBER OF AREA UNITS IS ' F10.5)

PZERO = EXP((-X))

DO 4018 K = 1,100

IF (K-1) 4010,4010,4011

4010 TQ (1) = X

GO TO 4012

4011 TQ(K) = (X/K)*TQ(K-1)

4012 CONTINUE

PROB(K) = EXP(-X)*TQ(K)

IF(K-1) 4013,4013,4014

4013 CUM(1) = PZERO + PROB(1)

GO TO 4015

4014 CUM(K) = CUM(K-1) + PROB(K)

```

4015 CONTINUE
      SQNUM = CUM(K) * AREA
      SQLEF = AREA - SQNUM
      IF(1.0-SQLEF) 4016,4016,4017
4016 CONTINUE
4018 CONTINUE
4017 AVAIL = K
      WRITE(3,4030) AVAIL
4030 FORMAT( ' NUMBER OF PP SPORES AVAILABLE = ' F5.1)
      IF(T-21.7) 3052,3052,3053
3052 KNEED = 1000.
      GO TO 3081
3053 IF(T-25.) 3054,3054,3055
3055 KNEED = 1.
      GO TO 3081
3054 Z=(T-21.7)*10.
      I = Z
      KNEED = NEED(I)
3081 CONTINUE
      WRITE(3,3056) KNEED
3056 FORMAT( ' KNEED = ' F7.1)
      IF(KNEED - AVAIL) 4050,4050,4051
4050 CONTINUE
      WRITE(3,4052)
4052 FORMAT( ' PP WILL FRUIT ' )
      GO = 1.0
      GO TO 4053
4051 CONTINUE
      WRITE(3,4054)
4054 FORMAT( ' PP WILL NOT FRUIT ' )
      GO = -1.0
      GO TO 4053
4053 CONTINUE

```

```

C      THE FOLLOWING COMMENT CARDS IDENTIFY THE SYMBOLS USED
C      T = TEMP.
C      TL = TEMP. LOW
C      TO = TEMP. OPTIMUM
C      TH = TEMP HIGH
C      K = CONSTANT
C      C = CONSTANT
C      LDA = LAG FOR DD AMOEBAE
C      GDA = GROWTH INDEX FOR DD AMOEBAE
C      LDF = LAG FOR DD FRUITING BODIES
C      GDF = GROWTH INDEX FOR DD FRUITING BODIES
C      LPA = LAG FOR PP AMOEBAE
C      LPF = LAG FOR PP FRUITING BODIES
C      GPA = GROWTH FOR PP AMOEBAE
C      GPF = GROWTH FOR PP FRUITING BODIES
C      THE DATA THAT IS INPUT SO THAT THE SUBROUTINES CAN FUNCTION
C      IS CODED IN FOUR PARTS .....THE FIRST LETTER IS L FOR LAG
C      OR G FOR GROWTH .....THE SECOND ONE OR TWO LETTERS ARE TO,TH,
C      TL,K,C ALL OF WHICH HAVE BEEN IDENTIFIED BEFORE.....THE SECOND TO

```

```
C THE LAST LETTER IS P OR D STANDING FOR PP OR DD....THE LAST
C LETTER IS A OR F STANDING FOR AMOEBAE OR FRUITING BODY.....AN
C EXAMPLE..LTHPA..STANDS FOR LAG TEMPERATURE HIGH PP AMOEBAE..
C THIS IS THE TEMP. HIGH FOR THE PP AMOEBAE LAG SUBROUTINE
C ALL OF THE FOLLOWING INFORMATION IS READ INTO THE SUBROUTINES
C D.DISCOIDEUM AMOEBAE
  LTODA = 23.0
  LTLDA = 9.
  LTHDA = 27.5
  LKDA = 1.60137
  LCDA = 4.74403
  CALL DLAG (T,LTODA,LTLDA,LTHDA,LKDA,LCDA,LDA)
  GTODA = 21.5
  GTLDA = 9.
  GTHDA = 27.5
  GKDA = 0.80084
  GCDA = 0.79554
  CALL DGROW (T,GTODA,GTLDA,GTHDA,GKDA,GCDA,GDA)
C D.DISCOIDEUM FRUITING BODY
  LTODF = 24.
  LTLDF = 9.
  LTHDF = 27.5
  LKDF = 2.47681
  LCDF = 7.62542
  CALL DLAG (T,LTODF,LTLDF,LTHDF,LKDF,LCDF,LDF)
  GTODF = 21.
  GTLDF = 9.
  GTHDF = 27.5
  GKDF = 0.86318
  GCDF = -0.65368
  CALL DGROW(T,GTODF,GTLDF,GTHDF,GKDF,GCDF,GDF)
C P.PALLIDUM AMOEBAE
  LTOPA = 31.
  LTLPA = 18.
  LTHPA = 37.
  LKPA = 0.81132
  LCPA = 2.59356
  CALL PLAG (T,LTOPA,LTLPA,LTHPA,LKPA,LCPA,LPA)
  GTOPA = 31.
  GTLPA = 18.
  GTHPA = 41.
  GKPA = 1.62709
  GCPA = -13.59423
  CALL PGROW (T,GTOPA,GTLPA,GTHPA,GKPA,GCPA,GPA)
C P.PALLIDUM FRUITING BODY
  LTOPF = 30.
  LTLPF = 18.
  LTHPF = 37.
  LKPF = 1.28527
  LCPF = 3.76145
  CALL PLAG (T,LTOPF,LTLPF,LTHPF,LKPF,LCPF,LPF)
  GTOPF = 31.
  GTLPF = 18.
  GTHPF = 37.5
  GKPF = 0.78172
  GCPF = 0.97552
```

```
CALL PFGRO(T,GTOPF,GTLPF,GTHPF,GKPF,GCPF,GPF)
```

```
CALL DDFRU(DDCON,T,QUIT,DFRU,TQUIT)
WRITE(3,491)TQUIT
491 FORMAT( ' MAX TEMP FOR DD FRUITING IS ' F10.5)
WRITE(3,492) DDCON
492 FORMAT( ' D.DISCOIDEUM SPORE CONCENTRATION IS ' F10.5)
IF (QUIT) 484,484,485
484 WRITE(3,488)
488 FORMAT( ' DD WILL NOT FRUIT ' )
GO TO 468
485 WRITE(3,489) QUIT
489 FORMAT( ' DD WILL FRUIT BECAUSE QUIT IS ' F10.5)
468 CONTINUE
```

```
WRITE(3,309)
309 FORMAT( '          LDA          GDA          LDF          GDF          LPA
1GPA          LPF          GPF ')
WRITE(3,308) LDA,GDA,LDF,GDF,LPA,GPA,LPF,GPF
308 FORMAT(8F10.2)
WRITE(3,310)
310 FORMAT(1H0)
WRITE(3,301)
301 FORMAT( ' TIME          DDA          DDF          PPA          PPF          SUMA
1 SUMF ')
```

```
C      IN THE FOLLOWING AREA CALCULATIONS ARE MADE.....AREAS ARE
C      CALCULATED FOR DD AMOEBAE, PP AMOEBAE, DD FRUITING BODIES,
C      AND PP FRUITING BODIES.
```

```
SUMA(1) = 0.0
SUMF(1) = 0.0
APF(1) = 0.0
ADF(1) = 0.0
```

```
DO 1000 I = 1,50
IF(I-1) 78,78,79
79 IF(SUMA(I-1) - 1935.) 400,400,401
78 CONTINUE
C D.DISCOIDEUM AMOEBAE
400 DATIM      = I*.1 -LDA
IF(DATIM      ) 120,120,121
120 DATIM      = 0.0
```

```

121 ADA(I) = ( GDA*DATIM +2.0)**2.
ADA(I) = ADA(I)*6.4516
C P.PALLIDUM AMOEBAE
PATIM = I*.1 - LPA
IF(PATIM ) 140,140,141
140 PATIM = 0.0
141 APA(I) = ( GPA*PATIM + 2.0)**2.
APA(I) = APA(I)*6.4516
GO TO 936
401 ADA(I) = ADA(I-1)
APA(I) = APA(I-1)
936 SUMA(I) = ADA(I) + APA(I)
1000 CONTINUE

```

```

DO 1098 J=1,50
C P.PALLIDUM FRUITING BODY
IF(GO) 150,150,86
86 CONTINUE
IF (J-1) 81,81,82
82 IF(APF(J-1)-APA(50)/7.5) 503,503,504
81 CONTINUE
503 CONTINUE
PFTIM = J*.1 - LPF
IF(PFTIM ) 150,150,151
150 PFTIM = 0.0
151 APF(J) = ( GPF*PFTIM )**2.
APF(J) = APF(J)*6.4516
GO TO 727
C D.DISCOIDEUM FRUITING BODY
504 CONTINUE
APF(J) = APF(J-1)
727 IF(QUIT)130,130,695
695 IF(J-1) 83,83,84
84 IF(ADF(J-1)-ADA(50)) 510,510,501
83 CONTINUE
510 CONTINUE
DFTIM = J*.1 - LDF
IF(DFTIM ) 130,130,131
130 DFTIM = 0.0
131 ADF(J) = ( GDF*DFTIM )**2.
ADF(J) = ADF(J)*6.4516
GO TO 760
501 ADF(J) = ADF(J-1)
760 SUMF(J) = ADF(J) + APF(J)
1098 CONTINUE

```

```

DO 1032 K = 1,50
TIME(K) = K*.1
1032 CONTINUE

```

```
      WRITE(3,302)(TIME(L),ADA(L),ADF(L),APA(L),APF(L),SUMA(L),SUMF(L),  
1 L = 1,50)  
302  FORMAT(F5.1,6F10.2)  
      WRITE(3,303)  
303  FORMAT(1H1)  
      IF(T-38.) 99,99,98  
98   CALL EXIT  
      END
```

PROGRAM IX - COMPLETED MODEL

Program IX comprises the finished simulation model for D. discoideum and P. pallidum salvador growing together in the laboratory at temperatures ranging from 9°C to 37.5°C. The program is composed of six subroutines and a mainline program.

SUBROUTINES:

The first five subroutines have been explained in Appendix I - Program VI. The sixth subroutine describes P. pallidum's ability to inhibit D. discoideum. The inputs to this subroutine are: the total number of D. discoideum spores per plate (DDCON), the temperature (T), and the one dimensional array of temperatures above which D. discoideum will not fruit at any given spore concentration. The output is a positive or negative number (QUIT). Positive if D. discoideum can fruit, negative if not.

MAINLINE PROGRAM

The mainline program is composed of four sections: input, output, colony and fruiting body expansion, and a section which describes D. discoideum's ability to inhibit P. pallidum fruiting body production. P. pallidum interference has been explained in Appendix I - Program VIII, and expansion has been explained in Appendix I - Program VI.

The input is composed of the number of P. pallidum spores per plate, the number of D. discoideum spores per plate, the growth parameters for the two species, the interference data input, and amount of area covered by fruiting bodies and amoebae of both species at any time t .

The output is divided into thirteen sections:

(1) P. pallidum spore lag, (2) D. discoideum spore lag, (3) P. pallidum fruiting body lag, (4) D. discoideum fruiting body lag, (5) P. pallidum amoeba colony area, (6) D. discoideum amoeba colony area, (7) P. pallidum fruiting body area, (8) D. discoideum fruiting body area, (9) temperature, (10) time, (11) necessary clump size, (12) available clump size, and (13) D. discoideum fruiting body boundary temperature. From this information 95% confidence limits and spore numbers can be calculated.

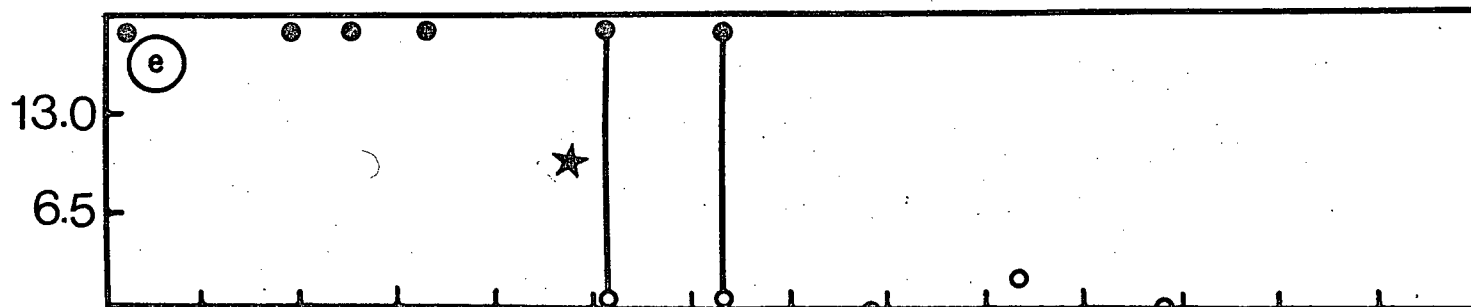
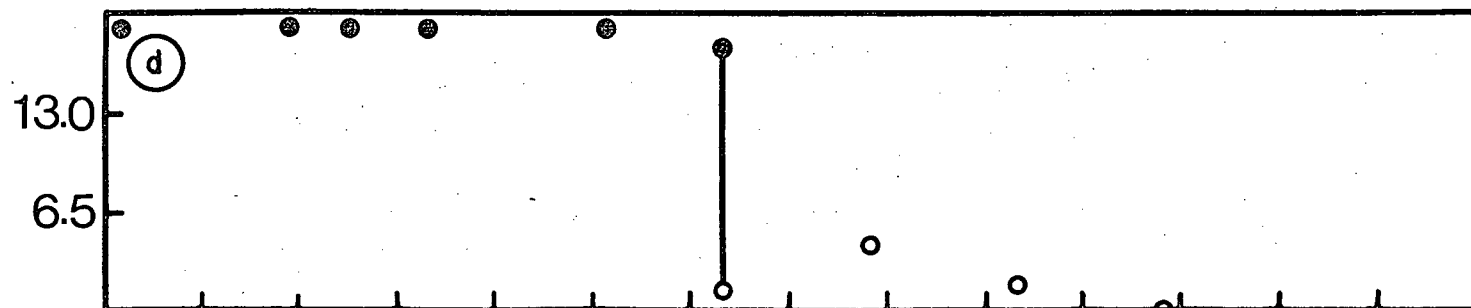
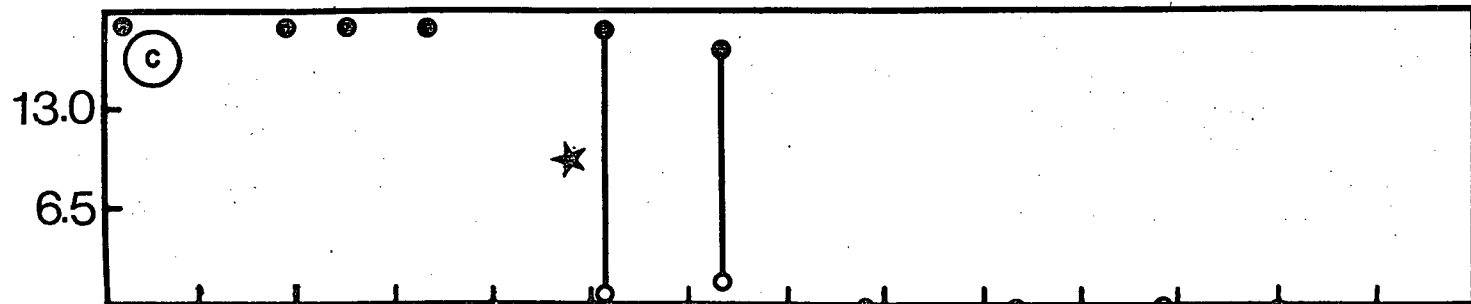
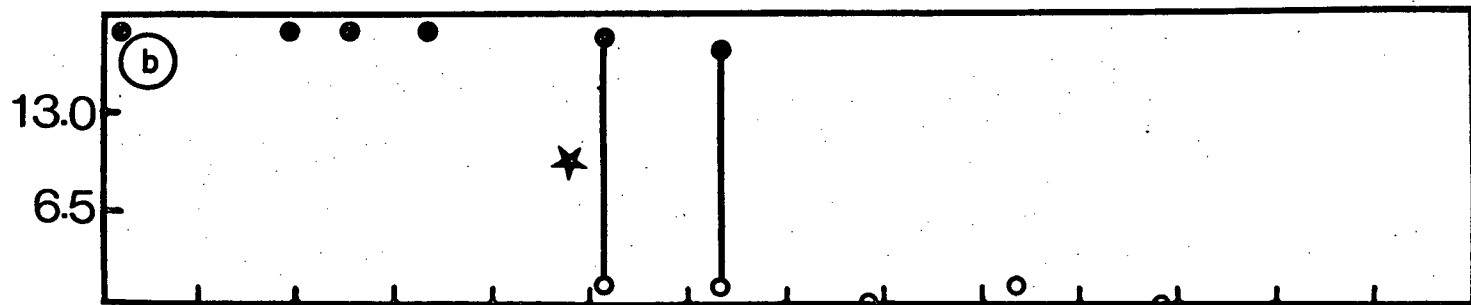
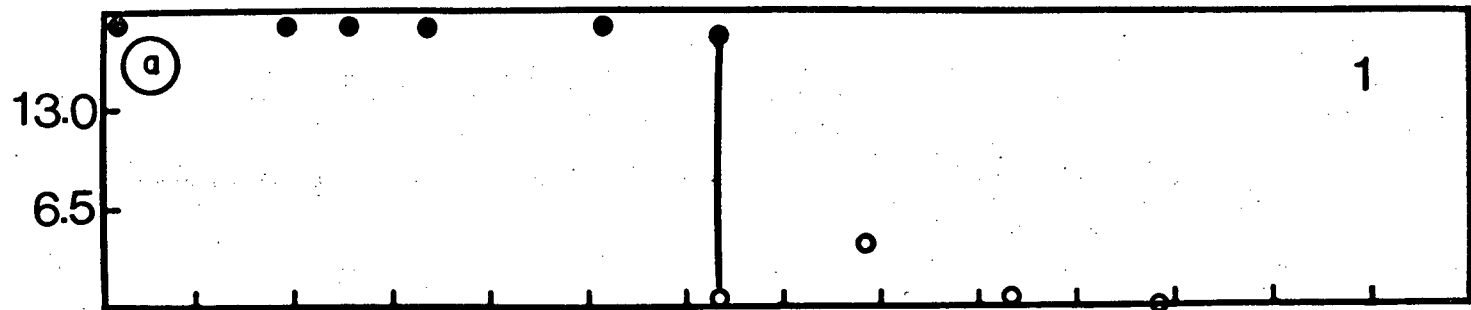
APPENDIX II

Figure 42

P. pallidum and D. discoideum V12 were inoculated together at five relative concentrations. Plates were grown at several temperatures, and when fruiting body expansion terminated, the total area occupied by fruiting bodies of both species was noted. Temperature is plotted along the x-axis (in degrees centigrade) and area covered is plotted along the y-axis. Black dots represent D. discoideum, open circles P. pallidum. A line joining a black dot and an open circle indicates that both species fruited together. The five experimental sets are listed (a) to (e) from the top to the bottom. The spore concentrations used are:

EXPERIMENTAL SET	CON PP.	CON Dd.
42-a	90	10
42-b	70	30
42-c	50	50
42-d	30	70
42-e	10	90

AREA CM²



19 20 21 22 23 24 25 26 27 28 29 30 31

TEMPERATURE

Figure 43

P. pallidum and D. discoideum V12 were inoculated together at five relative concentrations. Plates were grown at several temperatures, and when fruiting body expansion terminated, the total area occupied by fruiting bodies of both species was noted. Temperature is plotted along the x-axis (in degrees centigrade) and area covered is plotted along the y-axis. Black dots represent D. discoideum, open circles P. pallidum. A line joining a black dot and an open circle indicates that both species fruited together. The five experimental sets are listed (a) to (e) from the top to the bottom. The spore concentrations used are:

EXPERIMENTAL SET	CON PP.	CON Dd.
43-a	600	66
43-b	466	200
43-c	333	333
43-d	200	466
43-e	66	600

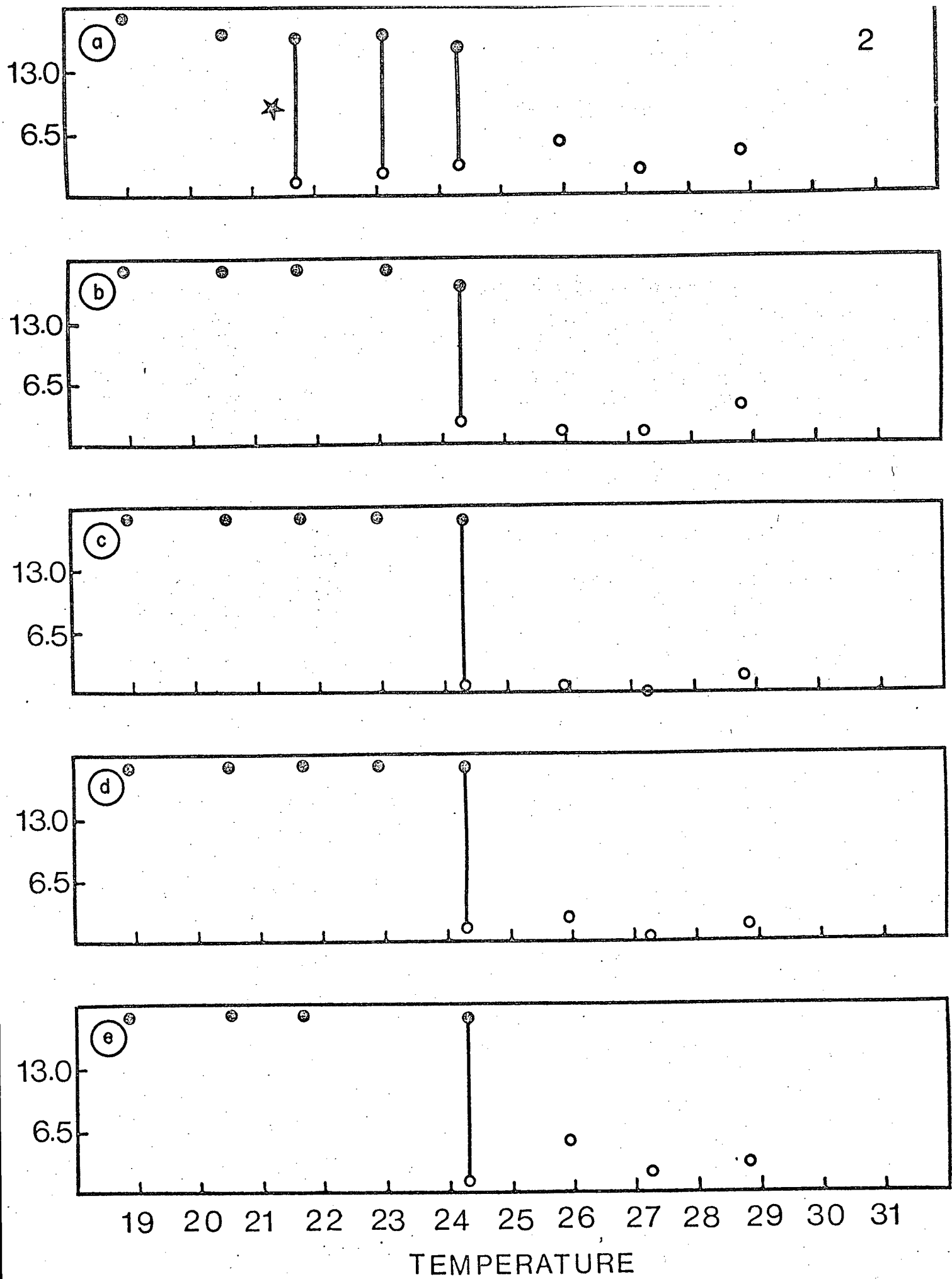
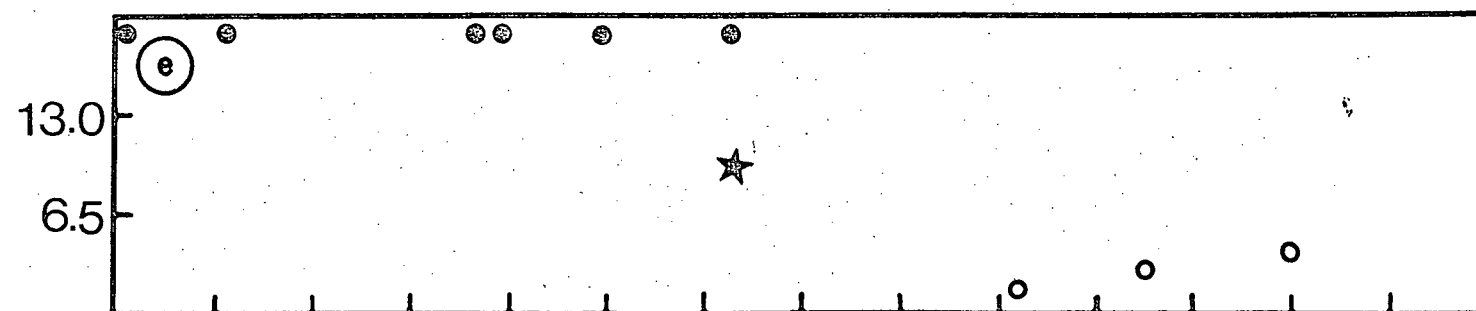
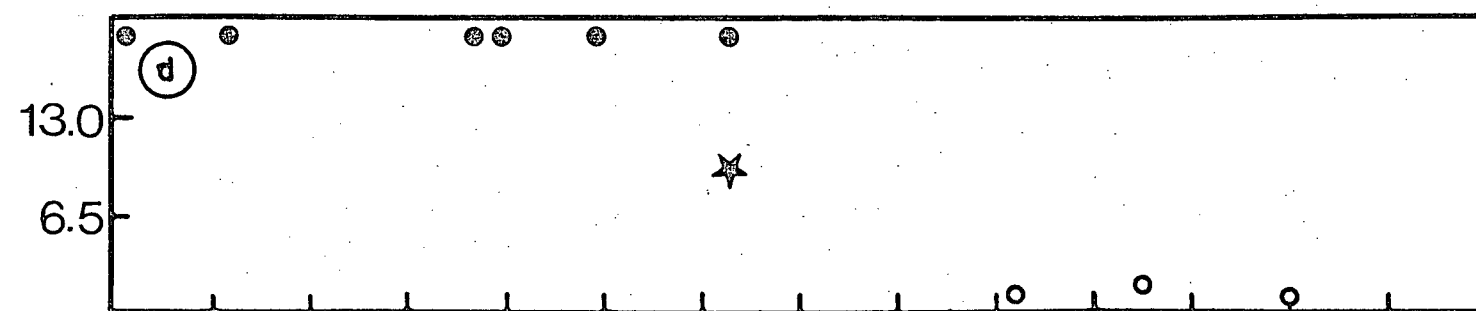
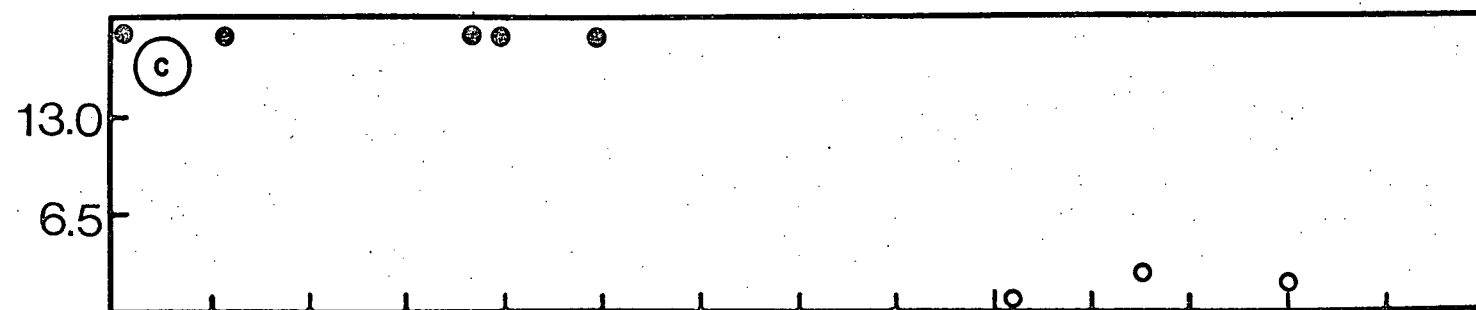
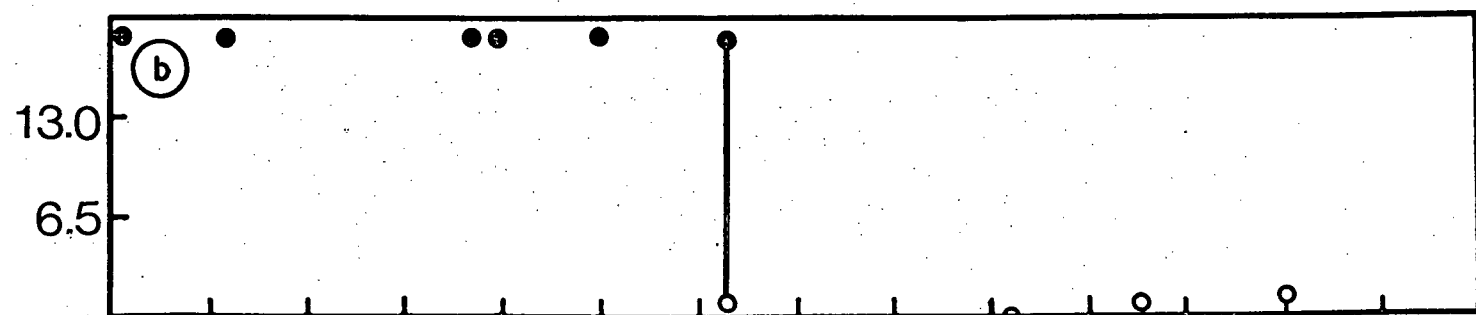
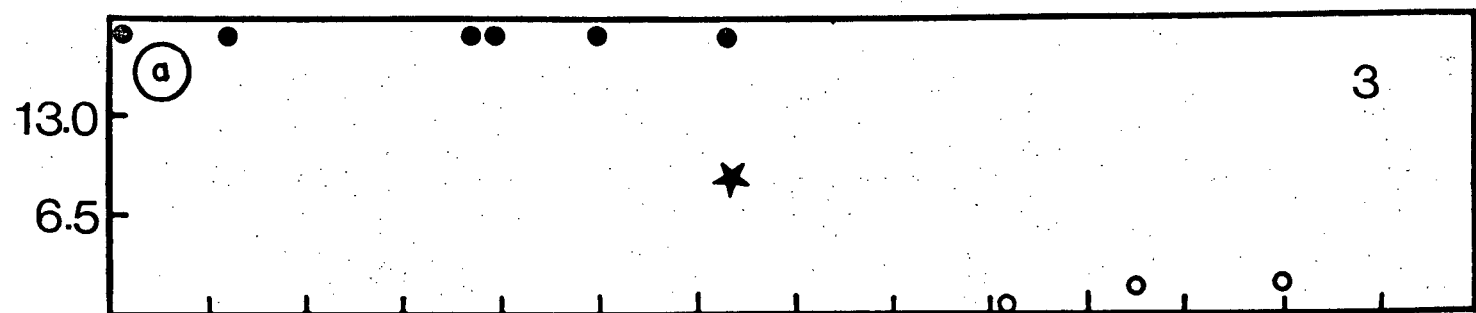


Figure 44

P. pallidum and D. discoideum V12 were inoculated together at five relative concentrations. Plates were grown at several temperatures, and when fruiting body expansion terminated, the total area occupied by fruiting bodies of both species was noted. Temperature is plotted along the x-axis (in degrees centigrade) and area covered is plotted along the y-axis. Black dots represent D. discoideum, open circles P. pallidum. A line joining a black dot and an open circle indicates that both species fruited together. The five experimental sets are listed (a) to (e) from the top to the bottom. The spore concentrations used are:

EXPERIMENTAL SET	CON PP.	CON Dd.
44-a	2394	266
44-b	1862	798
44-c	1330	1330
44-d	798	1862
44-e	266	2394



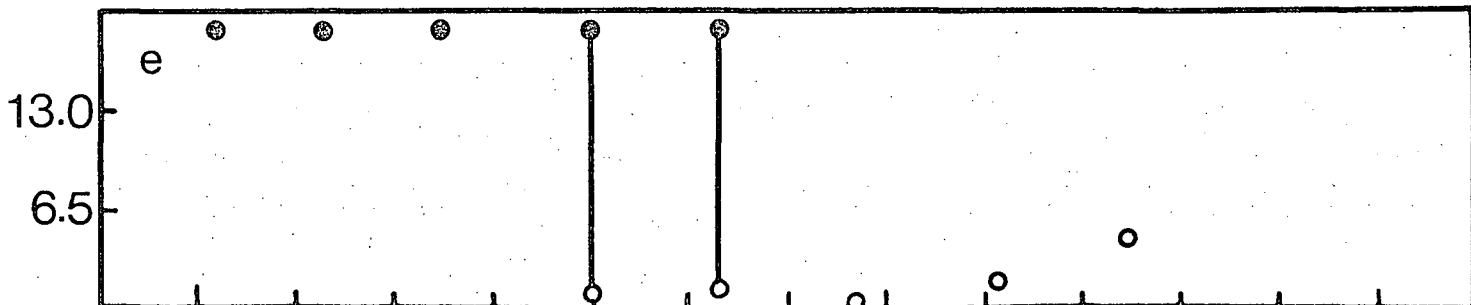
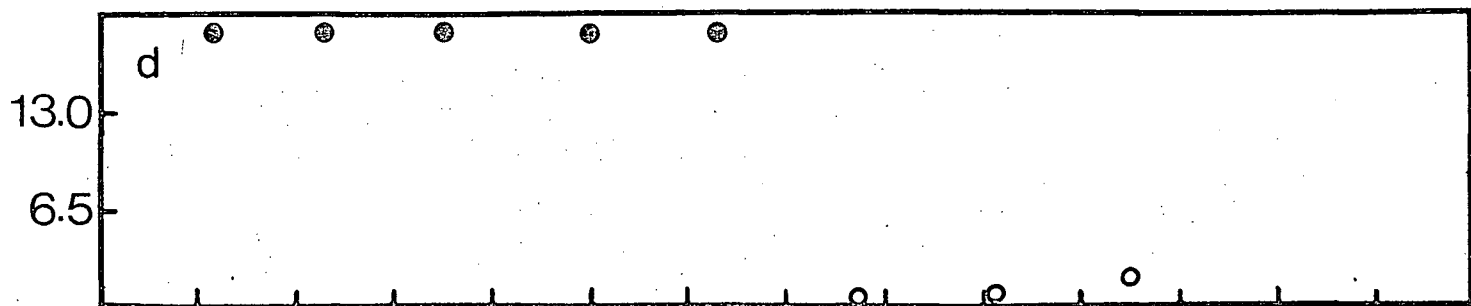
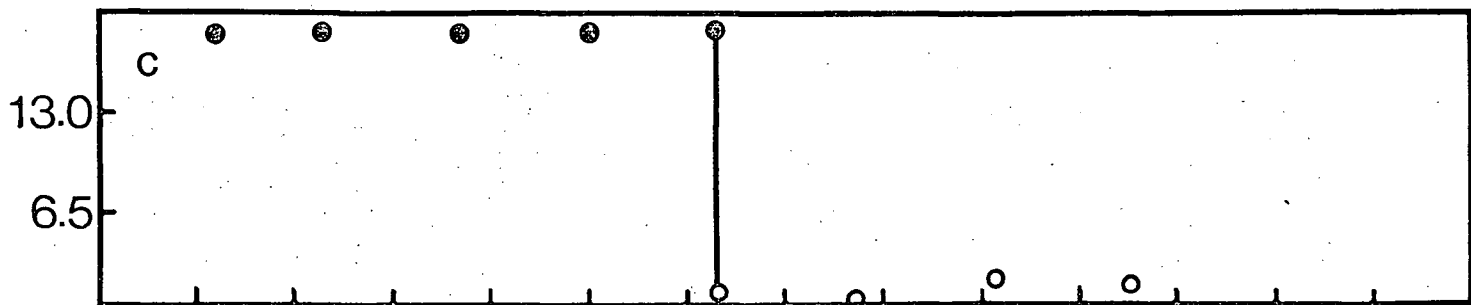
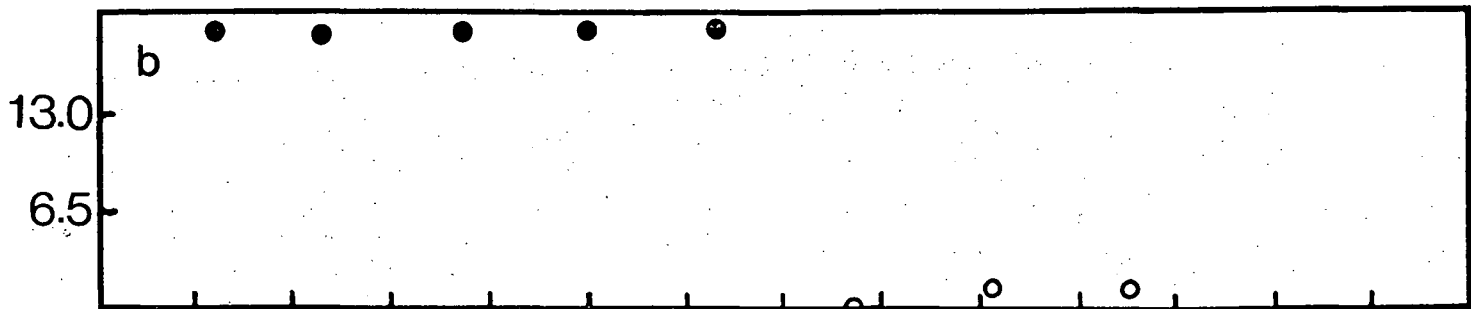
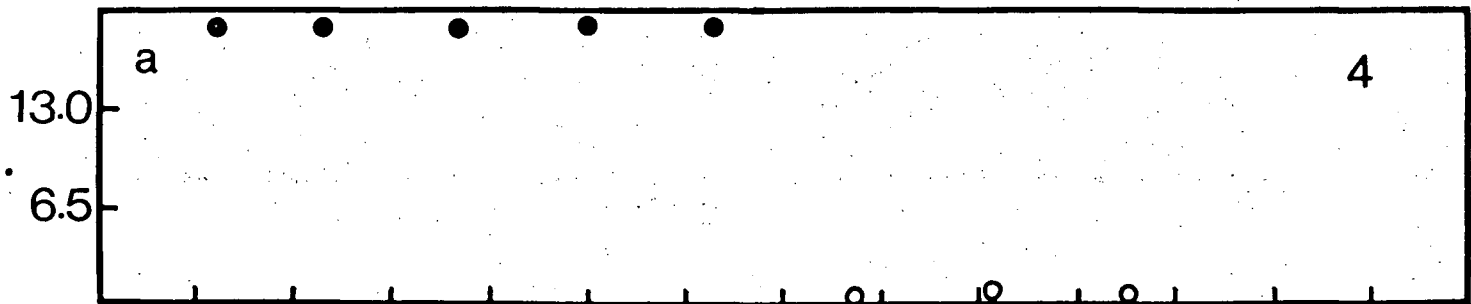
19 20 21 22 23 24 25 26 27 28 29 30 31

TEMPERATURE

Figure 45

P. pallidum and D. discoideum V12 were inoculated together at five relative concentrations. Plates were grown at several temperatures, and when fruiting body expansion terminated, the total area occupied by fruiting bodies of both species was noted. Temperature is plotted along the x-axis (in degrees centigrade) and area covered is plotted along the y-axis. Black dots represent D. discoideum, open circles P. pallidum. A line joining a black dot and an open circle indicates that both species fruited together. The five experimental sets are listed (a) to (e) from the top to the bottom. The spore concentrations used are:

EXPERIMENTAL SET	CON PP.	CON Dd.
45-a	14400	1600
45-b	11200	4800
45-c	8000	8000
45-d	4800	11200
45-e	1600	14400



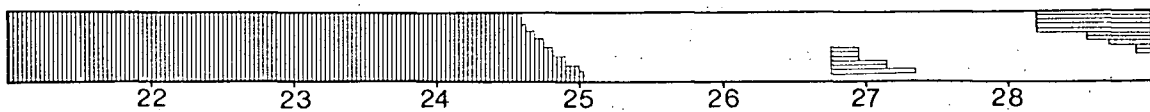
19 20 21 22 23 24 25 26 27 28 29 30 31

TEMPERATURE

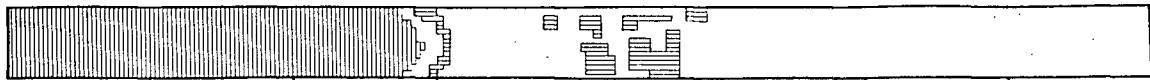
Figure 46

Culture gradient II. Gradient drawings showing the change in fruiting ability experienced by P. pallidum. The horizontally shaded areas were occupied by P. pallidum fruiting bodies, the vertically shaded areas by D. discoideum fruiting bodies. The areas shaded with horizontal and vertical lines were occupied by fruiting bodies of both species. The unshaded areas were unoccupied. Temperature is marked at intervals under each diagram.

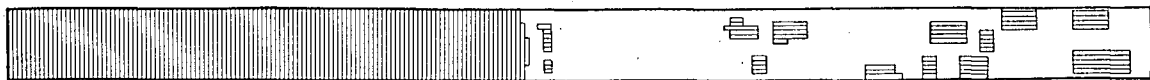
A



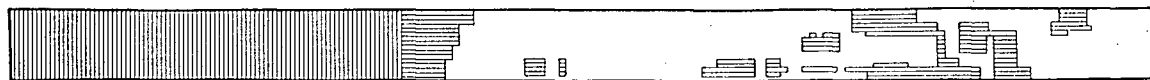
B



C



D



E



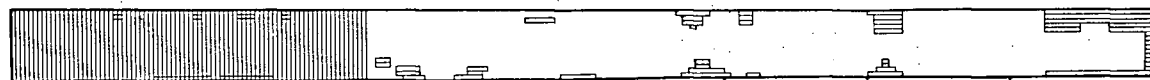
F



G



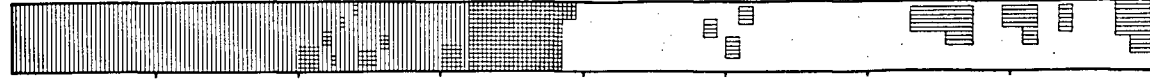
H



I



J



K



L

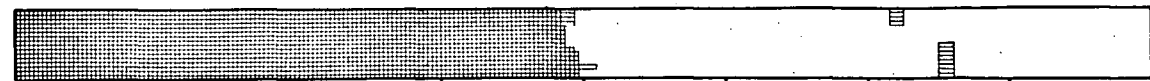
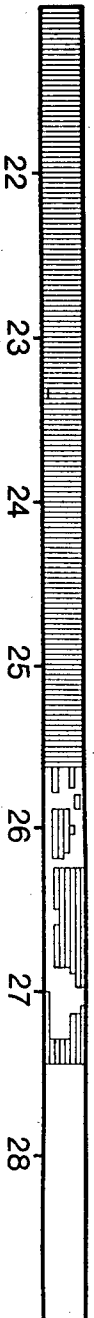


Figure 47

Culture gradient III. Gradient drawings showing the change in fruiting ability experienced by P. pallidum. The horizontally shaded areas were occupied by P. pallidum fruiting bodies; the vertically shaded areas by D. discoideum fruiting bodies. The areas shaded with horizontal and vertical lines were occupied by fruiting bodies of both species. The unshaded areas were unoccupied. Temperature is marked at intervals under each diagram.

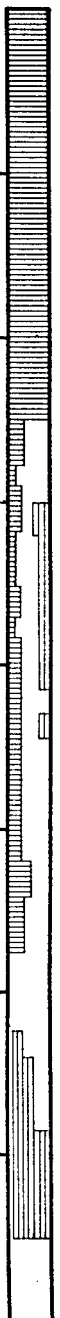
A



B



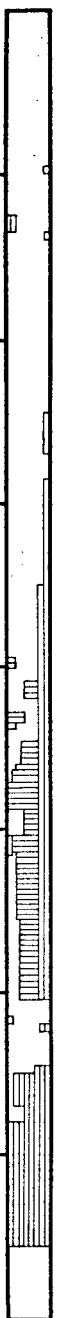
C



D



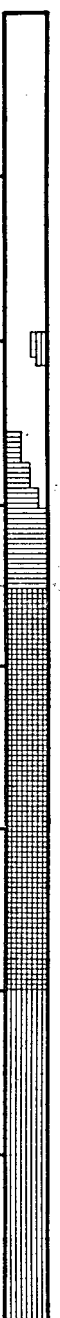
E



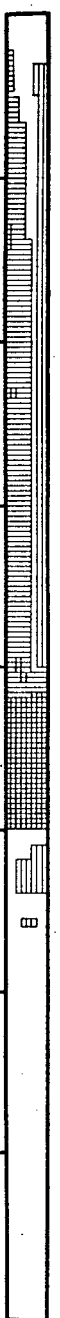
F



G



H



I



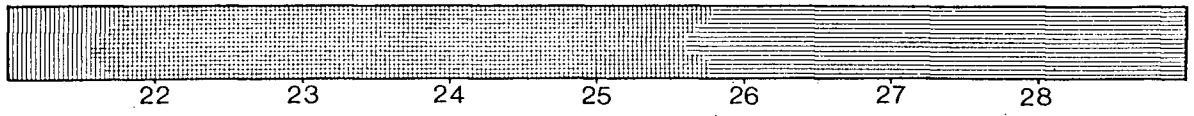
J



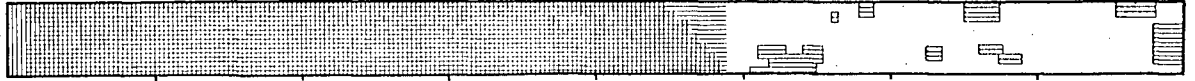
Figure 48

Culture gradient IV. Gradient drawings proving that after continued competition P. pallidum and D. discoideum fruited together. The horizontally shaded areas were occupied by P. pallidum fruiting bodies; the vertically shaded area by D. discoideum fruiting bodies. The areas shaded with horizontal and vertical lines were occupied by fruiting bodies of both species. The unshaded areas were unoccupied. Temperature is marked at intervals under each diagram.

A



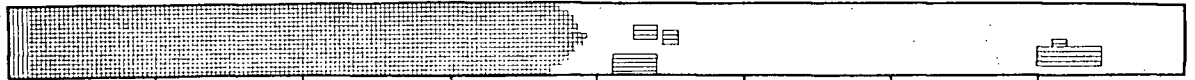
B



C



D



E



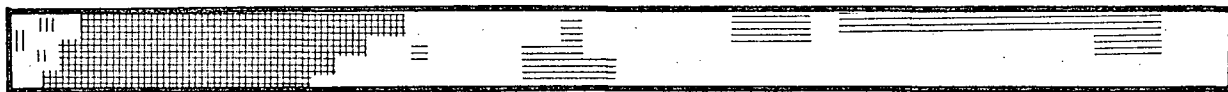
Figure 49

Culture gradient drawings demonstrating that after continued competition P. pallidum could co-fruit with D. discoideum. D. discoideum did not change in response to continued competition.

The horizontally shaded areas were occupied by P. pallidum fruiting bodies. The vertically shaded areas by D. discoideum fruiting bodies. The areas shaded with both vertical and horizontal lines were occupied by fruiting bodies of both species. The unshaded areas were not occupied by any fruiting bodies. Temperature is marked at intervals under each diagram. The sets were made up of the following:

SET #	GRADIENT #	P. PALLIDUM	D. DISCOIDEUM
1	A	grad. 1-K	VC4 stock
1	B	stock	grad. 1-K
2	A	grad. 1-L	DF stock
2	B	stock	grad. 1-L
3	A	grad. 2-K	DF stock
3	B	stock	grad. 2-K
4	A	grad. 2-K	VC4 stock
4	B	stock	grad. 2-L

A



A'



B



B'



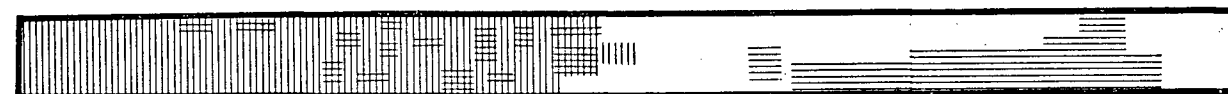
C



C'



D



D'



22

23

24

25

26

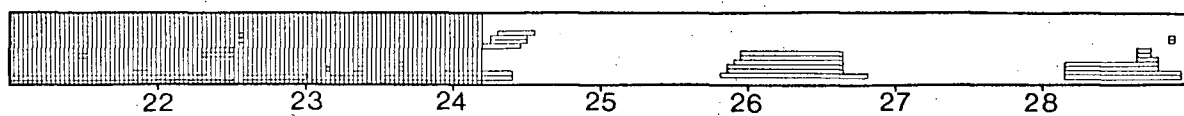
27

28

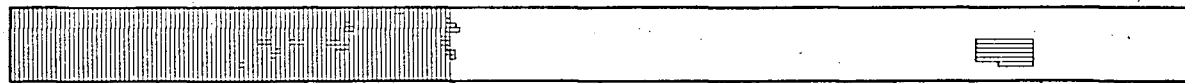
Figure 50

Culture gradient drawings demonstrating that P. pallidum (changed) retained it's co-fruiting ability when grown alone (A, B, C) but lost this ability when grown with P. pallidum (stock). Horizontally shaded areas contain P. pallidum fruiting bodies. Vertically shaded areas D. discoideum. Areas shaded both ways contain both species. Areas unshaded contain neither. Temperature in degrees centigrade is measured along the x-axis.

A



B



C



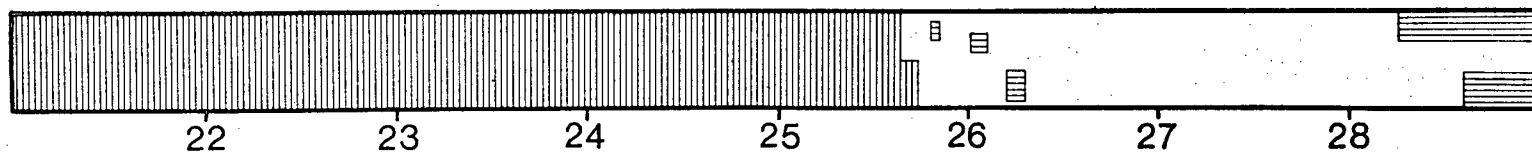
D



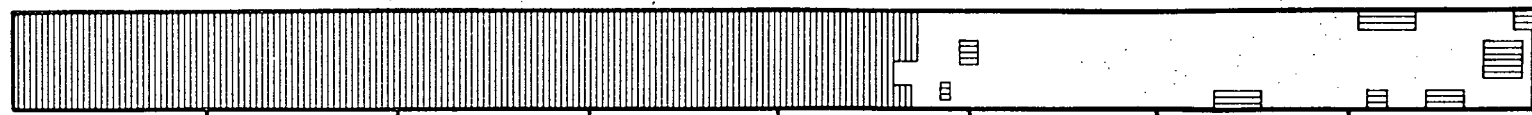
Figure 51

Culture gradient drawings demonstrating that some P. pallidum clones have co-fruiting ability (E) and others do not (A, B, C, D). The cloned spores came from a stock culture. Horizontally shaded areas contain P. pallidum fruiting bodies. Vertically shaded areas D. discoideum VC4. Areas unshaded contain neither. Temperature in degrees centigrade is measured along the x-axis.

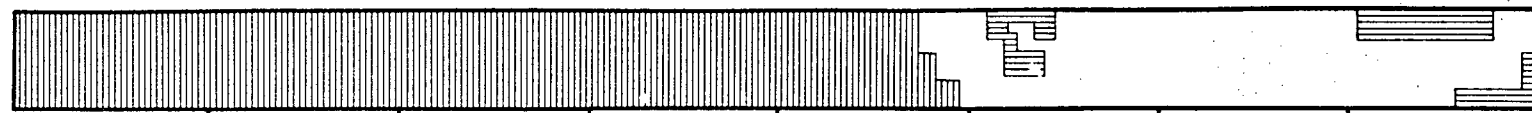
A



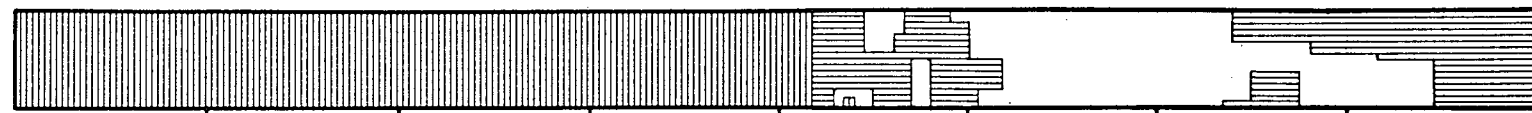
B



C



D



E

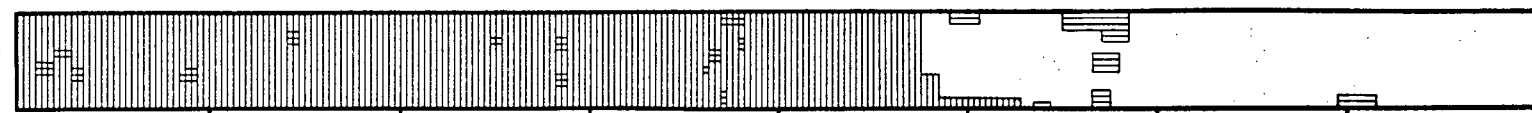
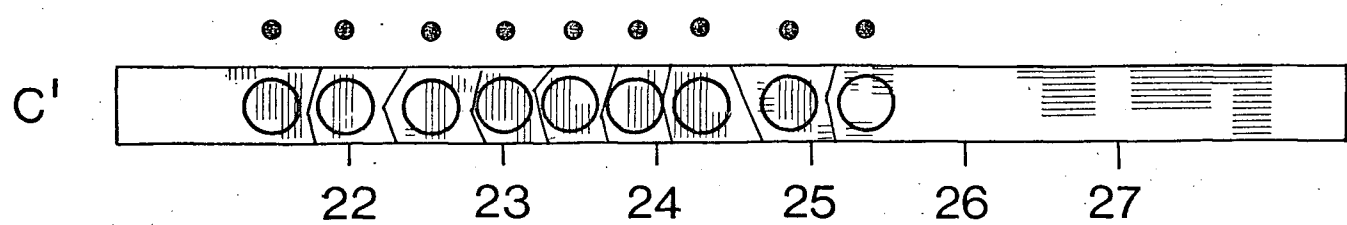
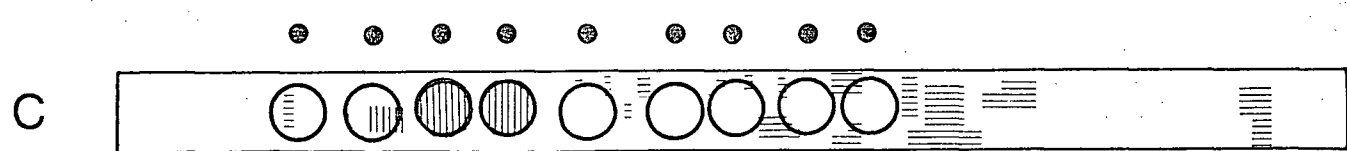
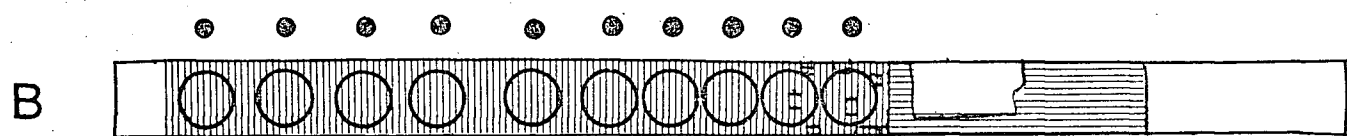
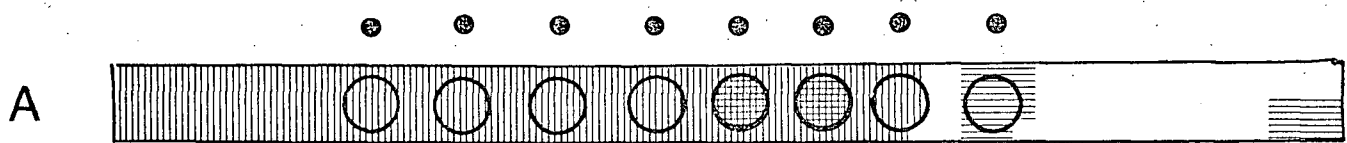


Figure 52

Culture gradient drawings demonstrating that D. discoideum amoebae are produced and grow above 24°C even though fruiting body production stops at about 24 - 25°C. Horizontally shaded areas contain P. pallidum fruiting bodies. Vertically shaded areas D. discoideum V12 fruiting bodies. Areas shaded both ways contain both species. Unshaded areas contain neither species. Temperature in degrees centigrade is measured along the x-axis. The circles along the length of the gradients represent areas in which agar blocks were placed. Stars above the circles indicate the agar blocks that contained D. discoideum vegetative amoebae.



APPENDIX III

GOODNESS OF FIT OF EQUATION (1c)

If amoebae and fruiting body colonies expand at rates which are proportional to the colony circumference then equation (1c) should describe the way in which colonies expand. The applicability of equation (1c) was tested by growing cultures, noting the area covered at various time intervals and plotting the square root of area against time. Both amoeba and fruiting body colonies yielded straight line relationships between the square root of area and time. Example cultures were run and plotted (Fig. 11, 17) in the text, but since one culture can only be measured four or five times during its growth period only four or five data points are available. It might be possible that the straight line relationships resulted from the fact that only a few data points were used to fit each regression line.

To prove that this was not the case, and that straight line relationships do exist between the square root of area and time, the data from several cultures was plotted together. Since each culture that was used was grown at a different temperature, and since data from both P. pallidum and D. discoideum were included; each data point had to be transformed with respect to area and time.

It was arbitrarily decided that the data would be transformed to fit a straight line passing through zero and having a slope of three. This relationship is described by:

$$A_T^{\frac{1}{2}} = 3t \quad (7)$$

where A_T is the transformed area and t is time. The equation for a regression line resulting from any one culture was:

$$A^{1/2} = \frac{a}{b} + b \cdot t \quad (8)$$

where a is the y intercept and b is the slope. To transform equation (8) to equation (7), a/b must be subtracted from the right hand side, and both sides must be divided by $3/b$, producing:

$$\frac{3}{b} \cdot A^{1/2} = 3 \cdot t \quad (9)$$

and substituting equation (7) into (9):

$$A_T^{1/2} = \frac{3}{b} \cdot A^{1/2} \quad (10)$$

The transformed square root of area was plotted against the transformed time ($t - a/b$). Both D. discoideum and P. pallidum amoeba colony expansion data (Fig. 53) and fruiting body colony expansion data (Fig. 54) conformed to the straight line relationship predicted by equation (1c). These data represent final proof that (1c) does describe both amoeba and fruiting body colony expansion and that the straight line relationships presented in the text (Fig. 11, 17) are not the result of a small number of data points.

Figure 53

The square root of amoeba colony area is plotted against time for both D. discoideum (solid dots) and P. pallidum (open circles). All data is transformed to conform to the line described by $A^{1/2} = 3t$, where A is area and t is time.

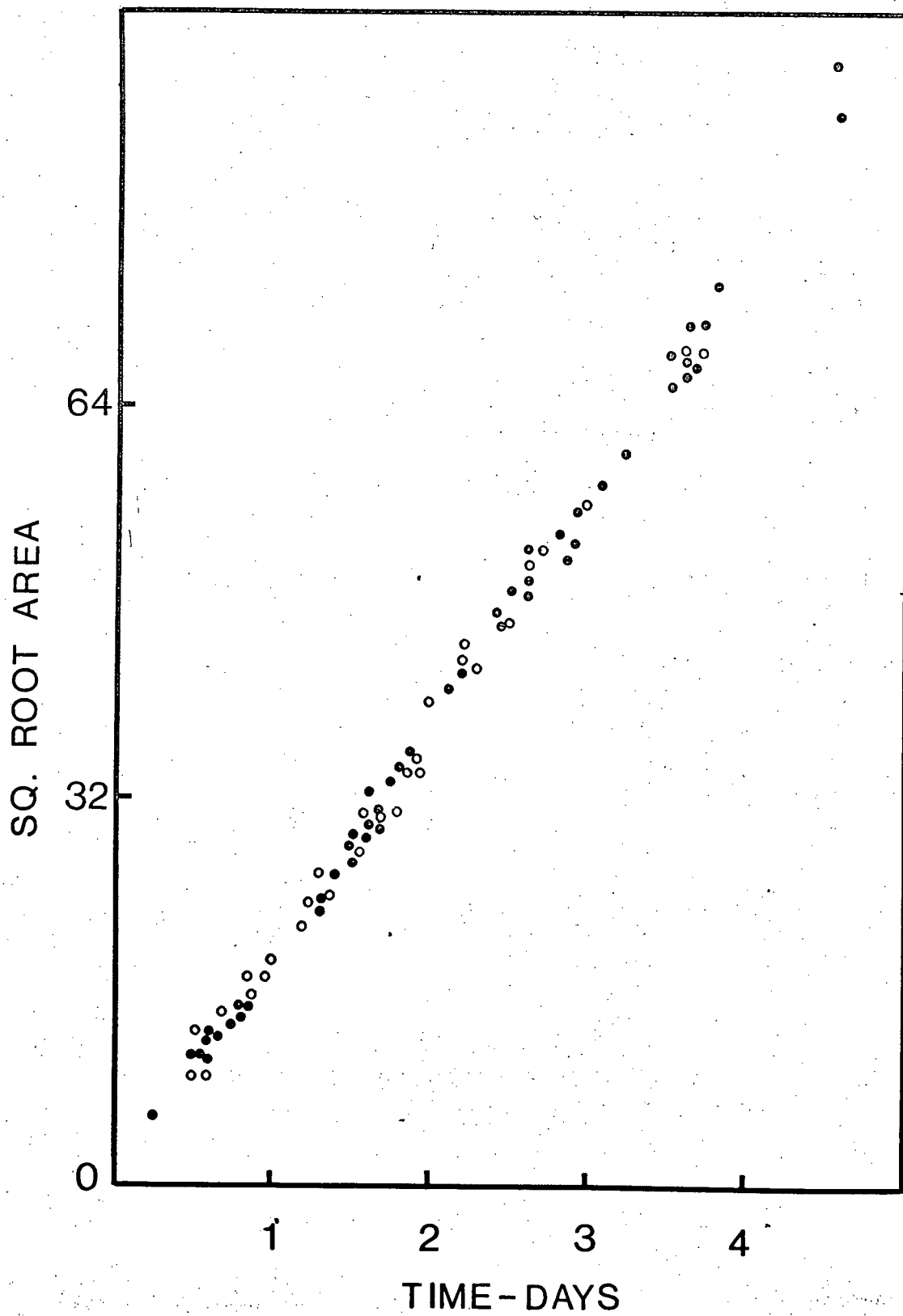
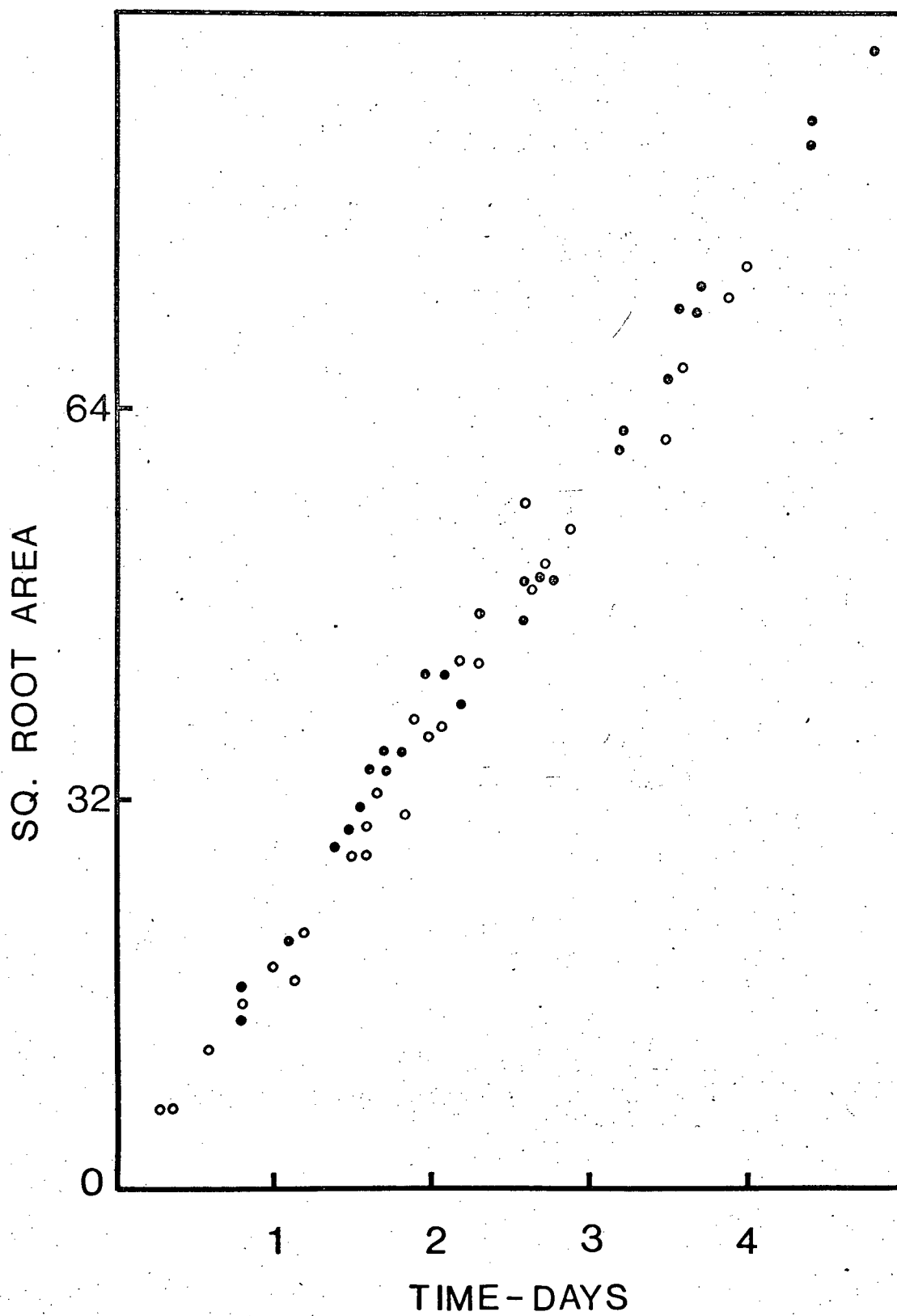


Figure 54

The square root of fruiting body colony area is plotted against time for both D. discoideum (solid dots) and P. pallidum (open circles). All data is transformed to conform to the line described by equation (7).



APPENDIX IV

STATISTICAL SIGNIFICANCE OF EQUATIONS (2b), (3c), AND (4b)

Throughout results section I equations (2b), (3c), and (4b) were fit to the amoeba colony expansion data, the fruiting body expansion data, the spore germination lag data, and the fruiting body lag data. The least of squares method (Appendix I, Programs I, IV, V) was used to ensure that the best line from the family of lines described by (2b) or (3c) or (4b) was used for each set of data. But this procedure did not attach any statistical significance to the descriptive ability of the line chosen.

This second step was achieved by calculating the total deviation around the mean y value, the deviation unaccounted for by the descriptive line, and finally the deviations accounted for by the line. From this the correlation coefficients were calculated (Table XIV). In every case except one the observed data and the fitted line were in agreement at the 95% level of confidence. The one case (Fig. 20) that failed was replaced by Figure 21 in which a special equation (4b) was used to describe the P. pallidum fruiting body expansion data.

TABLE XIV

The proportion of variability accounted for by equations (2b), (3c), and (4b) and the significance of the fit is listed for the figures in which lines were fit to experimental data.

FIGURE NUMBER	r^2 PROPORTION VARIABILITY ACCOUNTED	r CORRELATION COEFFICIENT	df	SIGNIFICANT AT 95% LEVEL
6	0.75	0.86	3/15	Yes
7	0.88	0.93	3/27	Yes
12	0.94	0.96	3/10	Yes
13	0.56	0.75	3/22	Yes
14	0.71	0.84	3/13	Yes
16	0.96	0.98	3/11	Yes
17	0.97	0.98	3/14	Yes
19	0.69	0.81	3/11	Yes
20	0.00	0.00	3/14	No
21	0.75	0.86	3/14	Yes
34	0.76	0.87	3/7	Yes
35	0.84	0.91	3/11	Yes
36	0.94	0.97	3/8	Yes
37	0.87	0.93	3/9	Yes
38	0.67	0.81	3/13	Yes
39	0.91	0.95	3/9	Yes
40	0.93	0.96	3/8	Yes