HATCHING AND SURVIVAL OF EGGS OF *HETERODERA AVENAE* WOLLENWEBER, 1924

by

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DECLARATION

I hereby declare that the work in this thesis has been carried out by myself except where due reference has been made in the text. This dissertation has not previously been submitted in full or part to any University in application for a degree or a diploma.

TABLE OF CONTENTS

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SUMMARY

The feeding mother was necessary for survival of most young eggs and for differentiation to the second larval stage. Fully differentiated eggs survived in water and underwent two essential phases before hatching. Phase 1 began after the first moult, and involved physiological changes which proceeded most rapidly at about 10°C, leading to activation of the larva from the dormant state. It varied considerably in duration between individuals and this variation was thought to be hereditable. Phase 1 development was not influenced by any inherent seasonal rhythm. Higher osmotic pressures retarded its rate but it continued in 1M glycerol. The second phase was of relatively short and uniform duration, involving active eclosion. It had an optimum of about 20°C which was similar for other activities, and was more sensitive to higher osmotic pressures than phase 1. Over the range of temperatures tested, the suggested optima applied irrespective of the temperature regimes used. Both phases proceeded over a similar wide temperature range and so low temperature was not essential for hatch. A minimum period of low temperature for substantial hatching could not be stated because hatching depended on the extent of completion of phase 1 in the field.

Although eclosion is a physical process, some denaturation of the eggshell occurred just before larvae fully "awakened" as indicated by the greater tendency for these larvae to be released from eggs under pressure. Granules appearing in the subventral

pharyngeal glands prior to hatching may be involved.

Eggs can be induced into a state of dormancy (the "plateau phase") depending on the physiological stage of larvae at the time of exposure to a rising temperature. Evidence for this phenomenon in the field is given, and its significance to survival discussed.

Response to temperature by eggs in the field and laboratory were similar; phase 1 development was slowest during the hot summer, but most rapid, once soil temperatures dropped to about 10°C in late autumn and winter. Responses to equivalent osmotic potentials were also similar in the field and laboratory; when soil conditions were dry enough, development of phase 1 was retarded and eclosion inhibited. Under conditions of rapid development and inhibited eclosion a population becomes increasingly hatchable and a "mass hatch" in a short time will follow rain. Such conditions occur periodically in South Australia and result in severe damage to germinating cereal crops.

Hatching responses to temperature by eggs produced under controlled conditions and from the field were similar, but more "hard to hatch" eggs were produced under a controlled environment especially at a low temperature. Length of life-cycle was related to rate of maturation of the host which exerted a greater influence on total numbers of eggs produced than did rate of egg production.

Infective larvae underwent periods of rest and activity, but at low and high temperatures (viz. 5° and 25°C) there was a tendency for motility to be arrested. Resting, immobile larvae

could be stimulated into activity for a short period by disturbance or by a period of low temperature. The significance of this behaviour to hatching, infection and survival is discussed.

Survival mechanisms, the relation of dormancy to diapause and the possible application of results to control under local conditions are discussed.

I. INTRODUCTION

Members of the genus Heterodera, the cyst-forming nematodes, seriously affect many agricultural and horticultural crops, but the host range of the various species are relatively narrow (Franklin, 1951). H.avenae, the oat cyst or cereal cyst nematode, is confined to cereals and certain grasses (Franklin, 1951; Winslow, 1954), and records outside the family Gramineae are considered doubtful (Franklin, 1951). Confusion regarding host status of some plants probably arose because of early confusion in the nomenclature of the nematode. First thought to be a form of H.schachtii, Schmidt 1871, it was later recognised as a sub-species and given the name of either avenae or major, but it was not until 1959 that the name was stabilised as H.avenae Wollenweber, 1924, based on the brief description of the cyst form and knowledge of the host (Franklin, Thorne and Oostenbrink, 1959).

The nematode is widespread in Europe, occurring in Germany, Italy, Sweden, Denmark, Holland, Norway and the British Isles, and has been known to cause substantial damage since 1874. Outside of Europe it has been recorded in India, South Africa, Russia, Japan, Israel, Canada and Australia.

In Australia, the first report of the nematode (Davidson, 1930) indicated its existence as early as 1906 in South Australia and Millikan (1938a, b) gave a detailed description and account of

its life history, though this differs considerably from other accounts (Franklin, 1951). Since these early recordings, the disease has spread alarmingly and now assumes great economic importance in both Victoria and South Australia (Banyer, 1967; Meagher, 1968). It has been recorded recently in New South Wales (McLeod, 1968), and in Western Australia surveys have revealed a wider distribution than previously known; resultant crop losses have created a greater awareness of this parasite as a potentially major economic pest in that State also (Parkin and Goss, 1968).

Because cereals are a low value crop per acre, there are economic limitations to the development of suitable fumigants for control. Instead, efforts have been directed, particularly in recent years, toward the finding of resistance and its incorporation into commercial varieties (Andersen, 1959, 1962; Cotten, 1963), although certain limitations in this approach also exist. For example, resistance amongst commercial cultivars of wheat is more limited than in barley and oats (Andersen, 1961), a conclusion which Millikan (1938b) also reached after testing 200 cultivars of wheat, oats and barley in Australia. He also failed to find complete immunity in any cultivar. Another limitation of resistance as a means of control, could be the development of new aggressive races under intensive systems of monoculture (Cotten, 1964). The main basis for control in Australia is rotation with the inclusion of non-host crops, principally annual legumes, in the cereal programme (Garrett, 1934; Millikan, 1938c; Meagher and Rooney, 1966),

but the recommended rotations are not always compatible with the grower's situation either for economic, environmental or personal reasons. Even when these recommendations are followed accurately, serious crop damage due to H.avenae sometimes arises. Thus, present control measures are unsatisfactory and growers have tried such deviations as early and late sowing, hard, quick grazing of crops before they reach the tillering stage, and the use of nitrogenous fertilisers, in an attempt at better control. Resowing of severely infected wheat or oat crops with barley, which is more tolerant of damage, is also commonly practised in South Australia with some success.

In the Northern Hemisphere, hatching of those Heterodera species which respond to host-root diffusates has been intensively studied and associated with this is a seasonal periodicity in hatching (Shepherd, 1962). Hatching of H.avenae in Europe, does not respond to host-root diffusates, (Winslow, 1955; Hesling, 1957) but proceeds in water and shows a type of seasonal dormancy as hatching either does not occur, or is diminished in the field at certain times of the year (Hesling, 1958; Duggan, 1961). A rise in temperature has been associated with a temporary stimulus to hatch (Winslow, 1955; Hesling, 1957). Cotten (1962) demonstrated that a period of low temperature stimulated hatching of H.avenae and subsequently a minimum period of 8 weeks at or below 7°C was suggested as necessary for substantial hatching to occur (Fushtey and Johnson, 1966). However, the nature of this response is yet

to be determined.

Under Australian conditions, there is a decided lack of knowledge on the general biology and ecology of cereal cyst nematode. In most cereal growing regions of Australia, cysts of H.avenae must survive hot, dry summers when soil temperatures at 6 inches may reach 30°C, and moisture content may be at wilting point for 3 months or longer, before hatching commences in autumn-winter when the crops are sown. Consistent damage to cereal crops indicates that the organism is well adapted to these conditions, but lack of moisture may restrict or check hatching during summer, and, in the surface layers of the soil, temperatures may be high enough to be lethal. The possible existence and relevance of dormancy and its arrest by low temperatures needs examination under our conditions.

The purpose of these investigations was to examine the effect of temperature on the survival and hatch of eggs over the late spring to winter period; to define the conditions causing the onset and arrest of dormancy if present; to elucidate, as far as possible, the underlying mechanisms, and to examine the effect of host roots and host-root diffusates on hatching of the local population. The examination of this part of the life-cycle was chosen with the ultimate aim of basing control measures on factual information.

II. GENERAL METHODS

1. Source of nematodes

As the intention was to obtain cysts from the field for hatching tests, considerable care was exercised in choosing a suitable sampling site. The factors considered important were the ease with which cysts could be isolated and a suitable environment for the multiplication of the nematode to ensure an adequate annual supply of new-season's cysts. For these reasons a sandy situation was preferred, and a typical cereal cyst nematode patch of stunted crop growth was selected on Mr. C.W. Duck's property at Pinery, about 60 miles North of Adelaide.

The rotation used by Mr. Duck since 1952 had been fallow - wheat or barley - pasture. The pasture consisted of volunteer annual medics and naturally occurring grasses, mainly Bromus spp. and Stipa spp. both of which were found to be infested with cereal eelworm cysts.

Pinery sand varies in depth from 2 to 5 feet with some marl occurring between 5 and 6 feet. Soil reaction is alkaline, pH 8.2 at 0 - 6 inches, and pH 8.3 at 6 - 12 inches. Table 1 details the physical characteristics and Figure 1 the soil moisture characteristic. Mean monthly rainfall figures over an 85 year period, and mean monthly maximum and minimum air temperatures, taken over 5 years from the nearest recording centre,

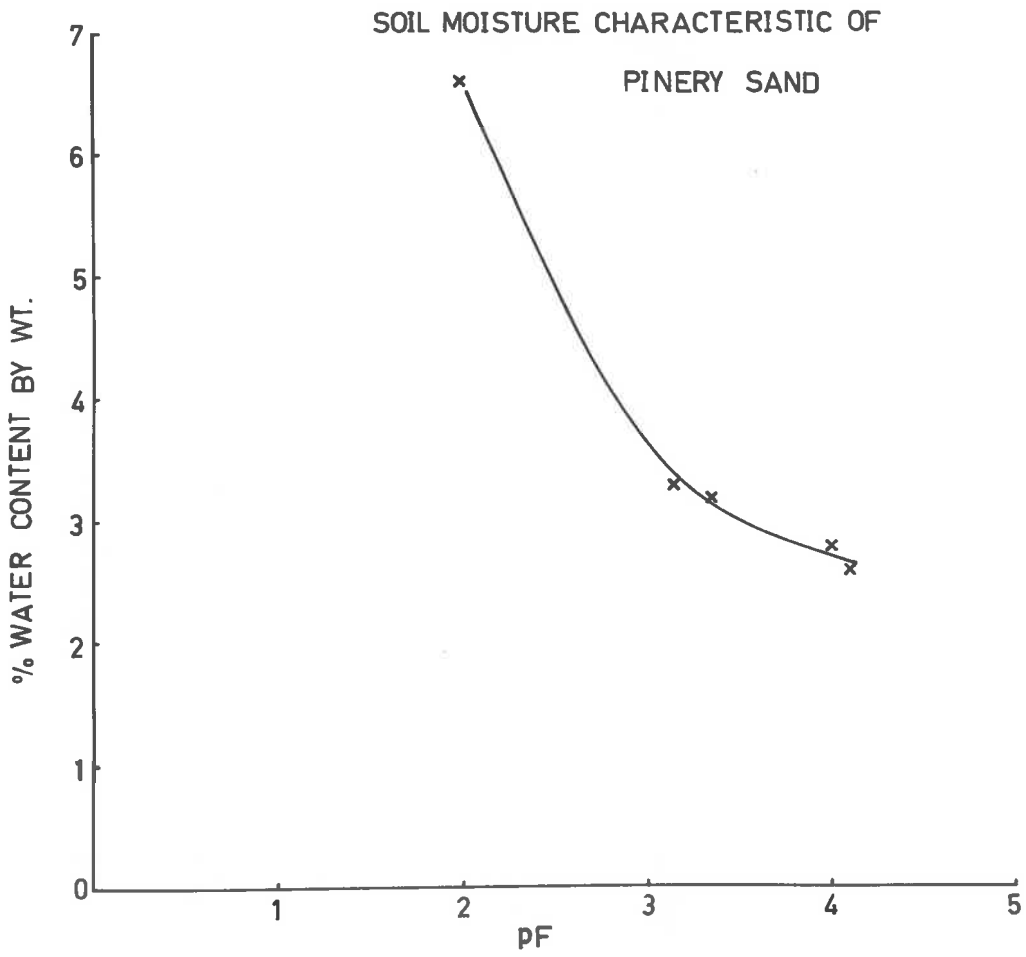
TABLE 1

Physical characteristics of Pinery sand

Particle size distribution - Hydrometer method (Bouyoucos, 1951)

	Depth 0 - 6"	6 - 12"
Sand	93.0%	92.5%
Silt	3.5%	2.0%
Clay	3.5%	5.5%
$\frac{1}{10}$ Atmosphere suction water content (Field Capacity) Pressure plate apparatus		
	6.6%	9.7%
15 Atmosphere suction water content (Wilting point) Pressure membrane apparatus (Richards, 1947)		
	2.6%	3.3%

FIG.1


SOIL MOISTURE CHARACTERISTIC OF
PINERY SAND
% WATER CONTENT BY WT.
0
1
2
3
4
5
6
7
1
2
3
4
5
pF

are shown in Table 2.

The site was evenly infested (Table 3) and the majority of cysts occurred on host roots.

2. Methods of sampling, isolating and storing cysts

It is not always clear whether previous investigators have sampled and used more than a single generation of cysts in various hatching and infection studies. Encysted eggs of H.avenae remain viable for as long as 4 years, and although there is only one new generation per year under South Australian conditions, it is possible to sample as many as four different generations in the field at any one time. Fenwick (1949); Anderseh, K. (1968) and others, emphasise the great variability between cysts in hatching and infection studies. In an attempt to reduce variability, and to maintain a consistent method which would allow more meaningful comparisons of results, only new-season's cysts were used in my experiments. Both Winslow (1955) and Hesling (1956), found that drying adversely affected hatching of cereal cyst nematode. Duggan (1960), suggested that the cysts were less sensitive to dry conditions, but nevertheless, care was taken to keep the cysts wet during separation. Cysts were always sampled from the same eel-worm patch and a cereal crop was sown each year to maintain continuity of supply of new-season's cysts.

TABLE 2

Mean monthly rainfall (points) and

Mean monthly maximum and minimum air temperatures (°C)

	Summer			Autumn			Winter			Spring			
	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Total
Rainfall	82	73	74	70	139	177	183	166	173	159	142	94	1532
Temperature													
Maximum	27.1	30.0	27.0	26.6	22.2	17.2	16.0	13.5	15.4	18.5	22.0	24.9	
Minimum	14.5	16.5	15.6	14.5	12.0	8.8	8.5	6.6	6.2	8.3	10.1	12.2	

TABLE 3

Cyst Recovery From Sampling Site at Pinery

Sample No.	No. of cysts from soil	Soil sample oven dry wt. gms.	No. of cysts from host roots	Host roots oven dry wt. gms.	No. of cysts per gm.dry wt. of host roots	% of total cyst popln. on host roots
1	47	2867	489	0.6	815	91
2	23	3574	514	0.9	570	96
3	63	3405	656	1.6	410	91
4	25	3794	355	0.4	890	93
5	79	3526	583	0.7	835	88
6	65	3874	315	1.2	260	83

A quick, efficient method of collection and isolation was developed as follows:-

As the greatest concentration of cysts on host roots appeared to occur 4 to 6 inches below the surface, samples were collected by digging the host roots along a row of cereal stubble to a depth of from 6 to 8 inches. Samples were taken in a grid fashion from a patch of stunted crop growth of approximately half a square chain and placed separately with adhering sand into airtight plastic bags for transporting. The sand was removed by wet or dry sieving through a 16 or 22 mesh sieve. Host roots were then placed in a 22 mesh sieve above a 60 mesh retaining sieve, and a firm jet of water applied to the roots forcing cysts into the sieve below. If the jet of water was applied correctly, an 80 to 90% recovery could be obtained. The cysts and debris were then separated using an eye dropper with a suitable sized aperture. Cysts remaining free in sand were isolated by first passing the sand through a 16 mesh sieve. The sand was then washed through a funnel, deep into a large cylinder and with an upward flow of water, cysts and lighter debris were floated off direct into a 60 mesh sieve. This method recovered 95% of the cysts.

Mature cysts which were stored for later hatches were collected in summer when soil moisture levels were near the wilting point for plants. They were sampled as previously described, and kept in airtight plastic bags at approximately 20°C. This method

was thought to be the most satisfactory, as even at the wilting point for plants, the relative humidity of air in soil pores is near 100%, and it was thought from Duggan's (1960) results that little or no hatching would occur, yet cysts would retain their viability this way at least for several months.

3. Standard hatching tests

Although eclosion of larvae from the eggshell and larval emergence from the cyst are two different processes, (Shepherd, 1962) in this thesis both are referred to as hatching.

(a) Encysted eggs

Fenwick (1949) concluded that it was imperative to use batches of at least 50 cysts of H.rostochiensis to secure reasonable accuracy in hatching tests, so replicates of usually 50 or 100 were placed in covered glass blocks (Goodey, 1963) containing 2 mls. of glass-distilled water from a 4 gallon reservoir. At each count, larvae were removed and the water changed.

(b) Free eggs.

Eggs from 100 squashed cysts were sub-sampled from a measuring cylinder after first vigorously bubbling through a wide aperture pipette, (Moriarty, 1963). To assist in preparing a clean sample of normal eggs, cysts were always squashed singly by hand. This also permitted any cyst abnormalities to be observed.

Batches of about 200 eggs were pipetted into glass blocks, and fragments of the cyst wall and other debris removed. The exact number of eggs per block was determined at the completion of each experiment. Although alternate methods were available which probably would have resulted in faster hatching rates (Shepherd, 1959; Curtis, 1965), the one described was preferred because of its simplicity and convenience, particularly when large numbers of treatments were required.

III. EXPERIMENTAL

1. SEASONAL CHANGE IN HATCHABILITY OF CYSTS

Introduction

In England, Hesling (1958) found no evidence for a "mass" hatch over a short duration, but larvae in soil emerged over an extended period between March and mid July. He suggested the reason for low emergence during the latter part of summer, was high soil moisture tensions, which Wallace (1956a) also considered responsible for low emergence of larvae of H.schachtii at that time of the year.

In South Australia, a "mass" hatch is often indicated by the mass invasion of young cereal seedlings, but hatching is probably retarded over the summer months when high soil moisture tensions prevail. Here, new-season's cysts may mature on host roots as early as October, but cereal for grain is not usually sown until May to July. Thus, encysted eggs must survive several months before suitable host roots are available. Factors besides high soil moisture tensions are probably involved in retarding hatching during this period, otherwise, substantial losses through untimely hatching could result from odd heavy summer thunderstorms and "false-breaks", when a prolonged dry period follows heavy late-summer or early-autumn rains. This could possibly be achieved by an inherent seasonal rhythm of hatch, or by some further maturation

requirement after the cysts become mature, similar to that suggested by Goffart, (1939).

To investigate these points, cysts were sampled at intervals beginning in January and tested for hatchability.

(i) Change in hatchability of new-season's free and encysted eggs from summer to autumn

Method

Although hatching of free and encysted eggs of either H.schachtii (Steele, 1962) or H.rostochiensis (den Ouden, 1963) did not greatly differ, the cyst wall has been implicated in hatching of H.rostochiensis (Onions, 1955; Kaul, 1962; Ellenby, 1965; Ellenby and Smith, 1967), and so both free and encysted eggs were used in the standard hatch tests.

Soil moisture determinations by means of tensiometers and gypsum blocks, and soil temperatures by means of thermistors were taken at the 6 inch level at about fortnightly intervals throughout the year (Table 4). Because of diurnal fluctuation of temperature, expected to be about 6°C in summer and 3°C in winter at 6 inches, readings were always taken at 10 a.m. Rainfall was also recorded.

To ascertain whether field hatching had occurred, about 2 Kg. of soil from the host-root zone was examined for the presence of free larvae, by mixing with water several times and decanting

TABLE 4

Rainfall, changes in soil moisture tension and soil temperature at the 6 inch level

Date	Points of rain	pF	Soil Temp.°C
Dec. 7th	0	4	23.6
Dec.23rd	0	4	26.5
*Jan.12th	37	4	26.5
*Feb. 9th	11	4	25.6
Feb.22nd	172	2.07	24.4
*March 13th	49	2.46	20
April 6th	16	2.46	20
*April 18th	14	3.3	14
*May 9th	111	2.03	13
May 31st	44	2.12	11.5
June 16th	54	2.11	10.2
June 28th	46	2.04	11.5
July 13th	87	2.03	8.7
Aug. 2nd	159	2.03	8.2
Aug.16th	42	2.22	8.4
Sept. 7th	36	2.90	9.7
Sept.22nd	91	2.8	12.6
Oct. 3rd	91	2.7	12.2
Oct.24th	23	> 4	18.8
Nov.16th	47	> 4	20.4
Dec. 8th	238	2.5	20

* = Time of sampling

into a series of sieves.

Batches of 100 cysts from each of twelve sampling sites were incubated at 20°C for 13 days. Batches of 100 cysts sampled from the same twelve sites were squashed, and four replicates of approximately 200 eggs sub-sampled from each batch, (giving a total of 48 replicates), were also incubated for 13 days at 20°C. Because of the time required for establishing the experiment, it was necessary to store cysts for 1 week prior to the cyst hatch, and for 2 weeks prior to the egg hatch at 7° ± 2°C in an attempt to retard hatching. At the completion of hatching, each of the twelve batches of 100 cysts was squashed and sub-sampled to determine the average egg content per cyst. Abnormal eggs were differentiated from normal by their dark, shrunken contents or indefinite larval shapes, and were confirmed by regular squashing of larvae from both types for the presence or absence of a positive internal hydrostatic pressure.

Results

When the first sample was collected, all cyst walls were brown in colour, and the eggs contained fully differentiated second-stage larvae. In all samples, most cysts contained some abnormal eggs, and about 18% of all cysts contained all or nearly all abnormal eggs or degenerate contents, (Table 5). Usually about 1% of cysts contained vesicles of Endogyne. The percentage of abnormal

TABLE 5

Average egg content per cyst; percentage of abnormal eggs and percentage of cysts with mainly degenerate contents, of each sample.

Mean of 12 replicates

Time of Sampling	Average Egg Content per cyst	% Abnormal eggs	% of cysts with mainly degenerate contents
January	202.2	10.9	22.2
February	267.1	9.5	16.4
March	252.7	9.3	16.5
April	224.8	10.1	21.1
May	189.4	7.8	19.0
	L.S.D. 1% = 33.4	N.S. C.V. = 10%	N.S. C.V. = 5%

N.S. = Not significant

C.V. = Coefficient of variation

eggs and cysts containing mainly degenerate contents, did not significantly increase with time (Table 5).

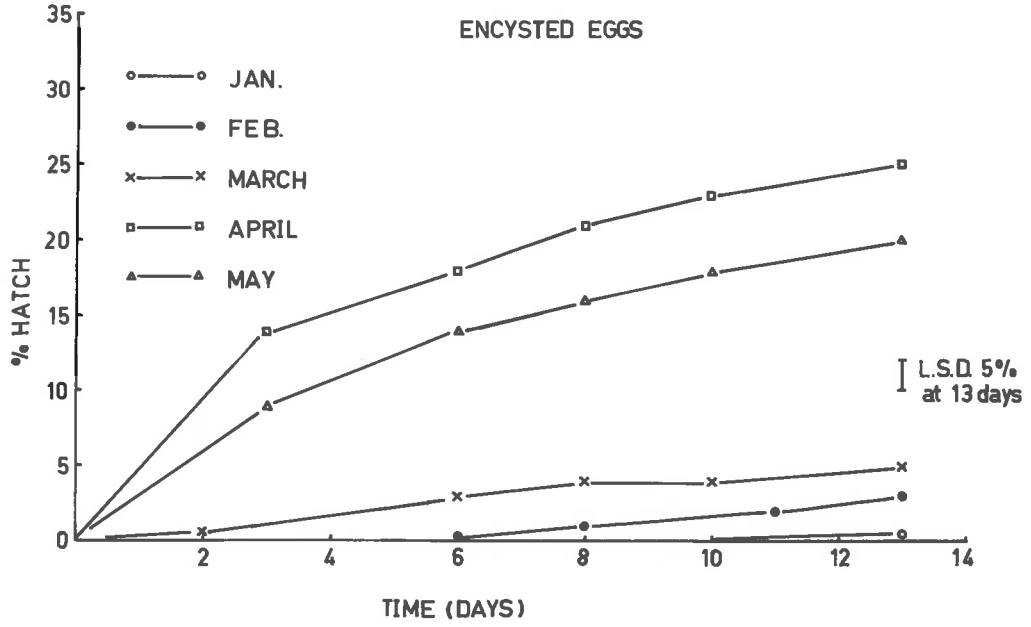
Hatchability of cysts assessed after 13 days increased significantly from less than 1% to 5% from January to March (Fig.2). Hatchability of free eggs was considerably greater than encysted eggs, but did not increase significantly over the same period (Fig.2). During this time, high soil temperatures and soil moisture tensions prevailed, except between February 9th and March 13th when 221 points of rain fell, resulting in a marked decrease in pF (Table 4). No free H.avenae larvae were found in soil sampled in January and February, but some were isolated from soil sampled in March. Little rain fell between March 13th and April 18th so soil moisture tension increased but soil temperature dropped from 20° to 14°C (Table 4). A marked increase in rate of hatching, particularly over the first 6 days, of both free and encysted eggs was recorded at this time. No free larvae, however, were found in soil sampled in April. Rainfall after April 18th marked the break of the season, and the fifth and final sample was taken on May 9th when 111 points were recorded. An abundance of free larvae in the soil at this time indicated a considerable field hatch.

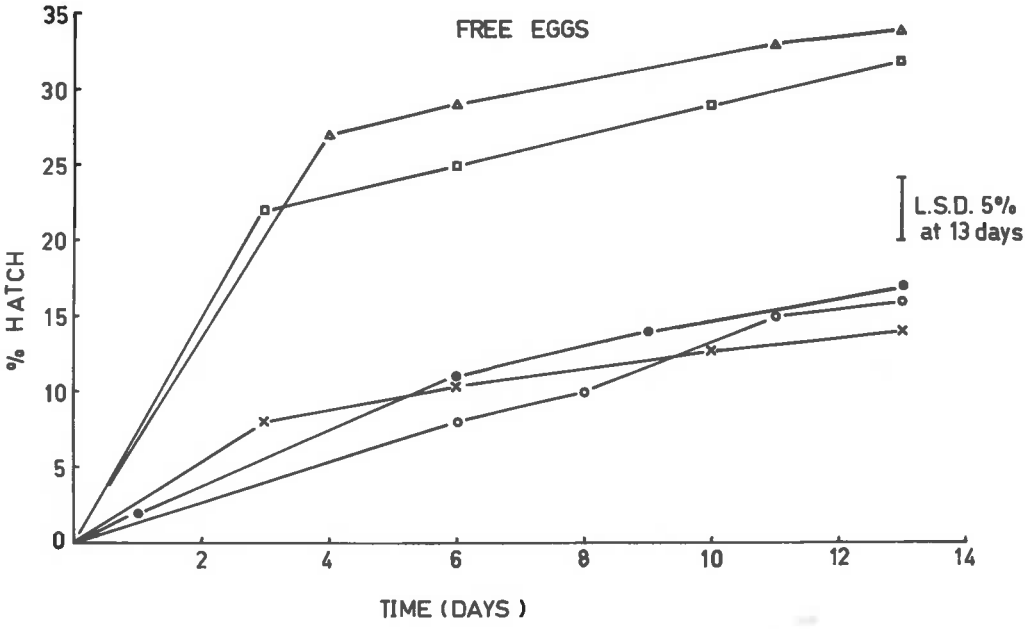
Discussion

The hatching patterns of both free and encysted eggs were similar except free eggs hatched more rapidly. The reasons

FIG. 2

EFFECT OF TIME OF SAMPLING (JAN. TO MAY) ON HATCH OF FREE AND ENCYSTED EGGS AT 20°C.





for this could be attributed to:-

(a) Better oxygen availability and no chemical or physical interference from the cyst wall or its degraded contents.

(b) The possibility that eggs in cysts are so close together that they interact through some inhibitory excretory product.

(c) Cysts from which free eggs were taken, were exposed to cold storage for an extra 7 days, and this may have resulted in some extra stimulus.

Little increase in hatchability occurred while soil moisture tensions and temperatures were high between January and March. Some free larvae were found in the soil in March, but the poor hatching response from cysts sampled then, indicated that field hatching would have been negligible. Thus, although soil moisture tensions reached sufficiently high levels to stop hatching during January to March, more importantly, the encysted eggs did not have the capacity to hatch freely even if moisture became available. Free larvae were not found in dry soil taken from the field in April, indicating that the few larvae which previously emerged failed to survive. Over the relatively short time of four weeks between March and April, despite the dry conditions, hatchability of both free and encysted eggs substantially increased, but the increased hatching response in the laboratory lasted only 3 to 4

days, then declined to a rate similar to that of the earlier samples. Reasons for this increased hatchability were not definitely established, but the most likely factor involved was a drop in soil temperature to 14°C, and wetting and drying of the soil over that period may also have contributed. Because hatching of free and encysted eggs responded similarly, the effect seemed to operate on the egg itself and excluded the cyst wall. Field hatching after April 18th probably accounted for a slight decrease in hatchability of cysts sampled in May.

The number of cysts containing all or nearly all abnormal eggs, and the percentage of abnormal eggs did not increase over the testing period, indicating that encysted eggs were very well adapted to survive the harsh summer conditions.

(2) Change in hatchability of new-season's cysts over the second summer to winter

The change in hatchability of new-season's cysts was investigated again in the second year in an attempt to repeat the same type of response already obtained; to relate the response more specifically to any environmental change, and to continue hatching for a much longer period in order to record any seasonal variation in rate, which might be associated with an inherent seasonal hatch cycle.

Method

The time and tedium involved in isolating 2400 cysts monthly and maintaining these as free as well as encysted egg hatches, made it impossible to extend the incubation period much beyond 13 days. It was decided, therefore, to incubate cysts only, and analysis of results from the previous experiments indicated that six replicates of 50 cysts would give sufficient accuracy.

To enable a strict comparison with the first year's results, batches of cysts were first held at $7^{\circ} \pm 2^{\circ}C$ for 8 days before incubating at $20^{\circ}C$. Since the initiation of these investigations, Fushtey and Johnson, (1966) had reported that a minimum of 8 weeks at 0° or $7^{\circ}C$ was required before substantial hatching of H.avenae could be obtained. Similar batches were therefore incubated for 8 weeks at $7^{\circ} \pm 2^{\circ}C$ before being placed at $20^{\circ}C$, while others were incubated at constant $20^{\circ}C$ without prior cold, for comparison.

In the first sample, the treatment of cysts to constant $20^{\circ}C$ with no prior cold was excluded, and cysts incubated at $7^{\circ}C$ were inadvertently moved to $20^{\circ}C$ after 7, instead of 8 weeks. Cysts sampled in July were incubated at $20^{\circ}C$ only, because they had been exposed to considerable cold in the field, and field hatching had commenced by that time.

Method of sampling and determinations of soil temperature and soil moisture tensions have already been described (pages 10 and 14). The first sample was taken in November and the sixth and final sample in July after the season's opening rains.

Two to three counts per week and weekly water changes were maintained for 293 days when the experiment was terminated. Incubation of cysts sampled in November and July and, those which had received 8 weeks cold initially, however, was continued for a much longer period.

Average egg content per cyst and final percentage hatches were determined at the completion of incubation. Field hatching was determined by the presence of larvae in soil, and was assessed by counting empty eggshells in excess of larvae hatched in the laboratory.

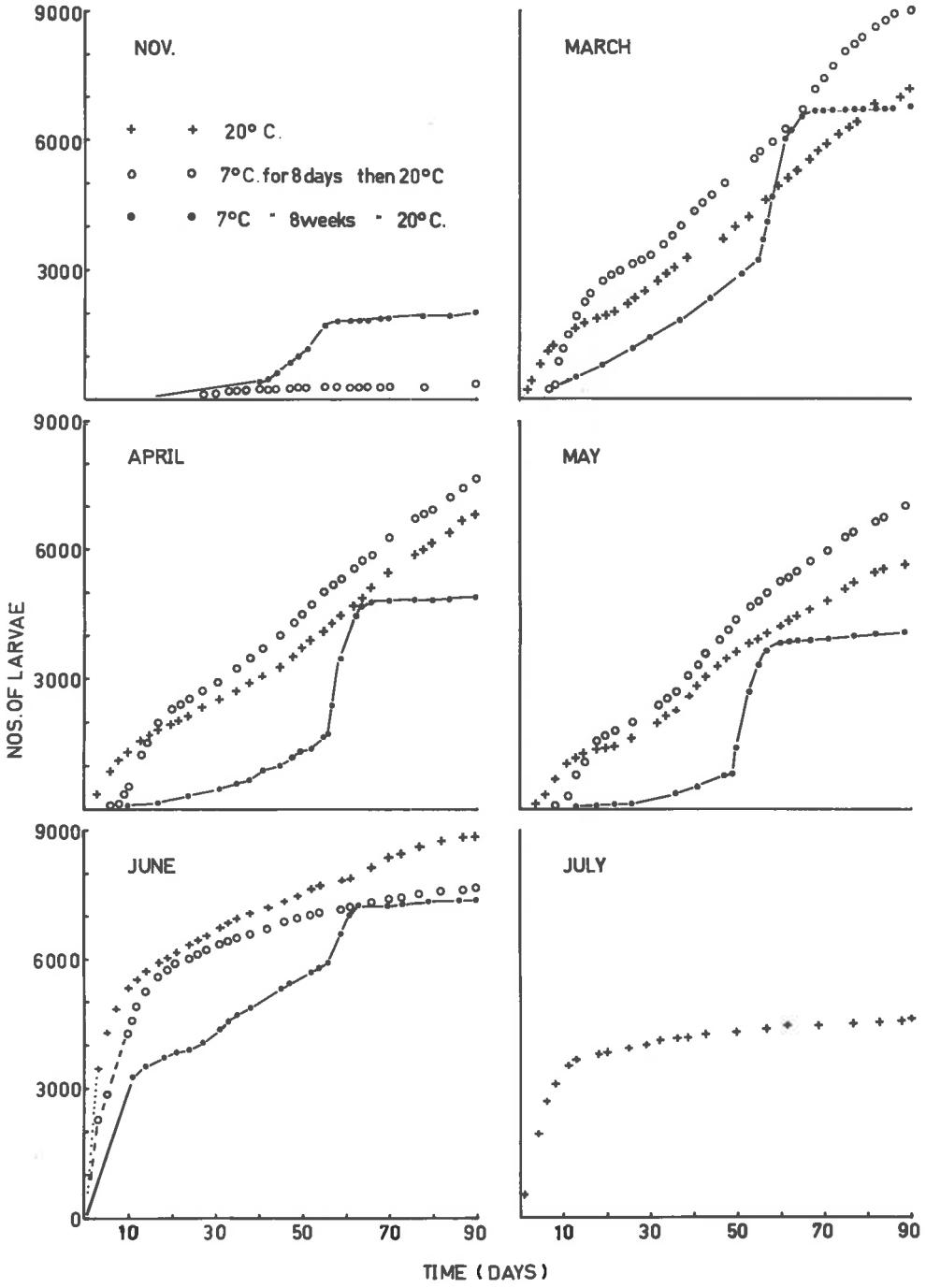
Results

Cysts from all samples were brown and eggs within contained apparently normal second-stage larvae.

Progressive hatching counts up to 90 days only have been presented (Fig. 3), because beyond this time similar hatching trends continued except in treatments where hatching had almost ceased. Further treatment and hatching of cysts exposed initially to 8 weeks cold, and the July sample, after 90 days is dealt with in sub-section(3).

FIG. 3

EFFECT OF TIME OF SAMPLING (NOV. TO JULY) ON HATCHING FROM CYSTS AT DIFFERENT TEMPERATURES



Hatching responses from cysts collected at different times could be divided broadly into four types (Fig. 3).

(a) Initial hatching from cysts sampled in November was negligible at 7°C and 20°C following 8 days cold. After about six weeks, rate of hatching increased at 7°C, and again slightly when cysts were moved to 20°C, but a few days after the temperature change, hatching practically ceased. In an attempt to induce further hatching, cysts from both temperature treatments were exposed to varying periods of cold and warm up to 8 months, during which time hatching proceeded either very slowly or not at all. Eventually, after a total incubation time of 26 months, the highest hatch reached was 60%, and would have been higher with more time as hatching was still continuing.

(b) Hatching from cysts sampled from March to May proceeded immediately at 20°C and was maintained at a steady rate similar to that from cysts which initially received 8 days cold. Cysts sampled at these times also responded similarly to a rise in temperature when moved to 20°C after 8 weeks cold; their rate of hatching increased markedly for about the first 10 days before entering a sharp plateau where hatching practically ceased.

(c) Initial hatching from cysts from the June sample showed

a marked increase in rate at all temperature treatments, but after 10 to 15 days the rates declined.

(d) Hatching from cysts sampled in July proceeded at a similar rapid rate to that from the June sample at 20°C, but after about 10 days, hatching almost ceased. This response was similar to that at 20°C following an 8 weeks cold period. Total hatch had not been reached because when field hatching was taken into account, only 51% of cyst contents had hatched altogether. For the first time, larvae were found in the soil in the July sample.

To test the validity of the initial hatching responses to both temperature and time of sampling, percentage hatch after 15 days was compared statistically (Table 6). Fifteen days was chosen because differences in rates of hatching were most marked at this time. Relatively small but significant differences occurred between the March to May samples at constant 20°C and 7°C, but when sample means were compared, taking all temperature treatments into account, these differences were not significant (Table 6). However, percentage hatch from the June sample was markedly higher than that from earlier samples at all temperature treatments. After 15 days, percentage hatch from the July sample was significantly less ($P = 0.01$) than from the June sample, when compared by a t test, but when field hatching was taken into account it was significantly greater, ($P = 0.05$).

TABLE 6

Effect of temperature on percentage hatch from cysts collected between March and June assessed after 15 days incubation.

Percentage hatch mean of 6 replicates

Temp.	Time of sampling				
	March	April	May	June	Temp. means
20°C	5.74	10.44	10.31	41.07	16.89
8 days at 7° + 7 days at 20°C	11.63	10.13	9.72	39.30	17.70
7°C	2.75	0.86	0.36	25.95	7.48
Sample means	6.71	7.14	6.80	35.44	

L.S.D. 5% Individual means = 2.2

Sample means = 1.3

Temp. means = 1.1

The associated changes in soil temperature and pF at 6 inches over the time of sampling (Table 7), show the very dry soil conditions existing from November to June; soil temperatures remained high from November to May, but then dropped markedly.

Hatching in all treatments was still continuing at 293 days, therefore, total hatch was not reached. Cysts of the March, April, May and June samples, which had not been treated initially to 8 weeks cold, reached a 72 to 83% hatch. The effect of the 8 weeks cold period in retarding subsequent hatch at 20°C was still evident at 293 days, as only a 35 to 56% hatch had been reached by these cysts.

TABLE 7

Changes in soil temperature and soil moisture tension at the 6 inch level over the period of sampling

Sampling time	Soil temp. at 6 inches	pF at 6 inches
Nov. 29th	21	4
March 9th	21	4
April 1st	21	4
May 12th	21	4
June 14th	14	4
July 14th	10	2.1

Discussion

A substantial increase in hatchability of cysts occurred later than in the previous year, but was considerably greater, and again coincided with a significant drop in soil temperature. The initial hatching response from the June and July samples, with no prior cold, was similar to that obtained when cysts were moved from 8 weeks cold to 20°C, which further suggested that the onset of low temperatures in the field was responsible for the increased hatch. High soil moisture levels were not essential for the increased hatchability, because soil moisture tensions in the field during the time of increase were sufficiently high to inhibit hatching. Negligible increase in hatchability occurred between March and May when soil temperatures were near 20°C, but more eggs hatched from the March sample in the laboratory at 20°C during the same time, suggesting that the changes leading to hatching were hastened in the presence of water. Hatching from the March to May samples proceeded at a slow steady rate at constant 20°C, and no marked deviations occurred throughout the 293 days, which suggested that no inherent seasonal hatch cycle was involved. However, this could not be entirely discounted on the basis of these results.

During the warmer months the inability of encysted eggs to hatch freely, even in the presence of adequate moisture, was

probably more important than high soil moisture tensions in controlling hatching in the field. As cysts became increasingly hatchable, however, high soil moisture tensions probably exerted a greater effect on field hatching. This was indicated by the "mass" hatch which was obtained in the laboratory from cysts collected in June (41% hatch at 20°C and 26% hatch at 7°C in 15 days), yet no field hatching at that time had occurred because of high soil moisture tensions. Provided the June sample of cysts responded similarly in the field to that in the laboratory once moisture became available, a "mass" field hatch would have occurred in a relatively short time, probably resulting in a heavy infestation of germinating cereal crops. This was supported by the severe damage to local wheat crops observed in early August.

The poor hatchability of cysts sampled in November did not appear to be due to poor viability, as encysted eggs appeared normal, and eventually one of the treatments yielded a 60% hatch. Initially, the inhibition was overcome sooner at the low temperature, but hatching ceased soon after the cysts were moved to 20°C presumably for the same reason that cysts sampled later, but receiving the same treatment also practically ceased hatching. Reasons for the poor hatching response initially were at the time obscure, but later investigations dealing with hatchability of immature and newly matured cysts offered an explanation (Section 5; page 113).

After November, low temperature was not essential for hatch, but the 8 week cold and not the 8 day cold period increased the rate of hatching markedly when cysts were first moved to the warm temperature. An initial period of 8 days cold was also insufficient to stop hatching after cysts were moved to 20°C, a phenomenon which invariably occurred after cysts had been subjected to 8 weeks of low temperature. A similar response was obtained from the July sample at 20°C with no prior cold, where hatching declined abruptly following a rapid rate for the first 10 days. The prolonged plateau could not be attributed to total hatch because when hatch from the field and laboratory were added, only 51% of cyst contents had hatched altogether. Whether eggs had been damaged or induced into a state of dormancy will be discussed after further investigations in the following sub-section. This phenomenon of cessation of hatching will be referred to throughout the thesis as the plateau phase.

Hatching was still continuing at 293 days when the experiment was terminated, and a total hatch of near 100% might have been attained had hatching been allowed to continue. Cysts which had been induced earlier into a plateau phase may have eventually reached the same total hatch but would have taken much longer, as the influence of this phase was still evident at 293 days. Further observations on the cysts in the plateau phase are discussed in the following sub-section.

(3) Investigation of the plateau phase

Neither constant 20°C nor 7°C were lethal to eggs of H.avenae, thus it seemed unlikely that the change in temperature after 8 weeks at 7°C, to 20°C damaged them, particularly as the plateau phase was not evident when cysts were moved to the warm temperature after 8 days cold. Nevertheless, several cysts in the plateau phase were examined for any abnormalities.

An attempt to induce normal hatching from these cysts was made by:-

(a) Alternating cysts between 7° and 20°C at 2 to 4 weekly intervals

(b) Adding wheat-root diffusate

(c) Subjecting cysts to partial desiccation by storing them in sand at the wilting point for 6 to 8 weeks before incubating in water at constant 20°C

(d) Temperature shock treatment, i.e. alternating cysts between -11° and 25°C at approximately 4 daily and weekly intervals

(e) Releasing eggs from the cyst.

For comparison, three replicates of 50 cysts in the plateau phase from each of the March, April and May samples, and cysts from the July sample were left in water at 20°C for up to 21 months, during which time regular weekly counts and water changes

were maintained.

Results

Cysts contained mostly normal eggs, several of which were squashed, demonstrating by their positive internal pressure that the larvae were alive.

The temperature shock treatment inhibited hatch completely, probably as a result of freezing damage. Other treatments failed to stimulate hatch, which continued at the very slow rate. However, hatching from cysts left at constant 20°C resumed at a faster rate about 4 months after they had been moved from the initial 8 weeks cold period. When the experiment was stopped, a 64% hatch had been obtained from the "plateau" cysts sampled in March, i.e. those which had received the longest incubation time of 21 months at constant 20°C.

Total hatch was not obtained as hatching was still continuing at the completion of the experiment.

Discussion

The phenomenon described as the plateau phase when hatching almost ceased after an initial response to an increase in temperature following 8 weeks cold, was not due to any apparent damage to eggs. Although a number of attempts to restart hatching

failed, the eventual resumption at a more normal rate, and the relatively high percentage hatch finally achieved, indicated that eggs had not lost their viability, but instead had undergone some change, whereupon their subsequent development or changes leading to hatching had been greatly retarded or stopped. Given sufficient time, the same final hatch as that reached by cysts which had not entered the plateau phase at constant 20°C (page 26) might have been obtained.

Cysts sampled in July and incubated immediately at 20°C responded in a similar manner to those in which the plateau phase had been induced artificially, suggesting the extra cold to which they would have been exposed in the field was sufficient to induce the plateau phase when incubated at 20°C in the laboratory. This suggested that the phenomenon might possibly occur under field conditions, and the following investigation was undertaken to gain some evidence for this.

(4) Evidence for the existence of the plateau phase in the field

Cysts in the field are subjected to warmer temperatures in spring following exposure to predominantly low temperatures in winter (Table 4), so it seemed that the phenomenon known as the plateau phase may occur in the field at this time.

Now-season's cysts were therefore sampled from the field in October when soil moisture tension at 6 inches was measured at pF 3.8, and their hatchability tested. The sample was mixed thoroughly and a sub-sample of six replicates of 50 cysts was isolated and incubated at constant 20°C in the usual way. The remainder of the sample was stored in soil in an airtight plastic bag at 22°C for 4 months, when a second sub-sample was isolated and incubated at 20°C in February. Cysts were also sampled from the field at the same time and similarly incubated. A time interval of 4 months was chosen as it took about this long for hatching from "plateau"cysts in the previous experiment to resume at a more normal rate.

Hatching from cysts incubated in February was stopped after 130 days, but those incubated in October were hatched until the same percentage hatch was attained.

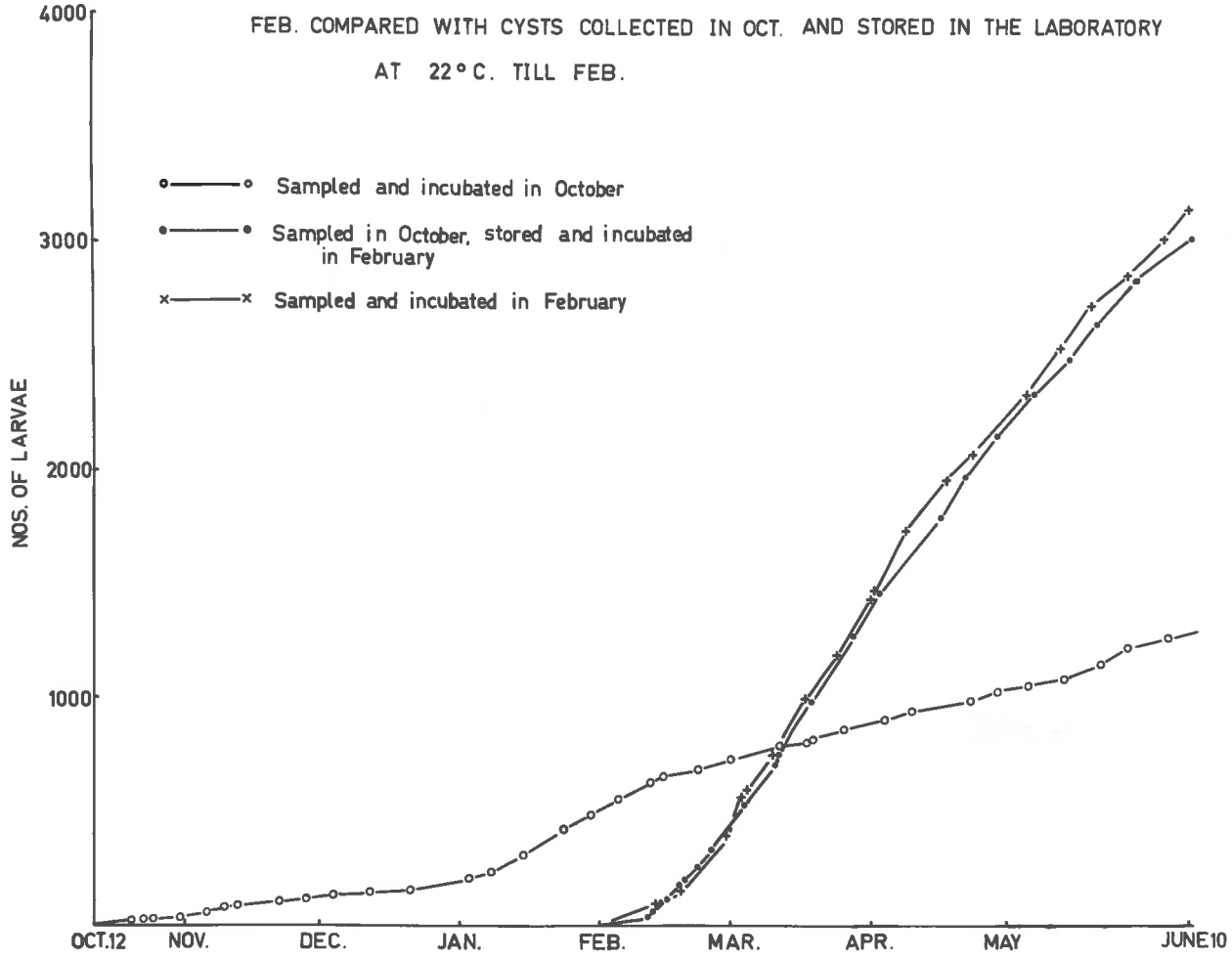
Field hatching was calculated by determining the percentage of empty eggshells in excess of the numbers hatched in incubation.

Results

Hatching from cysts sampled and incubated in October was negligible over the first 90 days (Fig. 4). Hatch from cysts of the same batch stored in soil or sampled from the field 4 months later, was not significantly different, but was considerably greater

FIG. 4

DIFFERENCE IN HATCHABILITY OF CYSTS COLLECTED FROM THE FIELD IN OCT. AND FEB. COMPARED WITH CYSTS COLLECTED IN OCT. AND STORED IN THE LABORATORY AT 22°C. TILL FEB.



than that from cysts incubated earlier (P=0.01), (Fig. 4). The same final percentage hatch of 50% was eventually obtained from cysts incubated in October, but it took 9 months longer.

In the field, 41% of eggs had hatched from the opening rains in winter to mid-spring (October), but no further field hatching was recorded over the following 4 months to February.

Discussion

Although only 41% of encysted eggs had hatched in the field by the time they were sampled in October, little further hatching in the laboratory at 20°C was obtained for about 90 days, and none was detected in the field over the next 4 months. In previous experiments when cysts of the same generation were collected prior to the onset of low temperatures, hatching at constant 20°C continued at a steady rate, until a final hatch of about 70 to 80% was obtained. This indicated that by October, unhatched eggs in the field had undergone some change which was responsible for the delayed hatching response to favourable conditions.

This inhibition was partly overcome by February, 4 months later, when hatching of cysts, either from the field or from cysts stored under simulated summer conditions, proceeded at a much faster rate. It took longer for the inhibition to be partly overcome in water than in field dry soil, which suggested

that partial desiccation or some soil factor may have hastened the processes involved.

The similarity between this result and those previously described for the onset and partial arrest of the plateau phase (page 31) suggested that the same mechanism was involved, and that exposure of cysts in the field to warm spring temperatures following predominantly low temperatures in winter, was probably responsible for inducing the plateau phase.

Hatchability of cysts greatly increased by early winter (Fig. 3), so that if hatching then proceeded uninhibited in the field whenever moisture was available, as it did in the laboratory at a constant temperature, 80% or more of encysted eggs could be expected to hatch over a period of 12 months. Very few individuals would therefore be left to perpetuate the species in the absence of a suitable host in some years. Thus the significance of such a mechanism controlling hatch, particularly in spring when hosts are rapidly maturing, is clear, as it would greatly enhance survival of the organism by allowing a greater carry over of individuals from one year to the next.

(5) Inherent seasonal hatch cycle

Since 1930, there have been conflicting results regarding the origin of dormancy in Heterodera and in particular the potato-root eelworm H.rostochiensis (Lownsbery, 1951; Fenwick and Reid,

1953; Ellenby, 1955; Winslow, 1956; Cunningham, 1960). Winslow (1956) found that winter dormancy varied in degrees with eelworm species, being slight in the case of beet-eelworm and more pronounced in the case of potato-root eelworm. He concluded that the dormancy was not wholly dependent on immediate environmental conditions but was possibly induced by previous conditions. More recently, evidence for the presence of an inherent seasonal hatch cycle in H.rostochiensis has been reported (Stelter and Meinl, 1962) but Shepherd and Cox, (1967) have interpreted the periodicity of hatching of the same species in terms of facultative diapause.

Previous results (page 27) indicated that no inherent seasonal dormancy existed in eggs of H.avenae, but further confirmation was sought by testing the hatchability of cysts immediately they were collected from the field in early autumn before the onset of low temperatures, and again after storage of this sample in soil until early winter. In early winter, cysts were taken from the field after the onset of low temperatures, but before hatching had commenced, and their hatchability tested for comparison. The early-autumn sample which was stored until early winter, was kept in field dry soil (pF 4) in an airtight plastic bag at 22°C. Six replicates of 50 cysts were isolated and incubated at constant 20°C in the usual way, and hatchability was assessed by the extent of hatching after 14 days.

Results

Soil was near the wilting point for plants (pF 4) when sampled in early winter, reflecting the dry conditions which prevailed since early autumn. Thus, temperature was probably the main environmental difference imposed on cysts stored in the laboratory, compared with those in the field.

Hatchability of cysts in the field increased from 6% to 35% ($P = 0.01$) over the early-autumn to early-winter period. If all or part of this increase was due to any inbuilt seasonal rhythm, then some increase in hatchability of cysts which were stored in the laboratory would be expected over the same period. However, no significant increase in hatchability of stored cysts occurred, indicating that changing environmental conditions (probably low temperatures) were mainly responsible for the increased hatchability of cysts in the field, rather than due to any inherent seasonal rhythm.

2. EFFECT OF TEMPERATURE ON HATCH

(1) Effect of temperature on encysted eggs in soil

Low temperatures have been associated with increasing hatchability of new-season's cysts of H.avenae (page 27) but thus far, there was no direct evidence. This was demonstrated by sampling new-season's cysts from Pinery in summer, and subjecting sub-samples to a number of temperature treatments.

The sample, containing cysts on host roots in soil, was mixed thoroughly and divided into four equal parts for storage in airtight plastic bags at constant 7°, 20°, 30°C and a temperature fluctuating between 7° and 20°C every 2 days. After 4 weeks storage, 100 current-season's cysts were isolated from each treatment and squashed. Replicates of approximately 200 free eggs were then sub-sampled from each of the four lots of squashed cysts and incubated at 15°C in the usual way.

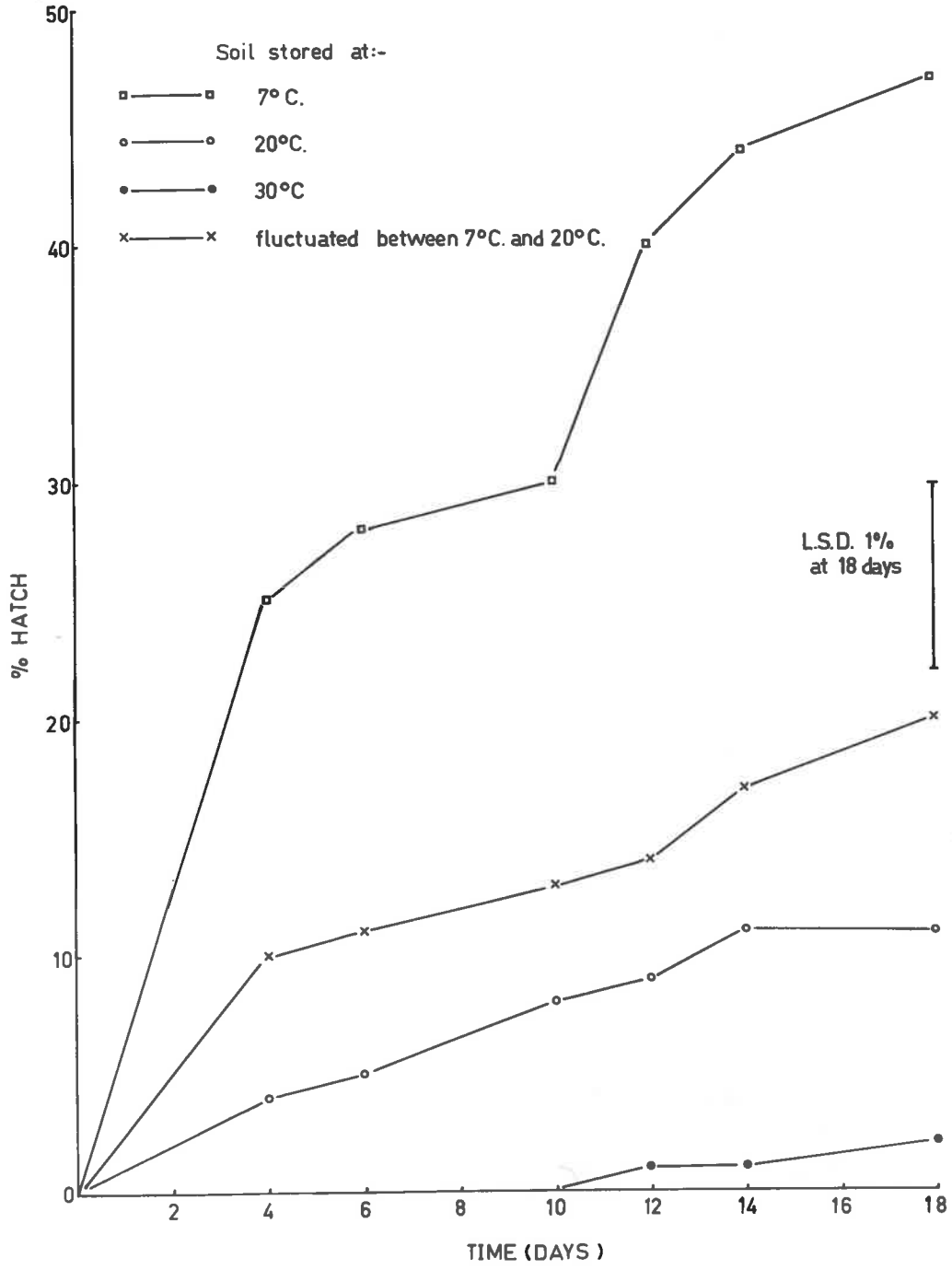
Hatching was assessed after 18 days.

Results

Eggs prestored at 7°C yielded the greatest hatch (P = 0.01) (Fig. 5). Hatching then decreased in the following order of pre-storage temperatures, 7° to 20°C fluctuating, constant 20°C, constant 30°C (P = 0.01).

FIG. 5

EFFECT OF STORING CYSTS IN SOIL AT DIFFERENT TEMPERATURES ON SUBSEQUENT HATCH OF FREE EGGS AT 15°C.



After the initial response to incubation at 15°C, hatching rate of eggs prestored at the low temperature decreased sharply for about 6 days before increasing again (Fig. 5). This check in hatching may have been due to some influence of the plateau phase.

The increased hatch following prestorage at the fluctuating temperature, compared with that following prestorage at constant 20°C, may have been due to either the fluctuation, or the reaction of eggs to the periods of low temperature only, or both.

This and other aspects of the hatching response to temperature, are investigated more fully in the following experiments.

(2) Optimum temperature for hatching of free eggs

Introduction

Optimum constant temperature for hatch of most other Heterodera species is somewhat higher than that reported for H.avenae, for example, 25°C for H.schachtii (Wallace, 1955), 25°C for H.rostochiensis (Fenwick, 1951), but 20°C for H.avenae (Winslow, 1955).

Fushtey and Johnson (1966) reported that a specific optimum temperature for H.avenae was difficult to establish as both initial rates over a short period, and final hatch over a prolonged

period, had to be considered. They concluded, however, that the optimum temperature lay in the vicinity of 15°C.

A minimum period of exposure to low temperature was not necessary for a substantial hatch of the local population, but increased hatchability of cysts was associated with the onset of low soil temperatures in the field (page 27), and hatching of eggs was more rapid following prestorage as encysted eggs in soil at 7°C than higher temperatures.

The object of the following studies was to relate more precisely the effects of temperature on hatch of the local population, and to determine the optimum temperature involved.

Method

In order to decrease or at least maintain the same variation without using large numbers of cysts, free eggs were used as they responded similarly to temperature, and differences in hatching could be detected using relatively small numbers. This was probably because the method of sub-sampling eggs was more accurate than that for cysts.

In the first experiment, eggs from 100 new-season's cysts collected in December were bulked and sub-sampled in the usual way to give about 200 eggs per glass block. Four replicates were incubated at 5°, 10°, 15° and 20°C for 8 weeks, while others were

kept four weeks at these temperatures then moved to 15°C for the remaining four weeks for further hatching.

Results

Hatching occurred during the whole period at all temperatures, but the overall hatch at 15°C following incubation at 10°C was greater than in any other treatment (Fig. 6). When constant temperatures were compared after 8 weeks incubation, hatching at 15°C was greater than at any other temperature (Fig.6).

In the second experiment, similar batches of eggs were incubated at 5°C for 7 weeks and were then moved to 5°C, 10°C, 15°C, 20°C and 25°C for a further week when hatching was assessed. Initial incubation at 5°C was adopted rather than 10°C because fewer larvae hatched at 5°C in the previous experiment.

Results

Rate of hatching increased when eggs were moved from 5°C to the higher temperatures, but the rate was greatest at 20°C (Figs. 7 and 7A). However, the plateau phase was reached by at least 3 days at 25°C and after 7 days at 20°C (Fig. 7A).

Discussion

Results of the two experiments indicated that hatching of eggs of H.avenae could be divided into two phases with widely

FIG. 6

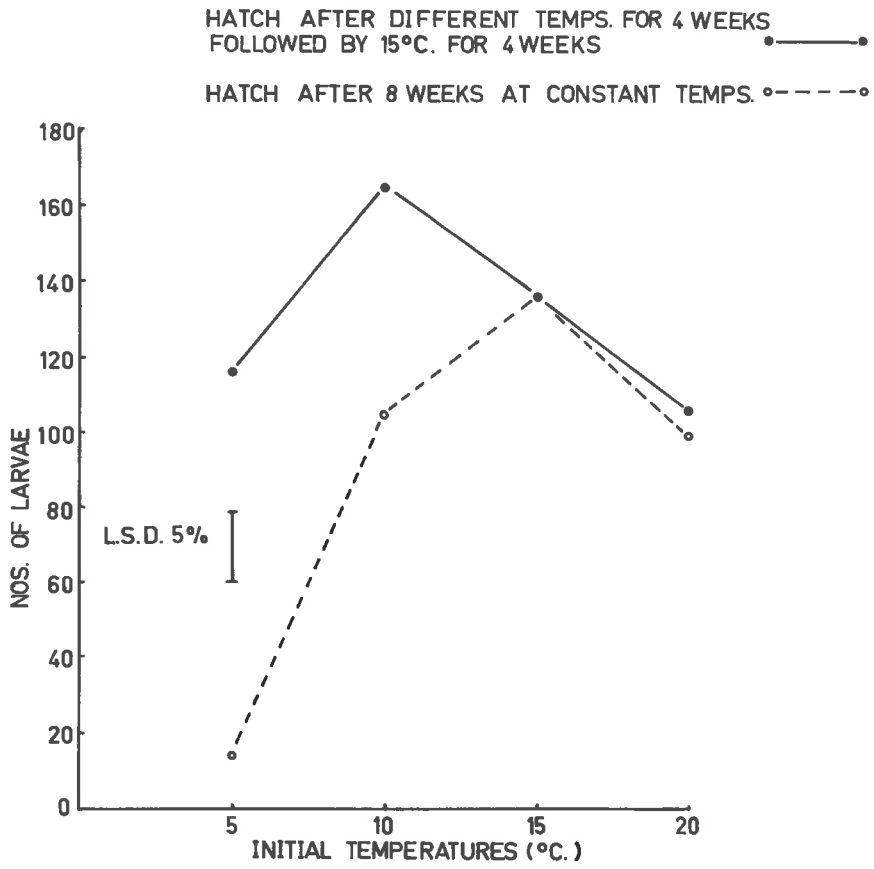

HATCH AFTER DIFFERENT TEMPS. FOR 4 WEEKS FOLLOWED BY 15°C. FOR 4 WEEKS
HATCH AFTER 8 WEEKS AT CONSTANT TEMPS.
180
160
140
120
100
80
60
40
20
0
NOS. OF LARVAE
L.S.D. 5%
5
10
15
20
INITIAL TEMPERATURES (°C.)

FIG. 7

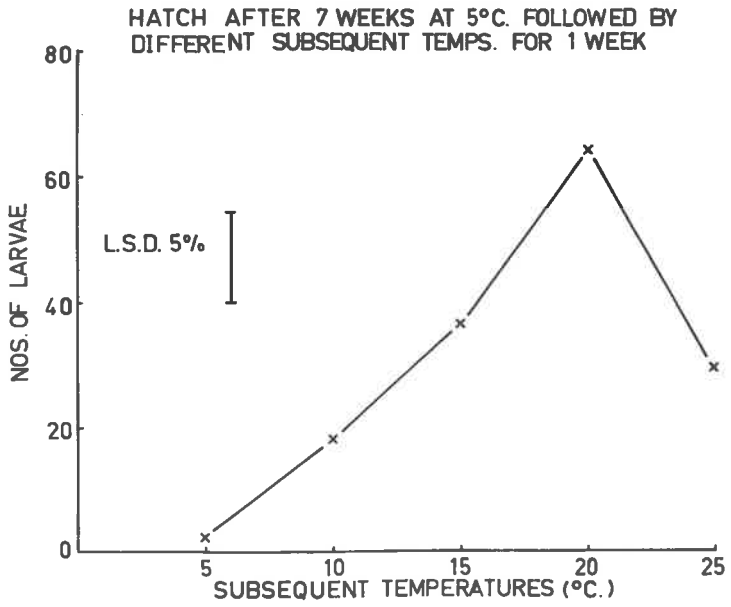
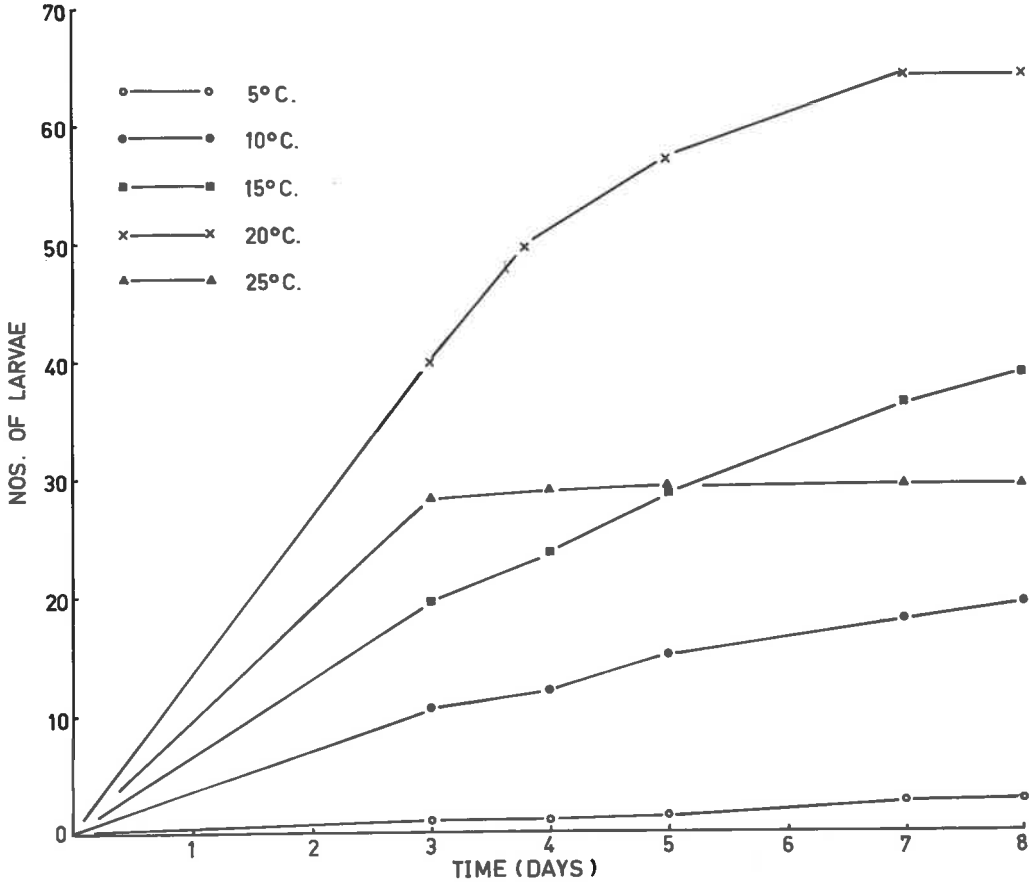

HATCH AFTER 7 WEEKS AT 5°C. FOLLOWED BY DIFFERENT SUBSEQUENT TEMPS. FOR 1 WEEK
80
60
40
20
0
NOS. OF LARVAE
L.S.D. 5%
5
10
15
20
25
SUBSEQUENT TEMPERATURES (°C.)

FIG. 7A

HATCHING AT DIFFERENT TEMPERATURES

FOLLOWING 5°C. FOR 7 WEEKS



separated optimum temperatures. The first phase (phase 1) proceeded best at a low temperature with an apparent optimum of about 10°C, while the second phase (phase 2) of shorter duration, had an apparent optimum of about 20°C. Thus, ideal conditions for hatch involved a changing temperature and not a constant temperature, but 15°C was the best constant temperature for hatch, because it was between the two suggested optima for both processes. This is probably why Fushtey and Johnson (1966) found 15°C near optimum for overall hatching. Hatching at constant temperatures was the resultant of the two different rates of the two phases. Hence it was necessary to determine the optimum temperature for phase 2 with least interference from phase 1 by comparing hatch soon after the 5°C cold period.

As both phases occurred over a similar wide temperature range, low temperature was not essential for hatch, but hatching proceeded more rapidly if the eggs were incubated first at a low temperature. These results differed to those described by Fushtey and Johnson (1966), who found a minimum period of low temperature essential for substantial hatching.

Hatching in water in blocks throughout the entire incubation period in preference to Fushtey and Johnson's method of pre-storage in wet soil, ensured that all eggs which hatched were recorded. With this method the best treatment yielded more than

an 80% hatch in 8 weeks, which is the fastest rate of hatching of H.avenae so far recorded in the literature.

The induction of the plateau phase by a temperature change in the second experiment was the first indication that this phase may be influenced by the size of the temperature change. The plateau phase which occurred when cysts were moved from 0°C after 12 weeks storage to 20°C (Fushtey and Johnson, 1966) was probably temperature-induced rather than due to freezing damage, as they suggested.

Presumably, if it was not for the induction of the plateau phase, rate of hatching of those eggs which had completed phase 1 would increase with increasing temperature, until a higher apparent optimum was reached, beyond which, temperatures may begin to have lethal effects. Mountford (1966) for example, suggested the reason why there is a limit to the hatching response to increasing temperature by insect eggs, is because beyond the apparent optimum, eggs metabolising the fastest (i.e. those nearest to hatching) are killed, and so the eggs remaining require longer to hatch. This results in an apparent decrease in rate of hatching at higher temperatures.

(3) Effect of duration of low temperature on subsequent hatch at a higher temperature

Previous results indicated that the size of the hatching

response to a rise in temperature may be dependent on duration of incubation at the low temperature (page 39). Two aspects needed further study:- (i) The relationship between duration of low temperature and subsequent hatch at a higher temperature, and (ii) the nature of the continuous hatching curves at the suggested optimum temperatures for both phases.

Method

Eggs were sub-sampled from 100 current-season's cysts collected from the field in January and again in March. Five replicates of about 200 eggs were incubated initially at 10°C for 1, 2, 4, 7 and 14 weeks, then moved to 20°C until hatching almost ceased, while others were hatched throughout at constant 10°C and 20°C. To determine the effects on phase 1 to the exclusion of phase 2, eclosion at the low temperature was stopped, using 0.4M NaCl which was known to inhibit hatching in other Heterodera species (Dropkin, Martin and Johnson, 1958; Wallace, 1956). Batches of eggs from the same cysts were similarly sub-sampled and incubated at constant 10°C in 0.4M NaCl for 1, 2, 4, 7 and 14 weeks. After immersion in NaCl, batches of eggs were rinsed twice, before moving to 20°C in distilled water. Regular weekly counts and liquid changes were maintained at 10°C and more frequent hatching counts made at 20°C, except when hatching was nearing completion.

Results

Similar responses to the various treatments were obtained with both the January and March collection of cysts (Figs. 8 and 9). Thus, description of results is based mainly on the January sample.

Incubation in water

Hatching was extended and slightly irregular at constant 20°C (Figs. 8 and 9), and was still continuing after 9 months; at constant 10°C, it was initially slower, but was sigmoidal with the maximum rate occurring between 8 and 9 weeks (Figs. 8, 9 and 10), and practically ceased after only 17 weeks (i.e. "total hatch").

In response to a rise in temperature, rate of hatching always increased for about the first week, then declined to approximately the same rate as that at constant 20°C except following 7 and 14 weeks cold when a sharp plateau was reached (Figs. 8 and 9). These responses increased with increasing time at the low temperature, except following 14 weeks cold (Table 8) when "total" hatch had almost been attained (Figs. 8 and 9).

Hatch at 10°C, plus each of the respective responses to 20°C after 1 week, were nearly always greater than that at constant 20°C over the same times, (Table 9).

FIG. 8

HATCHING OF EGGS IN WATER AT 20°C. AFTER DIFFERENT TIMES AT 10°C. IN WATER OR 0.4 M NaCl

A Incubation in water at 10°C.

B Incubation in 0.4M NaCl at 10°C.

—·—·— Constant 10°C. in water

———— " 20°C. " "

▲·····▲ 1 week at 10°C. then 20°C.

+·····+ 2 " " " " "

■·····■ 4 " " " " "

●·····● 7 " " " " "

○·····○ 14 " " " " "

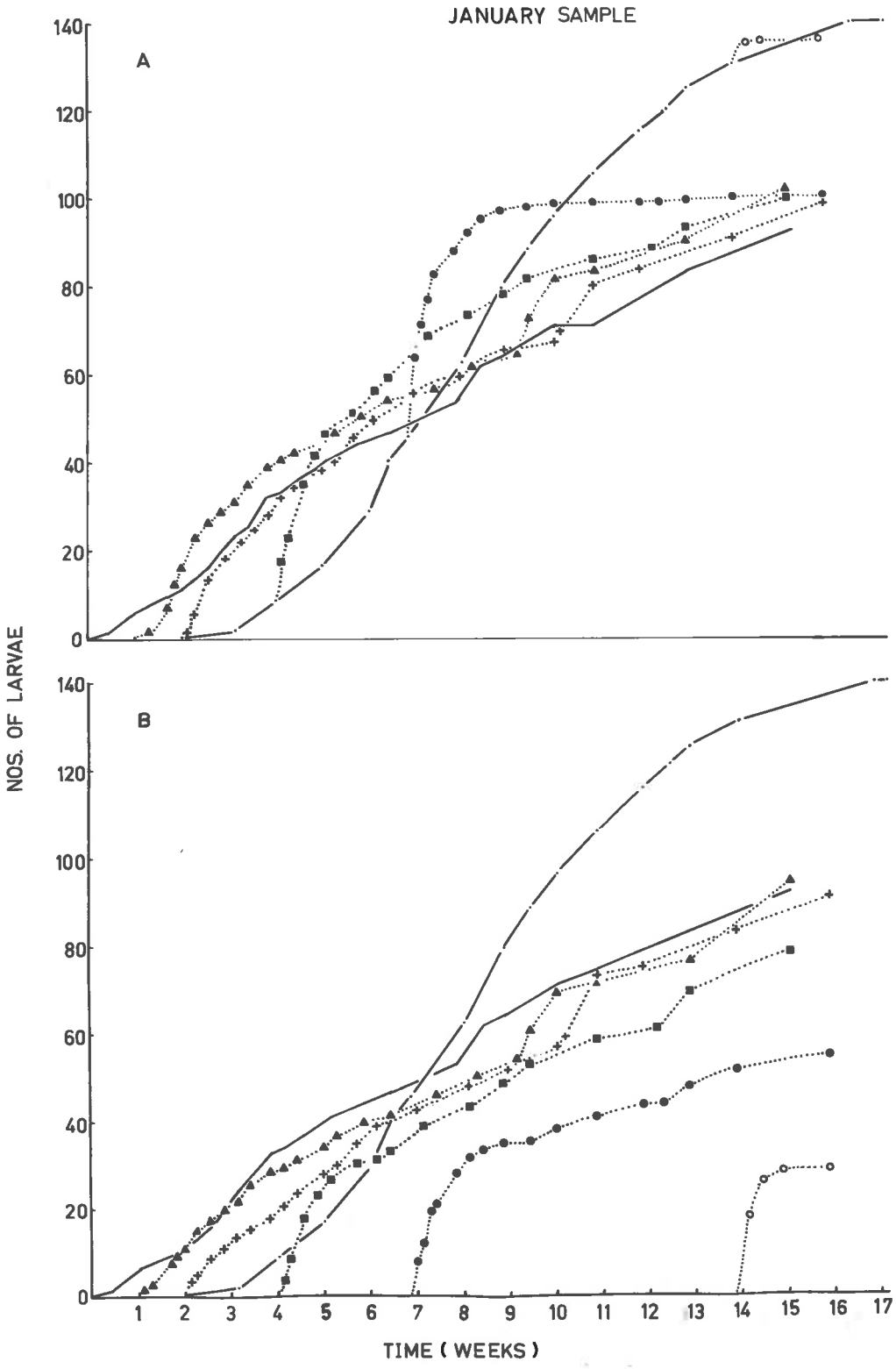

JANUARY SAMPLE
A
B
NOS. OF LARVAE
TIME (WEEKS)
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

FIG. 9

HATCHING OF EGGS IN WATER AT 20°C. AFTER DIFFERENT TIMES AT 10°C. IN WATER OR 0.4M NaCl

A Incubation in water at 10°C.

B Incubation in 0.4M NaCl at 10°C.

—·—·— Constant 10°C. in water

——————— " 20°C. " "

▲·····▲ 1 week at 10°C. then 20°C.

+·····+ 2 " " " " "

■·····■ 4 " " " " "

●·····● 7 " " " " "

○·····○ 14 " " " " "

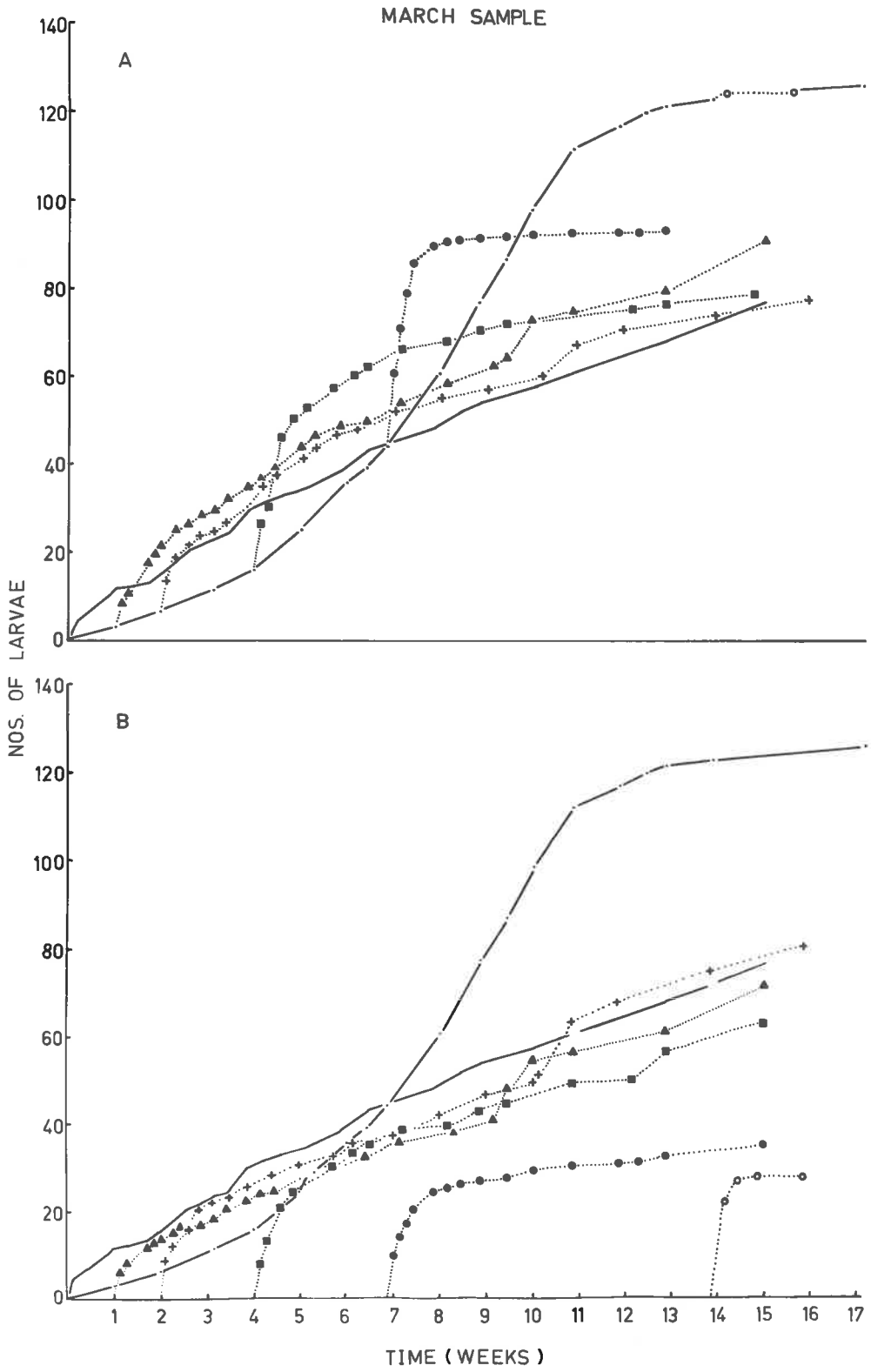

MARCH SAMPLE
A
B
NOS. OF LARVAE
TIME (WEEKS)
0
20
40
60
80
100
120
140
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

FIG. 10

FREQUENCY DISTRIBUTION OF EGG HATCH AT 10° C.

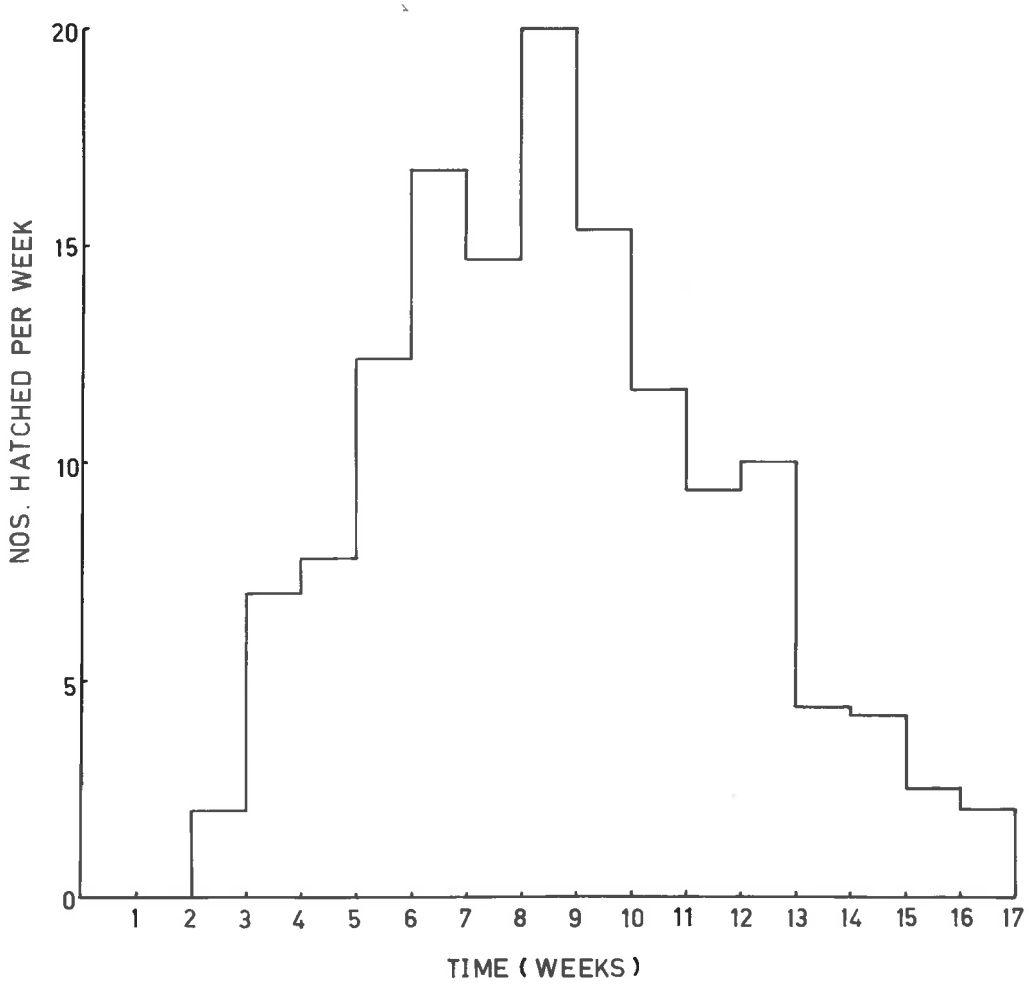


TABLE 8

Effect of different times at 10°C in water and 0.4M NaCl on the number of larvae hatched in the first 7 days following the change to 20°C.

Each number is the mean of 5 replicates

Time at 10°C in weeks	In water	In 0.4M NaCl
1	16.6	12.0
2	22.4	13.8
4	33.2	23.0
7	46.4	32.0
14	5.6	28.8

L.S.D. 5% = 4.0

TABLE 9

Numbers of larvae hatched after different times at 10°C in water and 0.4M NaCl plus those hatched in 7 days after the change to 20°C, compared with hatching at constant 20°C in the same time.

Each number is the mean of 5 replicates

Weeks at 10°C	Weeks at 20°C	JANUARY SAMPLE			MARCH SAMPLE		
		In water at 10°C	In 0.4M NaCl at 10°C	Const. 20°C in water	In water at 10°C	In 0.4M NaCl at 10°C	Const. 20°C in water
1	1	19.4	12.0		20.4	13.8	
0	2			12.0			15.6
2	1	20.4	13.8		23.4	18.4	
0	3			20.8			21.8
4	1	42.4	23.0		51.0	24.0	
0	5			39.4			33.4
7	1	92.4	32.0		92.2	24.2	
0	8			55.0			48.6
14	1	140.4	28.8		126.2	28.4	
0	15			92.4			77.0
			L.S.D. 5% = 7.9			L.S.D. 5% = 5.4	

There was a significant trend of decreasing final percentage hatch with increasing time of pretreatment at 10°C, except following 14 weeks, because "total" hatch had almost been reached by this time (Table 10).

Incubation in NaCl

The hatching responses to a rise in temperature, and change from NaCl to water up to and including 7 weeks cold pretreatment, increased similarly to those following incubation in water at 10°C, but they were always smaller (Table 8). The response following 14 weeks cold in NaCl did not differ significantly from that following 7 weeks pretreatment (Table 8), partly because no hatching occurred in NaCl, and because the influence of the plateau phase was apparent at about the same level following both periods (Figs. 8 and 9). Its induction, however, was more abrupt following 14 weeks cold.

Final percentage hatch following 0.4M NaCl and water at 10°C for the same times was about the same, except for the two longest periods of cold (viz. 7 and 14 weeks) when it was significantly less following incubation in NaCl (Table 10). Again, there was a significant trend of decreasing final percentage hatch with increasing time of pretreatment at 10°C in NaCl up to and including 14 weeks (Table 10).

All remaining eggs in all treatments appeared normal.

TABLE 10

Effect of different times at 10°C in water and 0.4M NaCl, followed by 20°C in water on percentage hatch after 9 months.

Mean of 5 replicates

Temp.°C	In water	In 0.4M NaCl
Const. 20°	75.8	-
Const. 10°	83.4	-
1 week at 10°	82.6	77.8
2 weeks at 10°	77.2	77.4
4 weeks at 10°	70.2	72.2
7 weeks at 10°	60.6	42.4
14 weeks at 10°	81.0	19.6

L.S.D. 5% = 5.0

Discussion

Increased hatching responses to a rise in temperature following progressively longer periods of cold, was simply an expression of the increasing number of eggs which had completed or nearly completed their phase 1 development, but whose second phase had been retarded by the sub-optimal low temperature. This confirmed that hatching involved two processes; the first, (phase 1) proceeding best at a low temperature must be completed before the second process (phase 2) can commence, which proceeded best at a warm temperature. Individuals varied considerably in the time they required to undergo phase 1. Some required a very short period as hatching commenced immediately at 20°C, while others remained unhatched, even after 9 months.

As all three parameters of the 10°C hatching curve, viz. (i) the mean hatching time derived from the formula:

$$\bar{x} = \frac{\sum_{i=1}^{n} f_i x_i}{\sum_{i=1}^{n} f_i}$$

where x_i = mid point of time interval

f_i = frequency in interval i

n = number of intervals

(ii) the median of the frequency distribution, i.e. the time taken for 50% of the population to hatch, and (iii) the mode of the frequency distribution, were approximately equal (8 to 9 weeks), the curve was symmetrical. Thus the point of inflexion,

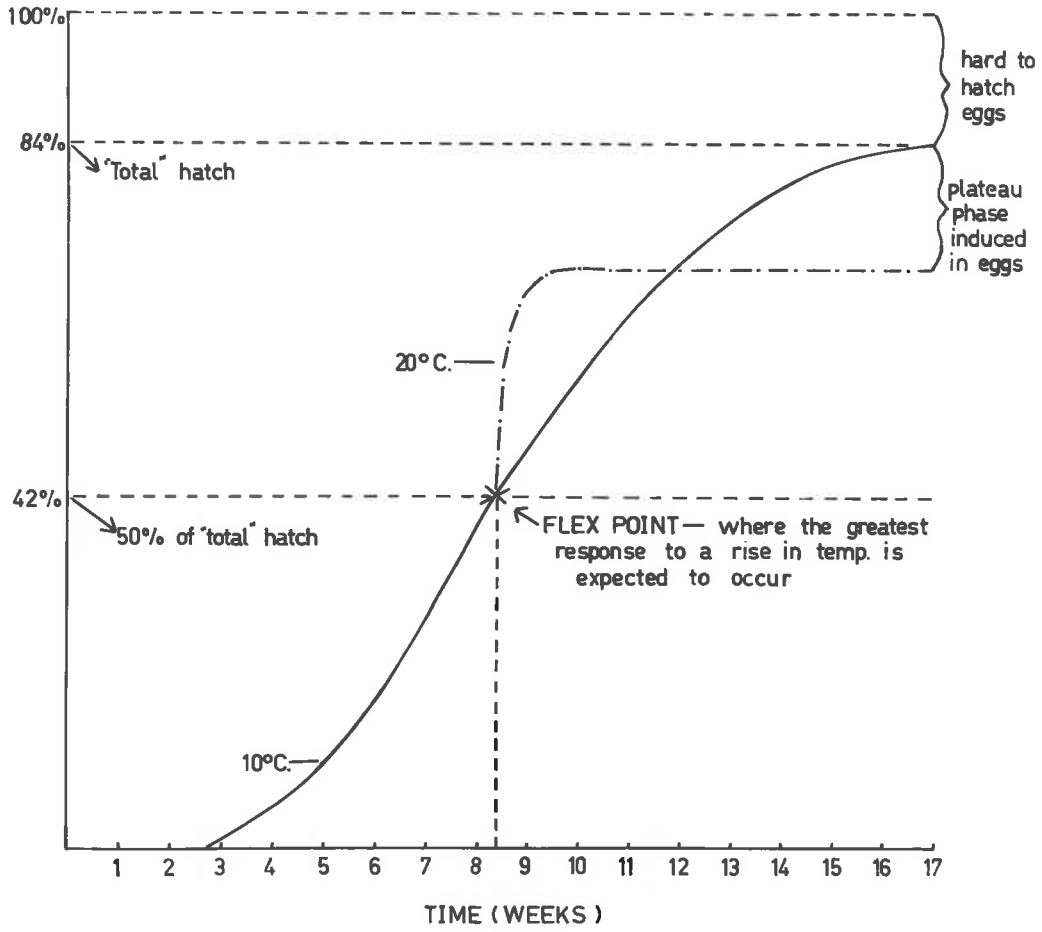
corresponding to the mode, coincided with 50% of "total" hatch. It is suggested, therefore, that at this point, the maximum number of eggs had completed their phase 1 development, and so the maximum response to 20°C would occur following 8 to 9 weeks at 10°C (Fig.11). This explains why in my results the maximum response was obtained following 7 weeks at 10°C, because this was closest to the time corresponding to the flex point. Beyond this point, the responses would become progressively smaller with time, as fewer eggs would be left to hatch; hence the smaller response in my results after 14 weeks. These conclusions contradict the conception that a period of low temperature is required to "trigger" some mechanism before hatching ensues, and the minimum period of 8 weeks cold suggested by Fushtey and Johnson, (1966) could be explained in the above terms.

Presumably, the same "total" hatch would have been reached at both constant temperatures had hatching continued, but it was reached much sooner at 10°C than at 20°C because 10°C is near optimum for the longest process (i.e. phase 1), and it may also be closer to the optimum for phase 2 than 20°C is for phase 1. Eggs remaining after "total" hatch at constant temperatures may have been in the plateau phase prior to incubation, or they might be inherently different requiring much longer to complete their development (Fig. 11).

The longer eggs were at 10°C (up to 7 weeks), the smaller was the final percentage hatch which followed at 20°C, indicating that progressively more eggs had been induced into the plateau phase

FIG. 11

HYPOTHETICAL REPRESENTATION OF HATCHING OF EGGS AT 10°C. AND THE RESPONSE TO 20° C. OBTAINED AT THE FLEX POINT



by the temperature change. Thus, up to 7 weeks the numbers of eggs induced into the plateau phase paralleled the responses to a rise in temperature, indicating that this phase was induced at a critical stage of development which was related to the completion of phase 1. Beyond the critical stage, hatching must follow. For similar reasons as outlined above, maximum numbers that could be induced into the plateau phase would therefore be expected at the flex point of the 10°C hatching curve. The hypothesis was supported in this experiment by the abrupt cessation of hatching after the response to 20°C following 7 weeks cold (Figs. 7 and 8), indicating that most, if not all the remaining eggs up to "total" hatch had been affected. Thus, responses to change in temperature were influenced by two opposing effects, (i) the extent of phase 1 development beyond the critical stage, and (ii) the number of eggs at the critical stage that could be induced into the plateau phase.

In Sodium Chloride at 10°C

Eclosion, but not the earlier processes leading to hatch was stopped by an 0.4M solution of NaCl which has an osmotic potential almost equal to the total potential existing in soil at the wilting point for plants (i.e. 15 atmospheres). This supported earlier evidence for lack of hatching under high soil moisture tensions occurring in the field (page 27).

Development of some eggs in 0.4M NaCl at 10°C proceeded

past the critical stage at which the plateau phase could be induced, because hatching occurred when eggs were moved to 20°C in water. However, if development beyond this critical stage was retarded by the high osmotic pressure, then those eggs which were potentially able to enter the plateau phase, would have accumulated with time at 10°C. This could account for the smaller responses being obtained at 20°C after incubation in 0.4 M NaCl than after incubation in water at 10°C, and may also be the reason why there was no significant increase in response to 20°C following 14 weeks, compared with that following 7 weeks at 10°C in NaCl.

Partial desiccation through loss of water by exosmosis, and the possible effect on larvae of Na and Cl ions which had passed through the cuticle, may have caused retarded development or even some damage which could have contributed to the smaller responses.

(4) Effect of different initial temperatures followed by different subsequent temperatures on hatch

Introduction

The apparent optimum temperatures for phase 1 (10°C) and phase 2 (20°C) were based on the responses to one pre-cold or one warm temperature, and so the effect of different initial and subsequent incubation temperatures on hatch was examined to verify these suggested optima.

Method

Four replicates of approximately 200 eggs were sub-sampled from 250 new-season's cysts collected from the field in early March, and were incubated initially at - 5°, 10°, 15° or 20°C for 4, 6 and 8 weeks, and then moved to 10°, 15° or 20°C until hatching had practically ceased. Similar batches of four replicates were incubated at constant 5°, 10°, 15° or 20°C as controls. Because of the time involved in maintaining regular counts for lengthy periods, the number of treatments was kept to a minimum by excluding incubation at 25°C and subsequent incubation at 5°C; these treatments being considered as the least important. One or more counts weekly were maintained until hatching slowed considerably, when counts at longer intervals were made. "Total" hatch was assumed when hatching practically ceased.

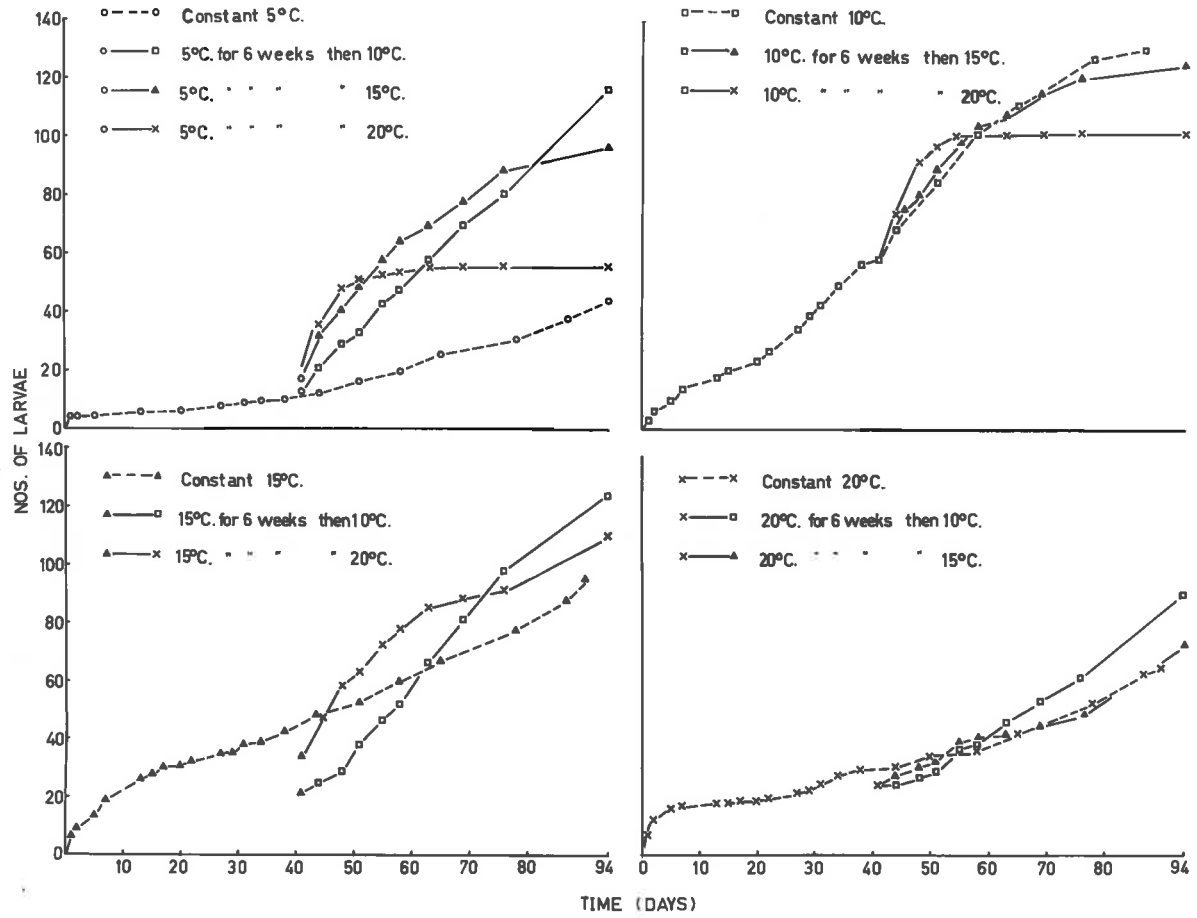
Results

As the continuous hatching curves for each temperature combination were similar following the 4, 6 and 8 weeks temperature changes, only those associated with the 6 week change are shown (Fig. 12).

Because of the design of the experiment, the use of different batches of eggs for each temperature regime meant that the initial number of hatched larvae at the time of each temperature

FIG. 12

HATCHING AT DIFFERENT INITIAL AND SUBSEQUENT TEMPERATURES COMPARED WITH HATCHING AT CONSTANT TEMPERATURES



change differed slightly due to random variation. This was most noticeable at 15°C after 6 weeks (Fig. 12).

The optimum temperature for phase 1 was obtained by comparing percentage hatches after 1 week at the same subsequent temperature following different initial incubation temperatures, while hatch assessed after 1 week at different subsequent temperatures following the same initial incubation temperature showed the optimum for phase 2.

These comparisons are made in Table 11 which shows that:-

(i) Percentage hatch was greatest at each subsequent temperature following initial incubation at 10°C, indicating that 10°C was about the optimum for phase 1 irrespective of the temperature for phase 2.

(ii) In almost every instance, percentage hatch increased with increasing subsequent temperatures following each of the initial incubation temperatures, indicating that 20°C was about the optimum for phase 2 irrespective of the temperature for phase 1.

(iii) Percentage hatch of eggs receiving a decrease in temperature, e.g. 15° to 10°C; 20° to 15°C and 20° to 10°C, was either not different from, or significantly less than hatch at the respective constant temperatures, viz. 15° and 20°C, so that a drop in temperature did not stimulate hatch.

TABLE 11

Mean percentage hatch of eggs after 5°, 10°, 15° and 20°C for 4, 6 and 8 weeks followed by hatching at 10°, 15° and 20°C for 1 week.

Initial incubation temp.°C for 4 weeks	Subsequent incubation temp.°C for 1 week			
	5°	10°	15°	20°
5°	9.3	17.3	18.8	21.8
10°	-	28.8	37.5	34.3
15°	-	20.0	26.3	25.3
20°	-	13.3	17.7	17.0
	L.S.D. 5% = 3.5			
Initial incubation temp.°C for 6 weeks				
5°	10.8	18.0	22.5	29.0
10°	-	38.3	43.4	51.8
15°	-	19.8	31.5	34.5
20°	-	17.0	20.0	18.8
	L.S.D. 5% = 4.1			
Initial incubation temp.°C for 8 weeks				
5°	18.0	18.3	30.3	39.8
10°	-	58.5	61.0	65.0
15°	-	37.5	41.3	51.0
20°	-	24.0	23.0	24.8
	L.S.D. 5% = 4.8			

More eggs entered the plateau phase at 20°C than at 15°C after eggs were moved from 5°C and 10°C (Fig. 12). A similar result was also obtained following 4 and 8 weeks at 5°C, and 8 weeks at 10°C.

Table 12 shows that:-

(i) By 6 months, "total" hatch had been attained at constant 10° and 15°C, but not at 5° and 20°C. It was reached first at 10°C, then 15°C, and appeared likely to be attained next at 20°C, then 5°C, had the experiment continued, as hatching at both these temperatures was still proceeding.

(ii) About the same "total" hatch was attained by eggs which received a decrease in temperature.

(iii) In most instances, "total" hatch was not reached following a rise in temperature, and final percentage hatch generally decreased with increasing size of the temperature change.

(iv) Following a rise in temperature, smaller final percentage hatches generally resulted from longer periods initially at the lower temperature, particularly 5°C.

Discussion

Over the range of temperatures tested, results indicated that irrespective of the subsequent incubation temperature, 10°C was

TABLE 12

Effect of changing temperatures at 4, 6 and 8 weeks and constant temperatures on percentage hatch after 6 months.

Mean of 4 replicates

Changing temperatures		Time of initial incubation		
Initial temp. °C	Subsequent temp. °C	4 weeks	6 weeks	8 weeks
20	10	70.0	70.3	69.5
20	15	73.0	71.3	70.0
15	10	70.8	75.8	73.8
15	20	74.0	76.0	73.0
10	15	75.0	67.3	65.0
10	20	62.8	57.5	65.0
5	10	74.5	70.3	60.3
5	15	55.5	50.5	43.3
5	20	41.8	33.8	40.5

Constant temp. °C	% hatch
5	58.0
10	75.5
15	70.5
20	68.8

L.S.D. 5% = 5.8

optimum for the cold process (phase 1), while irrespective of the initial incubation temperature, 20°C was optimum for the warm process (phase 2).

The percentage of unhatched eggs remaining at the completion of incubation as well as the time taken for hatching to cease after the temperature change, reflected the influence of the plateau phase. The results indicated that either at constant temperatures or decreasing temperatures, eggs did not enter the plateau phase. On the other hand, there was a tendency for eggs subjected to an increase in temperature to be induced into the plateau phase, the extent depending on the size of the temperature increase and the time when the temperature was changed. There was least tendency for the plateau phase to be induced by a 5°C increase, more by a 10°C and most by a 15°C increase. Thus, it appeared that the critical stage at which eggs could enter the plateau phase, or be influenced most by it , was dependent on the size of the temperature increase. This probably explains why Fushtey and Johnson (1966) found hatching over several weeks following 0°C pre-storage, was greatest at 10°C, less at 15°C, less still at 20°C and least at 25°C.

The results supported earlier evidence (page 51), that the effect of time at a low temperature on the subsequent influence of the plateau phase at a warm temperature, was an expression of the number of eggs at the critical stage which could be affected by the

temperature increase and was not a direct effect of time at cold per se on any one individual egg.

Eggs that failed to hatch after "total" hatch at constant temperatures, may have already entered the plateau phase in the field, perhaps by diurnal fluctuation of temperature.

(5) Effect of fluctuating temperature on hatch

Previous results in this thesis have demonstrated in some detail, the effects of constant or changing temperatures after relatively long periods of time, on three main aspects of hatching viz. phase 1, phase 2 and the plateau phase, but the effect of fluctuating temperatures on each of these was unknown.
The following experiment was designed primarily to determine whether development during phase 1 was influenced by fluctuating temperatures, and also to demonstrate any effect of fluctuating temperatures on the plateau phase.

Method

New-season's cysts were gathered from the field in February and eggs were sub-sampled into batches of four replicates, and incubated in the usual way.

To take into account degree of temperature fluctuation and frequency of temperature change, batches of approximately 200 eggs were subjected to fluctuations, 5° to $15^{\circ}C$, 5° to $20^{\circ}C$, 10° to

15°, and 10° to 20°C at daily and 4 - day intervals for 72 days. Similar batches were incubated at 5°C and 10°C for the first 36 days, then moved to 15°C and 20°C for the remaining 36 days. It was necessary to incubate eggs which were to receive only one temperature change at the lower temperature first, so that the extent of their development could be expressed by hatching when moved to the higher temperature. This then enabled comparisons with hatch of eggs receiving more frequent changes. Batches of eggs were similarly incubated at constant 5°, 10°, 15° and 20°C for the whole incubation period. Counts were maintained regularly every four days throughout the 72 day incubation period, and a final percentage hatch was determined after 167 days when hatching in most treatments had almost ceased. After 72 days, eggs which had received only one temperature change remained at the higher temperatures, while the daily and 4 - day fluctuations were maintained until 167 days.

Results

After 72 days incubation

Where there was least tendency for the plateau phase to be induced by a temperature change, viz. 10° to 15°C, frequency of temperature change did not significantly affect percentage hatch (Table 13). Where the tendency for eggs to enter the plateau phase was greater, viz. 10° to 20°C, and 5° to 15°C, about the same

TABLE 13

Effect of fluctuating temperature on hatch after 72 and 167 days

Each number is the mean of 4 replicates

Temp. °C	Fluctuation	% hatch after 72 days	Final % hatch after 167 days
5° to 15°	Daily change	74.5	82.0
5° to 15°	4 Daily change	70.3	83.3
Const. 5° for 36 days followed by const. 15°		39.3	42.3
5° to 20°	Daily change	74.0	83.3
5° to 20°	4 Daily change	54.0	86.3
Const. 5° for 36 days followed by const. 20°		13.5	18.3
10° to 15°	Daily change	71.5	82.3
10° to 15°	4 Daily change	69.3	82.3
Const. 10° for 36 days followed by const. 15°		71.8	77.0
10° to 20°	Daily change	69.5	83.5
10° to 20°	4 Daily change	69.8	86.5
Const. 10° for 36 days followed by const. 20°		61.0	65.3
Const. 5°		1.5	64.8
Const. 10°		67.0	80.0
Const. 15°		53.5	79.3
Const. 20°		30.8	60.8
L.S.D.	1%	6.97	5.25
	5%	5.2	3.9

percentage hatch was obtained whether eggs were exposed to daily or 4 - day fluctuations, but hatch of eggs which received only one change after 36 days was significantly less. Where the greatest tendency existed for the induction of the plateau phase viz. 5° to 20°C, percentage hatch decreased markedly with decreasing frequency of change.

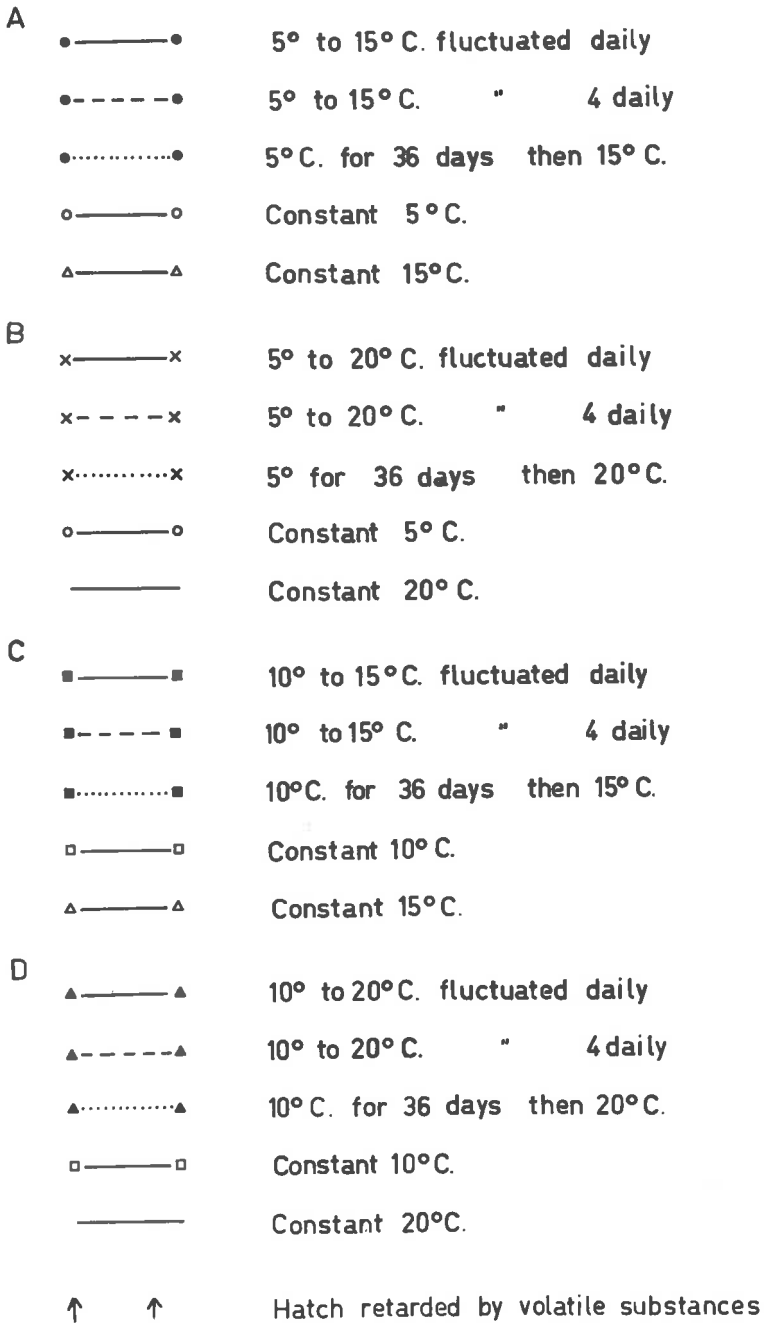
As eggs of most treatments had almost reached "total" hatch after 72 days, it was necessary to compare their respective hatching rates over this time to demonstrate any effect of temperature fluctuation. Rate of hatching was only slightly reduced by a 4 - day fluctuation compared with a daily change, but this was most evident in the treatment 5° to 20°C where the influence of the plateau phase was greatest (Fig. 13). As daily changes produced the fastest rates, the initial rates of hatching over the first 19 days were compared (Fig. 14). The fastest rate occurred in the 10° to 20°C treatment, followed by, in decreasing order, 5° to 20°C, 10° to 15°C then 5° to 15°C.

"Total" hatch after 167 days incubation

"Total" hatch of eggs receiving a daily or 4 - day fluctuation was about the same (Table 13). Where the temperature was changed after 36 days, "total" hatch was not reached and final hatch, which followed, decreased in the following order of changing temperatures; 10° to 15°C > 10° to 20°C > 5° to 15°C > 5° to 20°C (Table 13).

FIG.13

EFFECT OF FLUCTUATING TEMPERATURE ON HATCH OF FREE EGGS



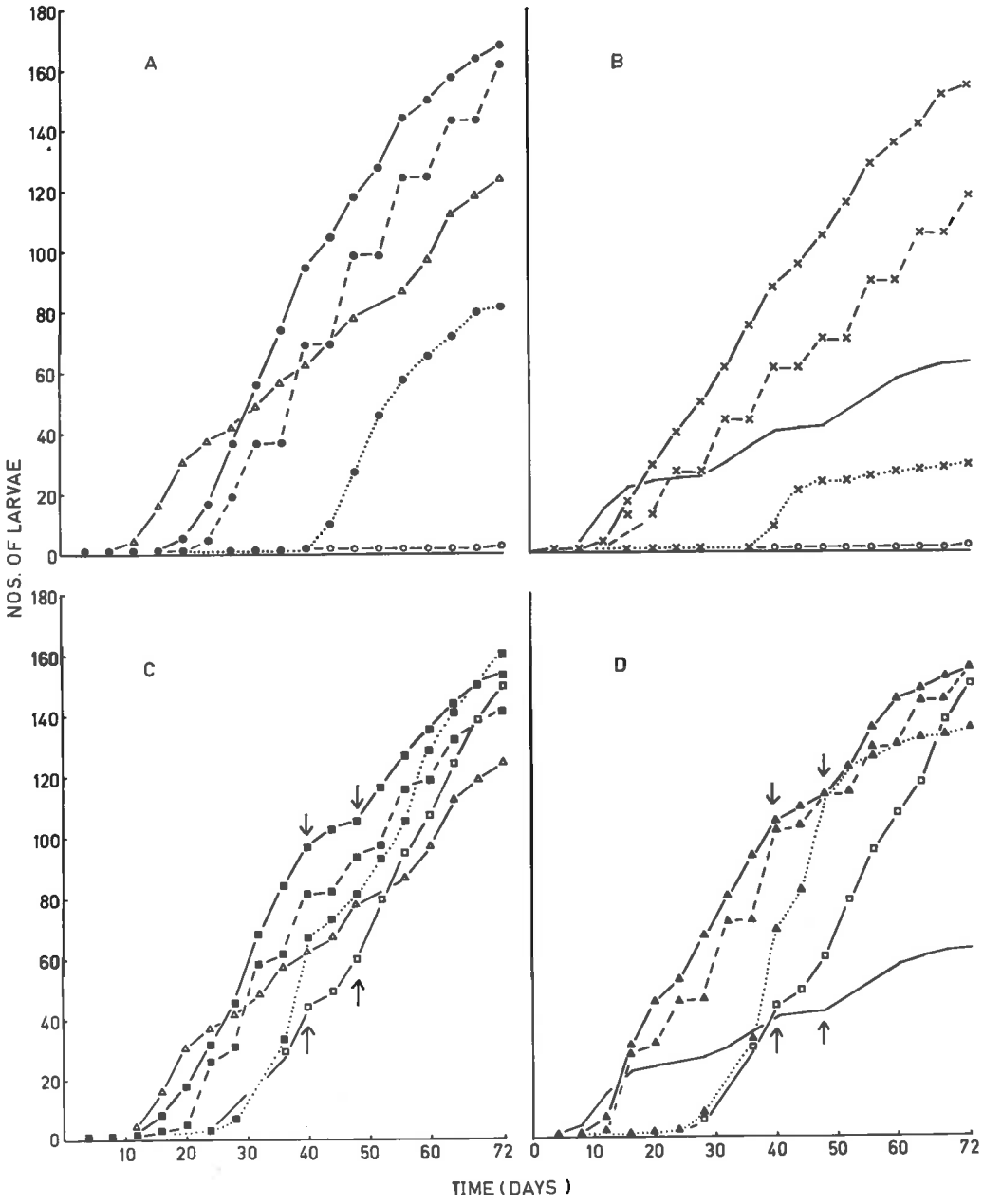
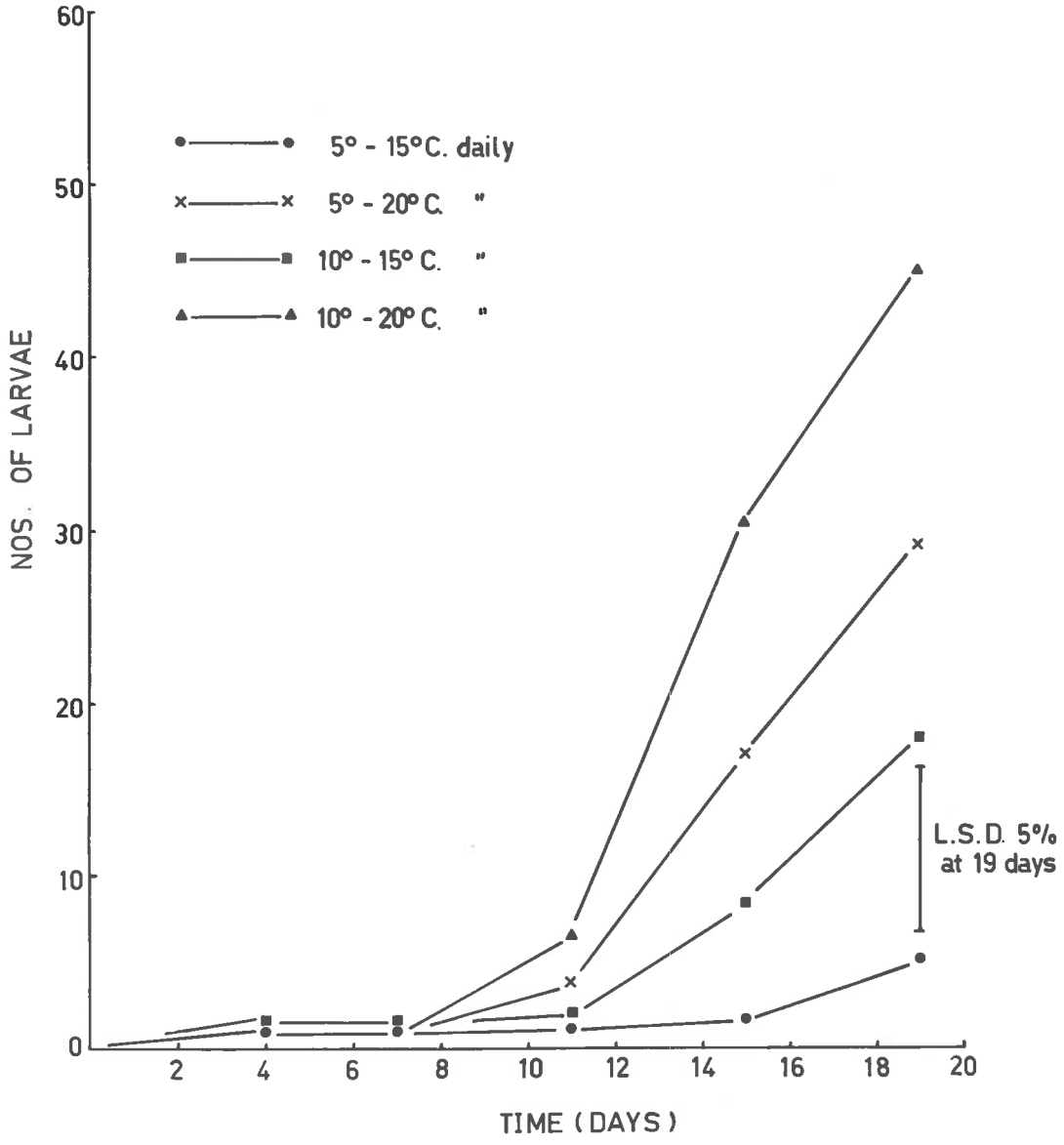

A
B
C
D
NOS. OF LARVAE
TIME (DAYS)
0
20
40
60
80
100
120
140
160
180
10
20
30
40
50
60
72

FIG. 14

EFFECT OF DAILY FLUCTUATIONS OF DIFFERENT TEMPERATURES ON HATCHING UP TO 19 DAYS



The same "total" hatch would presumably have been reached by eggs at all constant temperatures given sufficient time, as some hatching was still continuing when incubation was stopped.

Suspected volatile substances interfering with hatch

It was observed after the 40th day of incubation, that hatching rate of eggs, particularly those at the higher temperatures, suddenly decreased for no apparent reason for about 8 days, then resumed to the previous rate (Fig. 13). This was not so evident with eggs at 5°C.

About that time, a powerful and persistent odour of paint "thinners", was present, and these were being used to varnish benches close to the incubators. A few drops of the "thinners" in 2 mls. of water, anaesthetised active second-stage larvae, completely stopping motility. After 30 minutes, the larvae were washed and placed back into water where they again became highly active.

Discussion

Results of fluctuations from 10° to 15°C, where the influence of the plateau phase was least, indicated that rate of phase 1 development was independent of frequency of temperature change, but was an average of the respective rates proceeding at each

different temperature.

In previous attempts to establish the optimum temperature for phase 1, results were biased against 5°C. because of the greater tendency for the plateau phase to be induced when eggs were moved from 5°C to the higher temperatures than from 10°C. However, the initial rates of hatching at daily fluctuated temperatures, where the influence of the plateau phase appeared to be largely overcome, confirmed that 10°C was about the optimum for phase 1, and 20°C about the optimum for phase 2.

The same "total" hatch was reached by eggs fluctuated daily and every 4 days, and so the main effect of fluctuating temperatures was in overcoming the onset of the plateau phase, which was induced following a 10° or 15°C increase after 36 days. However, rate of hatching was much slower at the less frequent change between 5° and 20°C, which was probably due to some influence of the plateau phase. This suggested that subsequent development and hatch could be retarded to varying degrees by an increase in temperature, the extent depending not only on the physiological stage of development reached by eggs when exposed to the temperature change, (i.e. the "critical" stage) and the size of temperature increase, but also on the frequency of the temperature change. Thus, every time eggs receive an increase in temperature, there may be a tendency for all to enter the plateau phase with varying degrees of firmness, but more intensive study is required

to test this hypothesis, preferably with some physiological evidence.

Effect of volatile substances

It appeared very likely that highly volatile substances had diffused through the water and the eggshells, temporarily inactivating the larvae which were about to hatch. Eggs which were at 5°C were probably affected less because of a slower diffusion rate, and reduction in solubility of the volatile substances at low temperature.

No attempt at the time was made to find the most active constituent of the "thinners", but it is intended to investigate this further at some future date.

3. EFFECT OF TEMPERATURE ON OTHER ACTIVITIES LEADING TO HATCHING

Differences in the temperature optima have been found for different activities of nematodes, and it is possible that each stage of the life-cycle may have its own different optimum temperature (Bird and Wallace, 1965). Hatching of H.avenae has been divided into two phases requiring very different optimum temperatures. It was not known, however, whether either optimum was closer to that for other activities associated with hatching, such as, embryonic development and larval motility, or whether different optima were involved. It was decided, therefore, to determine the optimum temperatures for embryological development from the single or 2 - celled stage, and also for motility of hatched second-stage larvae.

(1) Optimum temperature for embryonic development

Barley plants (cv. Prior), infected with cereal cyst nematode, were brought in from the field and kept watered in pots at about 22°C. Sixteen undifferentiated eggs were selected at random from a number of white females, placed singly in distilled water in small syracuse dishes at 8°, 15° and 20°C, and examined daily.

Results

Eggs at the same temperature differed considerably in the time they remained undifferentiated, taking from 1 to 3 days to show the first cleavage. The stages during development most easily recognised, were the 2 - celled, 4 - celled and multi-celled stages, the gastrula stage (Hyman, 1951), the first outline of a larval shape, and finally a definite larval shape. Development through these stages was most rapid at the higher temperatures, but at all temperatures, most eggs did not reach a definite larval form and signs of deterioration were evident at different stages. Even eggs which developed through to a definite larval shape deteriorated and eventually disintegrated.

Using undifferentiated eggs from females feeding on wheat roots, embryos in double-glass-distilled water again died at all temperatures either before or soon after reaching an initial larval form.

To improve oxygen availability, eggs were placed on the surface of sterilised, distilled-water agar. It was observed previously that once cleavage started, development at the higher temperatures was rapid, and so in order to obtain all eggs as near as possible to the same stage of development, only those at the 2-celled stage were used. Eggs were selected from about 20 white females produced in the laboratory at 22^{o}C on wheat roots which had

been sampled earlier from the field. The remainder of the females were left on the host roots in pots to mature under laboratory conditions. Batches of 10 eggs at the 2 - celled stage were placed on the surface of the distilled-water agar, and incubated at 8°, 15°, 20° and 25°C. Observations were maintained until embryos had either fully developed or died.

Results

Rate of embryonic development increased with increasing temperatures up to the multi-celled stage (Table 14), when signs of deterioration first began to appear at the higher temperatures. Eggs which had a definite larval shape were squashed, and found to contain dead, first-stage larvae with no internal pressure, indicating that they had failed to undergo the first moult. Eventually all eggs at all temperatures died. Eggs containing first-stage larvae when removed from the feeding mother, also failed to undergo the first moult in water, and eventually died. Very few abnormal eggs were found in cysts which matured on the host in the laboratory at constant 22°C, indicating that a constant temperature was not associated with the cause of death.

It appeared that the feeding mother was essential for embryonic development to be completed. Possibly, extra nutrition or some other factor needed to be supplied by the living female for eggs to differentiate completely to the second-stage larva. This

TABLE 14

Effect of temperature on rate of embryonic development

of eggs developing from the 2 - celled stage

Days after incubation	Temp. °C	Stage of embryonic development					
		2-celled	4-celled	Multi-celled	Gastrula	Initial larval shape	Definite larval shape
1	8	-	10	-	-	-	-
	15	1	9	-	-	-	-
	20	1	9	-	-	-	-
	25	2	6	2	-	-	-
2	8	-	10	-	-	-	-
	15	1	3	6	-	-	-
	20	-	1	9	-	-	-
	25	-	2	8	-	-	-
3	8	-	10	-	-	-	-
	15	1	-	9	-	-	-
	20	-	-	10	-	-	-
	25	-	1	9	-	-	-
6	8	-	5	5	-	-	-
	15	1	-	9	-	-	-
	20	-	-	6	4	-	-
	25	-	1	2	1	6	-
7	8	-	-	10	-	-	-
	15	1	-	7	2	-	-
	20	-	-	3	7	-	-
	25	-	1	1	1	3	4
8	8	-	-	10	-	-	-
	15	1	-	7	2	-	-
	20	-	-	1	9	-	-
	25	-	1	1	1	2	5
17	8	-	-	10	-	-	-
	15	1	-	-	3	6	-
	20	-	-	-	4	1	5
	25	-	1	-	1	1	7

is not known for other nematodes.

Further information on the factors involved with survival of the embryo is discussed in Section 5.

(2) Effect of temperature on larval motility

(a) Standard sand column method

To establish the optimum temperature for motility of infective larvae, the time taken for larvae to migrate through a vertical column of sand was used (Bird and Wallace, 1965). Pieces of plastic tubing 2 cm. long with an 0.5 cm. internal diameter were filled with washed beach sand with particle sizes varying from 150 - 250 μ diameter. The column was sealed with a piece of fine nylon gauze on one end, and held vertically by a cardboard disc above a glass block containing water. It was pushed through the disc until the sealed end appeared just below the surface which allowed water to rise by capillarity. A complementary set of blocks with water were maintained at the various temperatures so that the column and disc could be readily transferred when each count was made. Larvae, hatched the previous day from cysts that had been incubated in water at 20°C for several months, were kept overnight at 10°C. They were exposed to 25°C for about 1 hour prior to a 15 minute pre-conditioning time at different temperatures.

After pre-conditioning, aliquots of 100 larvae were

pipetted on to the surface of the saturated sand in each column at 10°, 15°, 20°, 25°C and a temperature fluctuating between 10° and 20°C every 15 minutes. Hourly counts were made for the first 6 hours and a final count taken after 22 hours when larvae remaining in the columns were recovered.

Results

Maximum rate of migration occurred over the first 2 hours at all temperatures except at 25°C (Fig. 15). Migration at 10°C over the first 4 hours was significantly less than at other temperatures, except 25°C, where only about 10% of larvae had migrated. Migration at 15°C, 20°C and the fluctuating temperature was not significantly different throughout.

When the experiment was terminated after 21 hours, a final migration of 80 - 85% had been reached at all temperatures except 25°C at which 75% of the larvae had migrated. About 90% of the added larvae were accounted for at all temperatures, and all of those remaining in the sand column were motile and appeared normal.

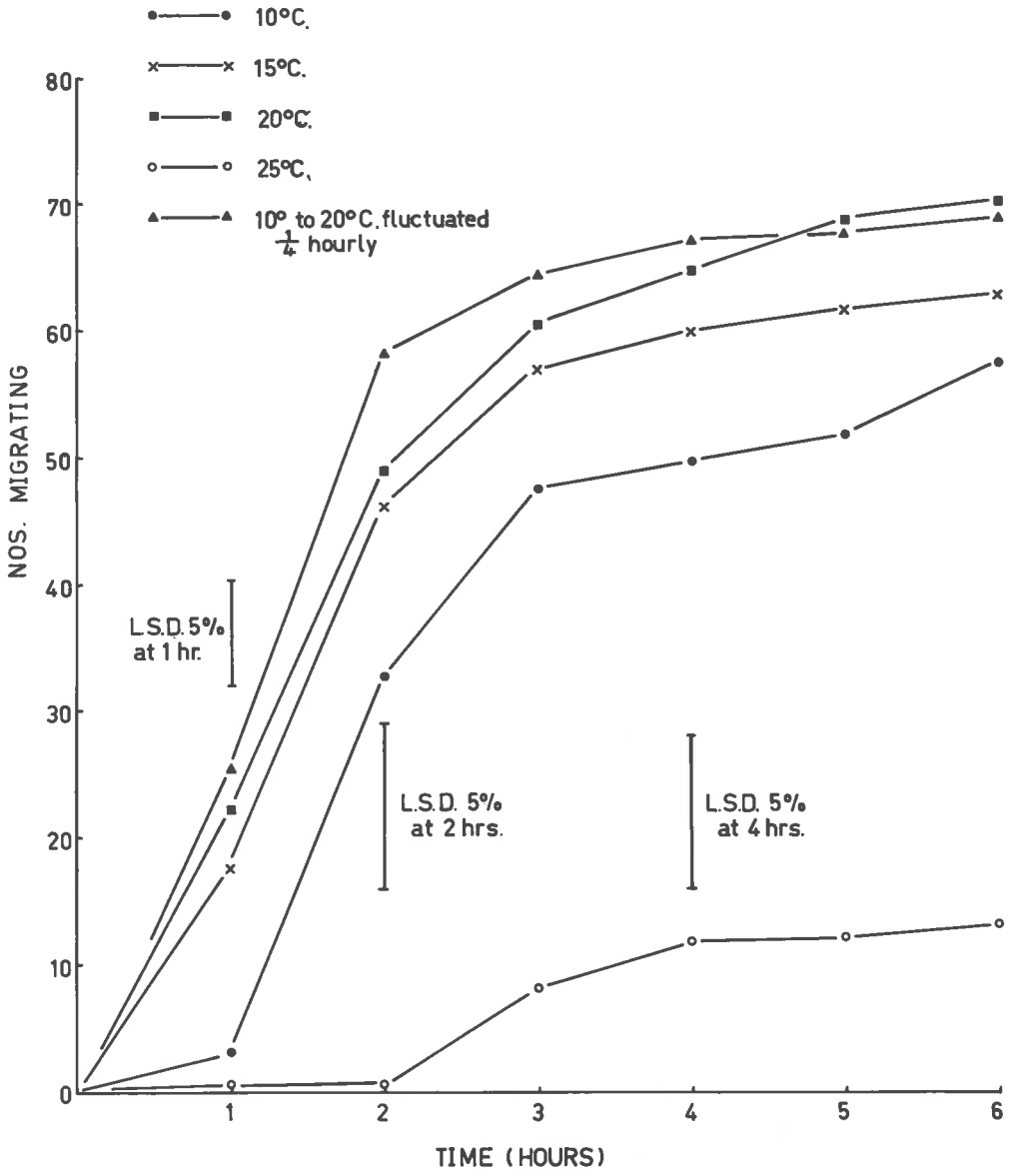
Variability within treatments was extremely high and the following factors were possibly responsible.

(i) The appearance of a number of large air bubbles in the sand columns indicated uneven distribution of sand particles.

(ii) The preconditioning time of 15 minutes may have been insufficient.

FIG. 15

EFFECT OF TEMPERATURE ON MOTILITY OF LARVAE USING THE SAND COLUMN METHOD



Thus it was considered necessary to repeat the experiment using certain modifications in order to check the validity of the results, particularly those at 25°C.

(b) Modified sand column method

Larvae were added carefully to the columns in the smallest possible amount of water, and the columns were lengthened to 3.5 cm. to reduce any effect of larvae being washed through. To obtain a more even distribution of sand particles, and to eliminate air bubbles, the columns were filled with sand under water using a large aperture eye dropper. Four batches of 100 larvae, hatched from the same source of cysts used previously, were kept at 10°C overnight for about 20 hours then pre-conditioned for 18 hours at the various temperatures. Motility was then tested at 5°, 10°, 15°, 20°, 25°C and a temperature fluctuated between 10° and 20°C every half hour. Counts were commenced after the first hour, and then half-hourly counts were made until $5\frac{1}{2}$ hours. Thereafter, they were taken every hour or two until $14\frac{1}{2}$ hours. A final count was taken after $29\frac{1}{2}$ hours when larvae remaining in the sand column were recovered. After $10\frac{1}{2}$ hours, larvae receiving the fluctuating temperature were left at 20°C for 2 hours then moved to 10°C for a further 2 hours, then 20°C for the remainder of the experiment.

Results

Migration of larvae was slower throughout at 5° and 10°C

than at the other temperatures (Fig. 16).

For the first 4½ hours there was no significant difference between rate of migration at 15°, 20° and 25°C, but migration was significantly faster at the fluctuating temperature over this time (Fig. 16). However, if the time taken for 50% of larvae to migrate was used as a criterion for motility, then it was significantly reduced only at 5° and 10°C. At 25°C, rate of migration decreased markedly after 7½ hours, and after 12½ hours had ceased altogether.

By the final count after 29½ hours, migration had ceased at 15°, 20°C and 10° - 20°C, but was still continuing at 5° and 10°C. Final counts at all but the two lower temperatures could therefore be regarded as total migration, which decreased with increasing constant temperatures, being significantly lowest at 25°C (Fig. 16).

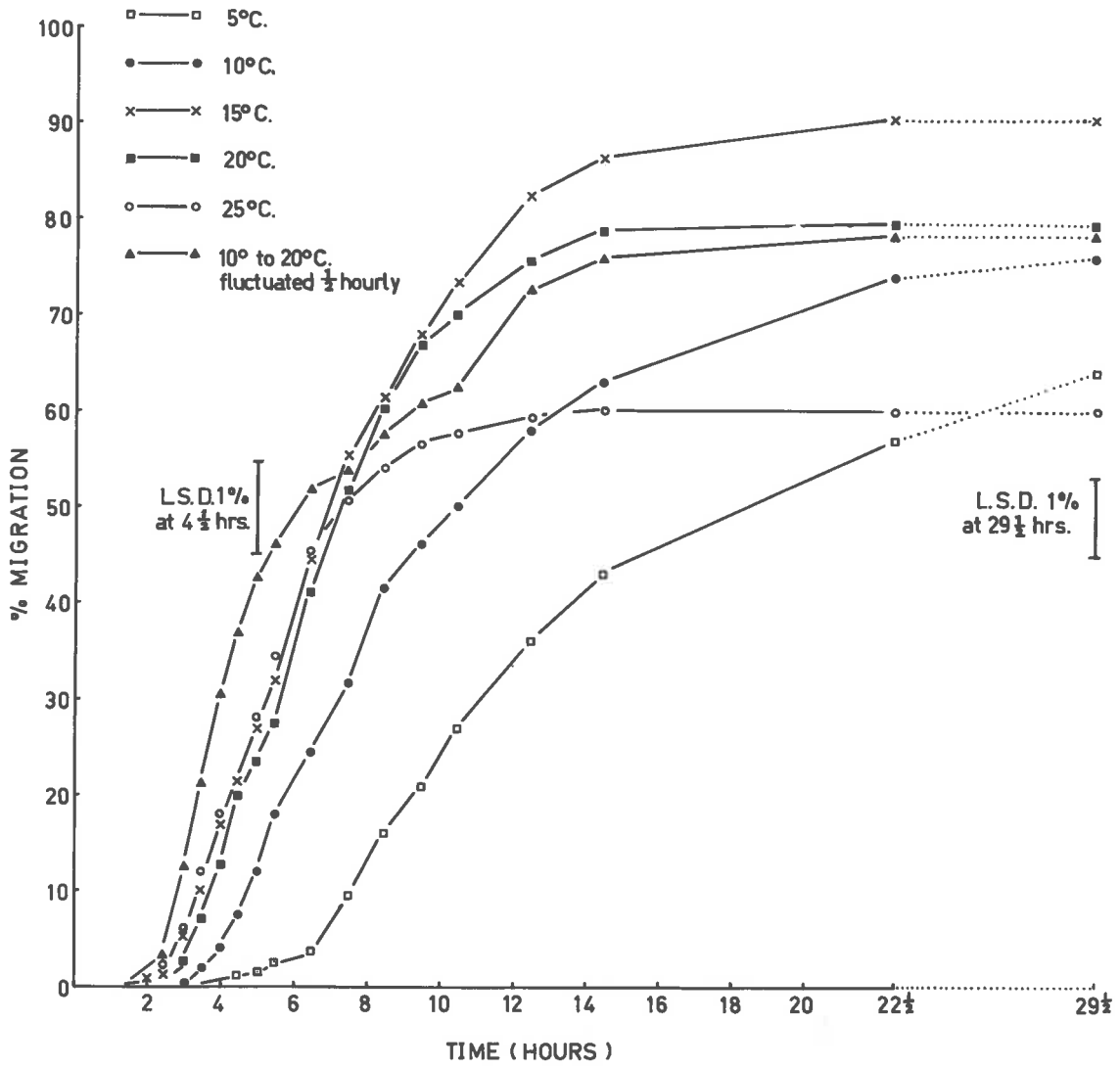
After recovery, 93 to 97% of the added larvae were accounted for, all of which appeared normal and motile. Variability was less than in the previous experiment.

Discussion

As temperature influenced rate of migration as well as total migration, both had to be considered in assessing its affect on motility. When total migration was taken as a measure of motility, 15°C was optimum. For the first 4½ hours, motility was greatest at the fluctuating temperature. If the time taken for 50% of added

FIG.16

EFFECT OF TEMPERATURE ON MOTILITY OF LARVAE USING THE MODIFIED SAND COLUMN METHOD



larvae to migrate was used as the criterion, the optimum constant temperature could not be defined between narrower limits than 15° to 25°C. This suggested, that for some reason the method was not sensitive enough, and that to define the optimum more precisely, an alternative method was required.

In all treatments, recovery was almost complete, and all remaining larvae appeared normal, so that decreasing total migration with increasing constant temperature could be accounted for by larvae becoming immobile sooner at the higher temperatures. That is, 25°C may be inhibitory after a given time, and at even higher temperatures motility might be greater than at 25°C initially, but final counts might be lower. Further information on this possibility is discussed in a subsequent experiment.

Although final migration counts at 25°C were lowest in both experiments, motility of larvae in the initial stages of this experiment at 25°C was considerably greater than in the previous one, possibly due to some difference in preconditioning.

(c) A comparison of motility of larvae hatched from cysts of different ages

In the preceding two experiments, larvae were hatched from cysts which were about 16 months old. A comparison of motility of larvae from younger (4-month-old) cysts was made using the modified sand column method, except that all larvae were hatched on the same

day at 20°C, and stored for 24 hours at 20°C before testing their motility at that temperature. Larvae remaining in the column at the completion of the test were recovered in the usual way. Four replicates of 100 larvae were used.

Results

Negligible migration occurred in the first 3½ hours, but after 7½ hours, more larvae from young cysts had migrated (mean 22%, range 12 - 28%) than from older cysts (mean 6%, range 1 - 11%), and after 23½ hours almost three times the number from young cysts had migrated (mean 38%, range 29 -42%) compared with those which had hatched from older cysts (mean 13.5%, range 7 - 20%). These differences were so marked that statistical analysis was not necessary. On recovery, over 90% of larvae were accounted for in all treatments.

Discussion

Measurements of rates of migration indicated that larvae from younger cysts were more motile than those from older cysts. Larvae from older cysts, therefore, might be less infective as infectivity has been shown to decrease with decreasing larval motility at certain temperatures in Meloidogyne javanica (Thomason, Van Gundy and Kirkpatrick, 1964) and in Tylenchulus semipenetrans (Van Gundy, Bird and Wallace, 1967). Compared with previous experiments, larvae took considerably longer to migrate. Here, larvae were hatched and stored at 20°C prior to use, whereas in

preceding experiments they had been stored for varying periods at 10°C before pre-conditioning, so that a period of low temperature might have stimulated their activity.

(d) Effect of different pre-conditioning temperatures on subsequent motility at 20°C

To investigate the possibility of a cold period stimulating motility, newly-hatched larvae were incubated at 10° and 20°C for 17 hours before testing their motility at 20°C using the modified sand column method.

Results from this experiment suggested that the 10°C pre-incubation period stimulated subsequent motility at 20°C, but only 10% to 20% of the larvae migrated after 7½ hours. Again variation within treatments was large. Further investigations of a possible cold stimulus were therefore carried out by observing directly the behaviour of larvae at different temperatures in water.

(e) Direct observations of larval motility in water

(i) Preliminary observations

Several larvae which had been kept in water at 5°C for 2 weeks were placed at 5°, 10°, 20° and 25°C. In the first few hours, most larvae appeared quiescent at 5°C, more were active at 10°C but their movement was very sluggish, while at 20° and 25°C all larvae initially were highly active. At the higher temperatures,

larvae appeared to gradually lose their activity and one by one became immobile, adopting a typically quiescent position, with the body slightly curved in a banana shape. This decreasing activity was demonstrated by recording at various times, commencing about half an hour after moving larvae from 5°C to 25°C, the time taken for 40 waves to pass through the body of one larva. Initially it took 2 minutes and 50 seconds for a wave to pass along the body; half an hour later the time had increased to 3 minutes and 45 seconds; after a further 2 hours, 4 minutes 12 seconds and one hour later still, 5 minutes 3 seconds. Amplitude of waves also appeared to gradually decrease before the larva became immobile, but this was not measured. Most larvae became immobile sooner at 25°C than at 20°C. Some remained in an immobile state for at least 2 to 3 hours and longer at 25°C, but eventually became active again for one to several hours.

Immobile larvae could be temporarily induced into activity by disturbing them, either by picking them up with an eye-dropper and squirting back into the block, or prodding them with a needle. When activity was renewed this way, it would last from half an hour to several hours at 25°C, before the larva again became completely immobile.

From these preliminary observations it seemed that the proportion of motile to immobile larvae at any one time could be a suitable means of recording any stimulus due to a period of low

temperature, or a change in temperature.

(ii) Effect of changing temperature on motility of larvae

Seventy larvae hatched from 16-month-old cysts over 2 weeks were placed in distilled water in a glass block for 17 hours at 25°C except for a three hour exposure to 10°C. Larvae were then alternated between 10° and 25°C for varying periods of time.

No observations were made during 10°C incubation, but larvae were examined just before moving to 25°C where observations were made at intervals commencing half an hour after each temperature change.

Because larvae alternated between an active and a resting state, a standard observation time of 30 seconds was adhered to. Larvae were kept in the dark except for the brief observation period.

Results

Initially, progressively more larvae lost motility at 25°C, and a three hour exposure to 10°C failed to re-activate any of the immobile larvae. After the first 17 hours at 25°C, about half had become immobile.

At the end of each cold period, larvae were either immobile or were very sluggish, but on each occasion that they were moved to

25°C, 90 to 100% became highly active within half an hour after the temperature change (Fig. 17). This increased activity was relatively short lived, as larvae became progressively more sluggish and eventually immobile with time at 25°C, and loss of activity was slowest following the longest exposure to cold (Fig. 17).

(iii) Effect of a period of low temperature on subsequent motility of newly-hatched larvae at 25°C

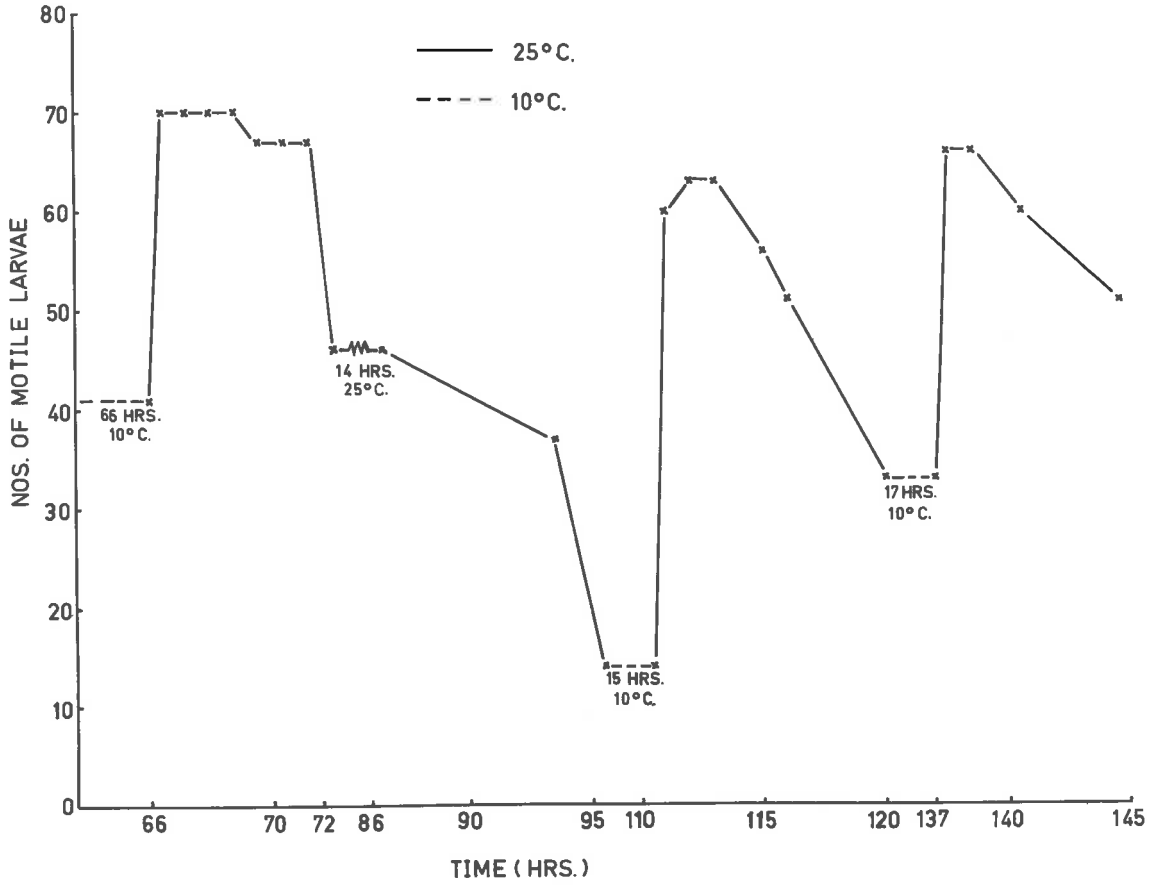
Larvae used in the previous observations were of different ages and were hatched from old cysts which had been stored for a long period, and so the effect of low temperature on motility was examined by a slightly different method using 1 and 2-day-old larvae hatched from new-season's cysts sampled direct from the field. After pre-conditioning for two days at 25°C, all immobile larvae were separated and sub-sampled into six batches of 13 or 14. Three batches were left at constant 25°C and three were incubated at 10°C for 16 hours, after which time the number of motile larvae was recorded, and the three batches at 10°C moved to 25°C. Numbers of motile larvae were then recorded at half hourly intervals for the first 2 hours after the change, then hourly for the next 3 hours, and the final observation made 8 hours after the temperature change.

Results

At the time of the temperature change only a small

FIG.17

EFFECT OF TIME AT 25°C. AND 10°C. ON MOTILITY OF LARVAE



proportion of motile larvae was recorded at both 10° and 25°C (Fig. 18). After the first half hour the mean number of motile larvae in the treatment which did not receive precold, decreased slightly, but markedly increased in that which was exposed initially to cold (Fig. 18). The stimulating effect of low temperature on motility was evident for about 4 hours after the temperature rise, but after 5 hours, mean numbers of motile larvae decreased, and by 8 hours had decreased further to the same number as that for the control.

(iv) Effect of conditions of temperature which normally induce the plateau phase in eggs, on motility of hatched larvae

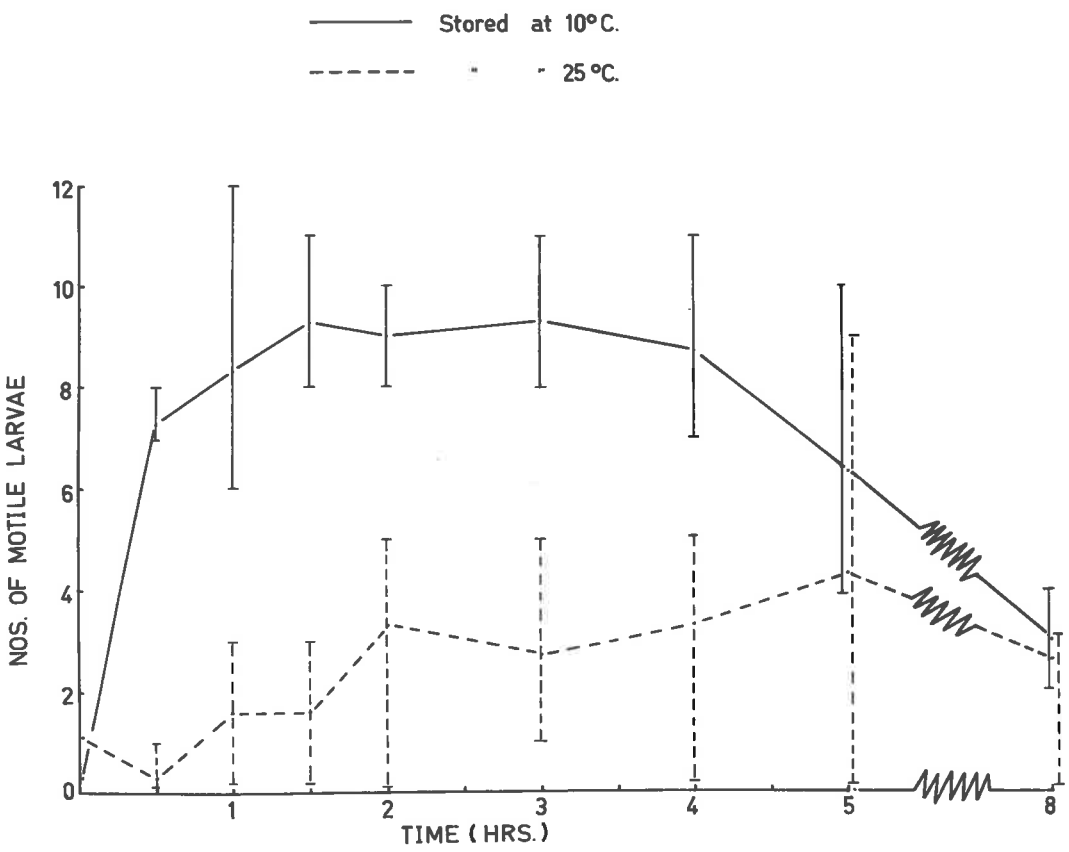
Fifty seven two-day-old larvae were hatched from new-season's cysts and placed at 5°C for 5 weeks then moved to 25°C. Within half an hour all the larvae became highly active and were still so 2 hours later. The number of immobile larvae observed at any one time gradually increased over the next 6 days to 34. Larvae were then placed at 10°C for 2 days, and all became active within half an hour after they were moved back to 25°C. The response obtained appeared normal and showed that hatched larvae could not be induced into the plateau phase.

Discussion

Motility of larvae of all ages decreased in water with time

FIG. 18

EFFECT OF DIFFERENT STORAGE TEMPERATURES FOR 16 HOURS ON SUBSEQUENT MOTILITY AT 25°C.



at warm temperatures, but could be re-stimulated by a minimum period of low temperature greater than 3 hours. The stimulating effect was short lived, but there was some evidence to suggest that it was longer with increased duration of the cold period, although more critical work is required for this to be substantiated.

Although the behaviour of larvae would undoubtedly be different in soil to that in water, the responses to temperature may be similar. For example, decreasing activity of larvae with time at higher temperatures in water, suggested that this was the reason for a decreasing total migration through soil with increasing temperature (page 74). Larval motility in both soil and water was less at 5° and 10°C than at higher temperatures, and there was a suggestion that a period of low temperature stimulated subsequent larval motility at a higher temperature in soil as well as in water. Although larvae for the sand columns were pre-conditioned at the respective temperatures, the effect of temperature on their motility may have been partially masked by a stimulus caused by disturbing them when they were placed into the columns.

If, as suggested by Van Gundy, Bird and Wallace (1967), general activity and metabolism increase with increasing temperature, food reserves would be exhausted sooner at the higher temperatures, and survival and possibly infectivity would accordingly be reduced.

Intermittent periods of rest, particularly at the higher temperatures, probably helped to conserve energy and food reserves, thus aiding survival for a longer period. Infectivity, as well as survival, may be prolonged at lower temperatures, and so if cysts are in short supply, it might be possible to accumulate sufficient numbers of larvae in a suitable condition for infectivity studies, by storing them at 5°C over an extended hatching period. Motility has been related to infectivity in Meloidogyne javanica, (Thomason, Van Gundy and Kirkpatrick, 1964). As motility of H.avenae can be stimulated by a period of low temperature, infection studies might therefore be best carried out at a warm temperature immediately following the cold period. If larvae responded similarly to diurnal fluctuations of temperature that occur in the field, they would be most active during the day when temperatures increased. Their motility may be thus regulated to coincide with the period of active host growth, when perhaps some attractant is being actively released from the roots, thereby increasing the chance of infection. More intensive studies are required to establish the conditions controlling the cold stimulus in water and soil, and then to establish its relevance to infection and survival in the field.

4. OTHER FACTORS AFFECTING HATCH

(1) Effect of host-root diffusates on hatching in vitro

Introduction

Although H.avenae does not respond to host-root diffusates in the Northern Hemisphere (Shepherd, 1962) its effect on the different hatching phases of the local population needed testing.

Method

Seeds of Insignia wheat were surface-sterilised in 20% Daizone (5% sodium hypochlorite) for 25 minutes and then washed three times in sterilised distilled water. The seeds were pre-germinated on moist filter paper at 25°C for 3 to 4 days. Six seedlings were then placed into each of a number of opaque test tubes containing 30 mls. of sterilised distilled water and were held in position by a plug of cotton wool. The roots had extended throughout the tube after 3 weeks when the root diffusate was collected, bulked and stored at 7°C in a brown glass bottle for future use. Distilled water for hatching was also kept at 7°C.

Cysts were collected from the field in early winter after the onset of low temperatures, but before the opening rains, and before any significant field hatching had occurred. They were sampled then as it seemed the most likely time when responses to host-root diffusates could be obtained, because it coincided with the

normal sowing time of the cereal host. Six batches of 50 new-season's cysts were incubated at 7°C and 20°C in water and wheat-root diffusate. After 56 days, cysts incubated at the low temperature were moved to 20°C. Regular counts and changes of liquid were made two to three times weekly throughout incubation.

Results

Hatching was most rapid during the first 14 days but was retarded by wheat-root diffusate at both temperatures (Fig. 19; Table 15). After 56 days, however, hatching in wheat-root diffusate and water at both temperatures was approximately the same (Table 15). Hatching practically ceased 14 days after cysts were moved from cold to warm (Fig. 19), but final hatch at this time was greater in wheat-root diffusate (Table 15).

Discussion

The initial faster rate of hatching indicated that a considerable proportion of eggs had completed phase 1 development before incubation was commenced. More eggs hatched in water after 20 days than in wheat-root diffusate, showing that root diffusate retarded phase 2. This might have been an osmotic pressure effect as the high osmotic pressure of concentrated beet diffusate retarded hatch of H.schachtii (Wallace, 1956).

The plateau phase appeared in both treatments following

FIG. 19

EFFECT OF WHEAT ROOT DIFFUSATE ON HATCHING FROM CYSTS AT DIFFERENT TEMPERATURES

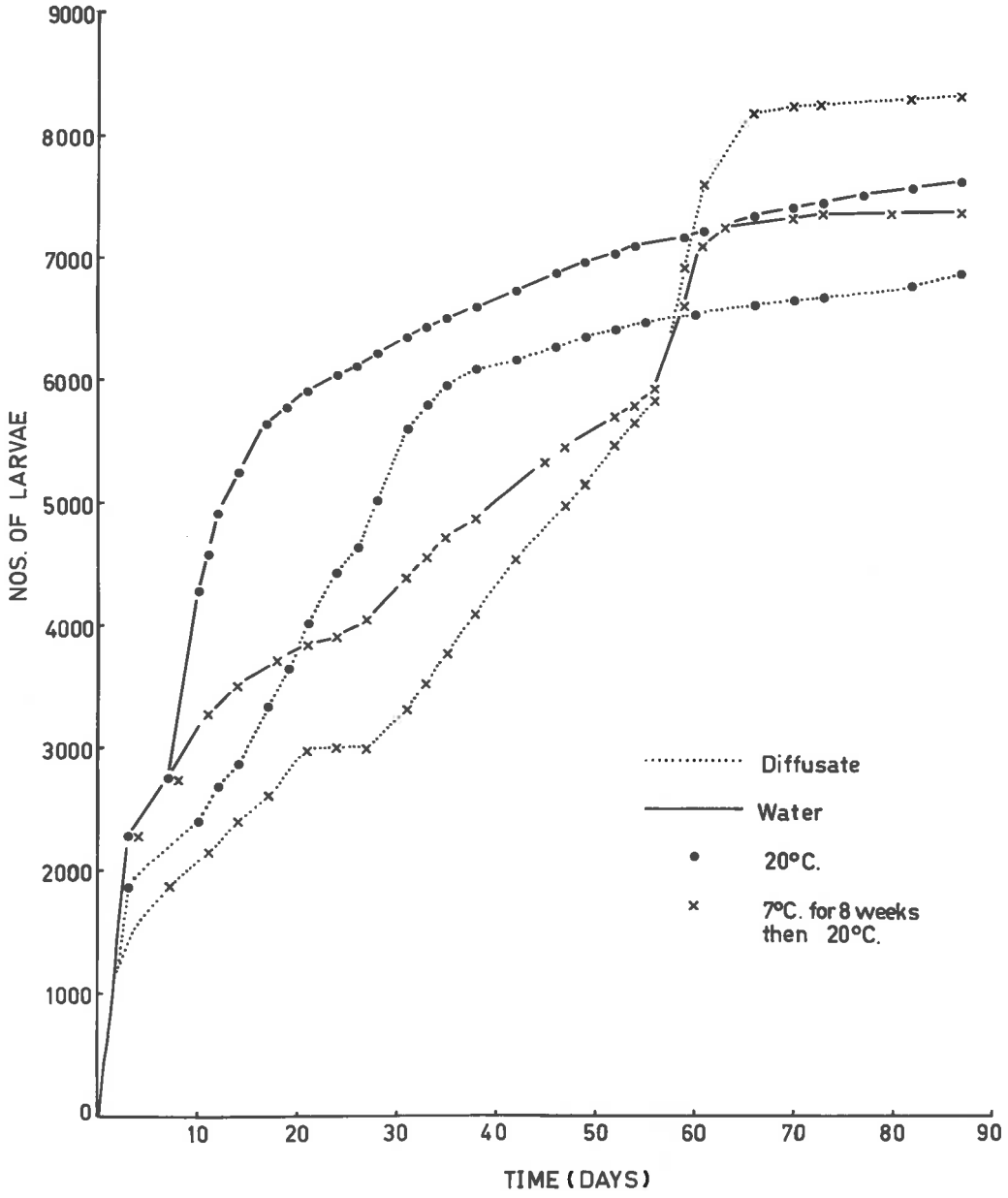


TABLE 15

Effect of wheat-root diffusate and temperature on hatch from 50 cysts after different times

Larval counts mean of 6 replicates

Treatment	After 14 days	After 56 days	After 70 days
In water at 20°C	5250	7098	7421
In diffusate at 20°C	2969	6478	6660
In water at 7°C for 56 days then 20°C	3512	5939	7389
In diffusate at 7°C for 56 days then 20°C	2395	5886	8239
	L.S.D. 5% = 398	L.S.D. 5% = 567	L.S.D. 5% = 432

7°C after eggs were moved to the higher temperature, but more eggs entered the plateau phase in water (Fig. 19). This suggested that wheat-root diffusate had affected the onset of the plateau phase.

On the basis of these results it was not known whether the responses obtained to wheat-root diffusate were due to an osmotic effect or specific chemicals, and so further investigations are required to clarify the nature of the responses and their significance.

(2) Effect of the presence of plant roots on hatching in soil

(a) Using cold-treated cysts

Batches of 50 new-season's cysts which had been incubated for 6 months in water at 7°C were placed in small sterilised terylene bags. Each bag was placed in 680 gms. of sieved Pinery sand, 2 inches from the bottom of 4-inch-deep waxed paper cups. Five replicate batches of cysts were placed in unsterilised sand or sand which had been autoclaved for 2 hours at 15 lbs. pressure per square inch. Twelve, 4-day-old Jemalong medic seedlings (Medicago truncatula Gaerpn.), or eight 4-day-old wheat seedlings (cv. Insignia), which had been surface sterilised before germination, were planted in each cup so that the roots were in close proximity to the terylene bags. Similar replicates were set up in sterilised or unsterilised Pinery sand, but without any

seedlings. All soil was brought to field capacity which was maintained every other day with sterilised distilled water. The experiment was carried out in an air-conditioned laboratory where temperatures remained near 23°C, and the plants were exposed to natural light. After 4 weeks the terylene bags were removed, and cysts recovered for squashing to determine the extent of hatching by assessing average number of eggs remaining per cyst.

Results

Wheat roots had grown around and beyond the terylene bags, and some fine laterals and root hairs had actually penetrated. Infection points on wheat roots were observed 1½ inches above the bag, indicating that infective larvae had travelled upwards by at least that distance. Infection appeared to be relatively severe, by the extent of tufting, and no marked difference was apparent in numbers of tufts on roots in sterilised and unsterilised soil. More eggs hatched in unsterilised soil and in soil in the absence of roots (Table 16).

Discussion

Presence of host or non-host roots did not greatly influence hatching in soil. More hatching occurred in unsterilised soil which may have been due to a stimulatory effect of soil micro-organisms which have been shown to influence hatching of H.rostochiensis (Giebel, 1963; Ellenby, 1963) or possibly accumulated

TABLE 16

Average numbers of eggs remaining in cysts after hatching for 4 weeks in sterilised and unsterilised soil in the presence and absence of wheat or medic

Species	Sterilised Soil	Unsterilised Soil	Means
None (control)	132.6	86.6	109.6
Wheat	132.4	101.6	117.0
Medic	139.6	94.4	117.0
	Means 134.9	94.2	

L.S.D. Individual means 5% = 31.2

Soil means 5% = 18.0

Species means 5% = 6.9

ammonia due to sterilisation may have retarded or slightly inhibited hatch. Conditions for infection appeared to be ideal and the severity of symptoms suggested that a "mass" hatch occurred in a relatively short time. This was to be expected as the cysts had been previously exposed to a long period of low temperature.

(b) Using cysts subjected to low temperature in the field

Cysts were sampled from the field in July after the onset of low temperatures, and when host crops would normally be germinating. Four replicates of 50 cysts were set up as in the previous experiment in sterilised and unsterilised Pinery sand from the same source as before. In this experiment, however, hatching in water was included instead of medic roots, and hatching was assessed after 3 and 6 weeks. Also, the experiment was carried out in the glasshouse where cysts were exposed to a diurnal fluctuation of temperature. At each harvest, cysts were removed, and the average egg content per cyst for each replicate was determined, and the percentage hatch in water calculated. Assuming that each treatment had the same total number of eggs, hatch in soil for each replicate was converted to a percentage by the following calculation:-

$$\frac{\text{average egg content per cyst remaining in water (mean of 4 reps.)}}{\text{average egg content per cyst remaining in soil of each replicate}} \times \text{Percentage hatch in water (mean of 4 replicates)}$$

Results

Infection appeared to be more severe on wheat in unsterilised soil than in sterilised soil, particularly after 6 weeks, but no attempt was made to measure this quantitatively. A substantial hatch occurred in the first 3 weeks as indicated by the extent of tufting on roots, but by the sixth week hatching had increased by not more than 6% in any treatment (Table 17). Hatching in water was about the same as in soil after 3 weeks, but after 6 weeks it was less than in unsterilised soil, but similar to that in sterilised soil. Percentage hatch in sterilised soil was slightly but significantly less than in unsterilised soil at both harvest times (Table 17).

Discussion

Presence of wheat roots did not influence hatching. Again, hatching in unsterilised soil was slightly greater than in sterilised, which could explain the apparent greater infection of roots in the former. However, further studies would be needed to account for other factors, such as, the presence of other damaging organisms, differences in host susceptibility and behaviour of infective larvae in both types of soil which may have influenced infection. Hatch in soil was similar to that in water. The marked decrease in hatch after the third week in both soil and water indicated the onset of the plateau phase which was expected at the

TABLE 17

The effect of wheat roots on hatching from cysts in sterilised and unsterilised soil after 3 and 6 weeks, compared with hatching in water.

Mean percentage hatch of 4 replicates

Time of sampling (weeks)	Water	Sterilised soil		Unsterilised soil	
		Wheat roots	No roots (control)	Wheat roots	No roots (control)
3	26.3	22.3	22.0	30.5	30.5
6	27.8	26.8	27.8	36.3	36.5

Individual means

L.S.D. (3 weeks harvest) 5% = 4.9

L.S.D. (6 weeks harvest) 5% = 7.1

higher temperatures following exposure of cysts to cold in the field.

(3) Effect of different osmotic potentials on phase 1 and phase 2 of hatching

Previous results indicated that phase 1, but not phase 2 of hatching, could proceed in soil near the wilting point for plants (page 27). Also, development, but not eclosion, proceeded in 0.4M NaCl (page 52), although the passage of ions through the eggshell and cuticle of the dormant larva may have had some deleterious effect. To establish more critically the relationship between osmotic potential and the processes leading to hatch, an experiment was designed in which the two processes were separated.

Method

Four replicates of 200 eggs isolated from new-season's cysts colleced from the field in early autumn before the onset of low soil temperatures, were used. They were incubated in solutions of different molarity at 10°C for 5 weeks to allow a reasonable proportion to complete phase 1. The eggs were then moved to water at 20°C for a further 2 weeks to allow phase 2 (eclosion) to proceed. The overall hatch at the end of this time was used to measure the extent of phase 1 development at each of the different osmotic potentials. Similar batches of eggs from the same source were incubated first in water at 10°C for 5 weeks, to ensure that an adequate number would hatch. They were then

transferred to solutions of different molarity at 20°C for 2 weeks. As the same amount of phase 1 development would have occurred in all batches at 10°C, the numbers hatching in 2 weeks at 20°C were used to measure the effect of osmotic potential on phase 2. After a further week, the different osmotic solutions were replaced with water for the remainder of incubation. To overcome any possible deleterious effect of electrolyte ions, glycerol as suggested by Wallace (1966) was used in de-ionised double-glass-distilled water to make up the different molar solutions, at the following concentrations:-

0.0 (water); 0.125M; 0.25M; 0.5M and 1M

Regular weekly counts and changes of all solutions were maintained for the first 7 weeks, after which less frequent counts were made until near "total" hatch. When eggs were moved from an osmotic potential solution to water they were washed twice in distilled water.

Results

When different osmotic potentials were applied to the first part of the hatching process, the total number hatched up to 2 weeks after eggs were moved to water at 20°C decreased with increasing osmotic pressure (Table 18). Approximately the same total number of eggs hatched by the end of incubation, except at 1M where fewer hatched than in continuous water (Table 18).

TABLE 18

Average numbers of larvae hatched after 5 weeks in different molarities at 10°C followed by incubation in water at 20°C for 2 weeks and 13 months.

Molarity	2 weeks in water at 20°C	13 months in water at 20°C ("Total hatch)
0.0 (water)	94.0	138.5
0.125M	81.0	129.0
0.25M	60.25	124.75
0.5M	57.75	131.5
1M	47.0	116.0

L.S.D. 5% = 8.47 L.S.D. 5% = 13.07

When different osmotic potentials were applied to the second part of the hatching process (following 5 weeks at 10°C in water), numbers hatched in the first 2 weeks at 20°C decreased with increasing osmotic pressure (Table 19A). Negligible hatching occurred in 1M glycerol, but when it was replaced with water after 3 weeks, the rate greatly increased for about the first 7 days, then decreased to parallel that in continuous water (Fig. 20). However, numbers hatched following the 1M treatment were fewer, even after 12 months in water (Table 19B). About the same "total" hatch was reached in the other osmotic potential solutions as in water.

Discussion

If the effects of osmotic potential and suction potential are regarded as interchangeable, these results indicated that although phase 1 was most rapid in water, it can proceed at much higher suctions than are ever likely to be encountered in soil under field conditions. Almost the same "total" hatch was eventually attained, except at 1M, which suggested that the effect of desiccation was through retarding phase 1 development rather than causing any loss of viability, except perhaps at 1M glycerol. The plateau phase may have been induced in more eggs at 20°C following 1M at 10°C, but this was not established as eggs remaining were not checked for viability.

TABLE 19

(A) Mean numbers of larvae hatched in 2 weeks at 20°C in solutions of different molarity following pre-cold for 5 weeks.

Molarity	Mean numbers hatched
0.0 (water)	51.75
0.125M	21.75
0.25M	19.25
0.5M	13.25
1M	3.75

L.S.D. 5% = 5.47

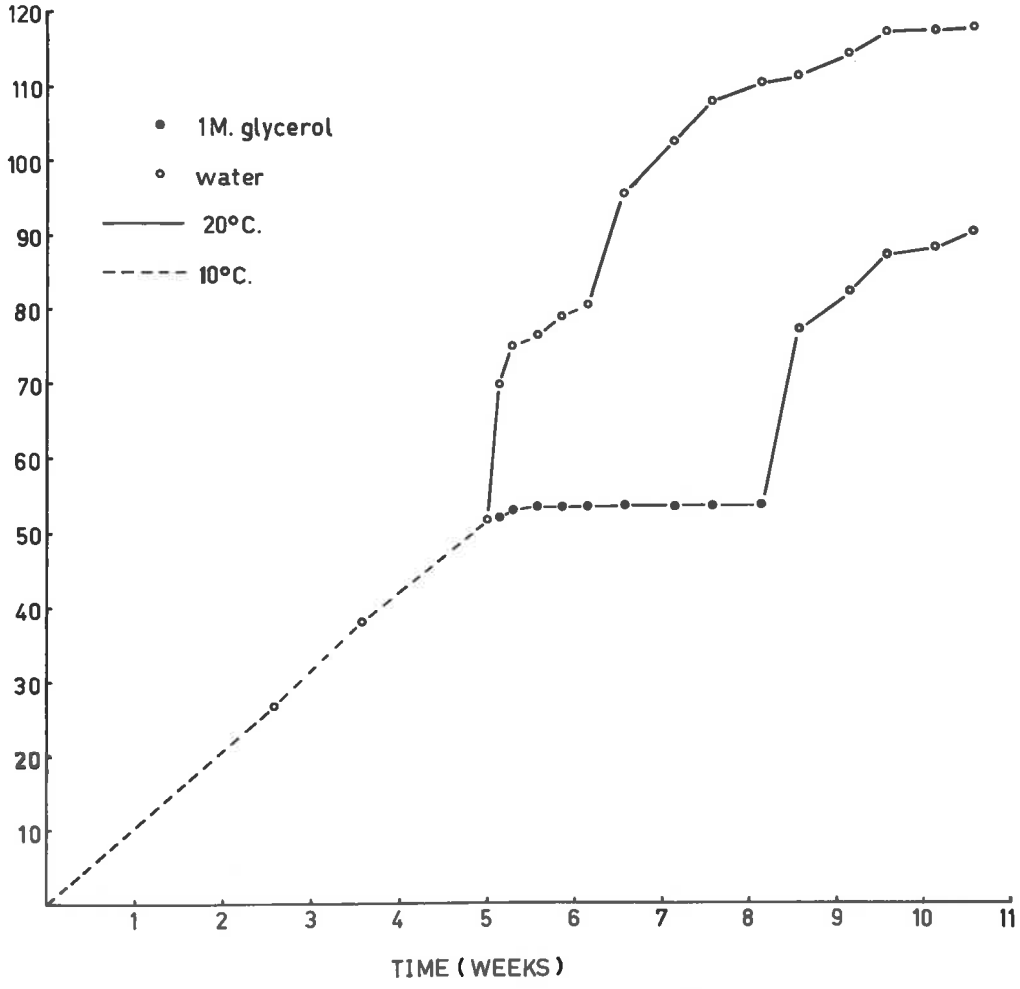
(B) Final hatch of above eggs after a further week in solutions of different molarity followed by 12 months in water.

Molarity	Final hatch
0.0 (water)	144.25
0.125M	134.25
0.25M	138.0
0.5M	146.75
1M	117.75

L.S.D. 5% = 7.64

FIG. 20

EFFECT OF 1M. GLYCEROL FOR 3 WEEKS FOLLOWED BY WATER ON HATCHING AT 20°C. AFTER 5 WEEKS IN WATER AT 10°C.



Phase 2 was also most rapid in water, but was more sensitive than phase 1 to higher osmotic pressures, being almost stopped at 1M, and its rate more than halved at 0.125M, presumably as a result of withdrawal of water from the nematode. This suggested that the change from phase 1 to phase 2 brought about an increase in permeability of the eggshell or cuticle of the larva. Not all eggs which had completed their phase 1 development in water at 10°C, hatched when moved back to water after 3 weeks in 1M glycerol, even after 12 months, suggesting that some had either been damaged or had entered the plateau phase.

If viability of eggs undergoing phase 2 is affected more by dry conditions, then survival of these eggs over summer would be poorer than those undergoing their phase 1 development. Thus, the slow development of phase 1 during this time of the year would permit better survival.

The response of eggs of H.avenae undergoing phase 2 to osmotic potential, appeared similar to that of encysted eggs of H.schachtii, in which larval emergence from cysts decreased rapidly as the solute concentration exceeded 0.01M regardless of the solute used (Wallace, 1956b). In most fertile soils, the salt concentration of the soil water does not exceed 0.05M (pF = 3.05) and so eclosion

in South Australian soils which are mainly sandy and low in fertility would normally be controlled by soil suction.

5. SOME BIOLOGICAL ASPECTS OF H.AVENAE PRODUCED UNDER CONTROLLED CONDITIONS

Introduction

Previous investigations define the type of dormancy which exists in eggs of H.avenae, and optimum conditions for its arrest. Apart from negative evidence for any influence of an inherent seasonal hatch cycle (page 35), nothing was known of the factors affecting its onset.

Cysts used in all previous work were collected from the field where infection, development and egg production probably occurred over a relatively long period of about 5 to 6 months. Eggs of the same generation varied considerably in age, hence they and their mothers were exposed to extremely variable environmental conditions during development. This probably accounted in part for the different times individuals took to undergo the first phase of development and hatch, although it is possible that rate of development is genetically controlled. Among some arthropods e.g. onset of diapause, a type of dormancy, is environmentally determined (facultative diapause), while in others it is heritable (obligatory diapause) (Andrewartha, 1952). Sometimes the environmental stimulus may be received by the mother and the "message" for the onset of diapause is passed on to her offspring during egg laying, while in other species the egg must be exposed to the particular environmental stimulus (Andrewartha, 1952)

for diapause to occur.

The main aim of the following investigations was to gain evidence for either an inherent dormancy or one which was induced by environmental conditions, by testing the hatchability of encysted eggs of about the same age, produced under controlled conditions. The opportunity was also taken to examine embryonic development of eggs within females feeding on and removed from roots, and of eggs removed from females. Also examined was the influence of the host and environment on rate of reproduction, length of life cycle and fecundity.

Method

Hosts used in decreasing order of time taken to mature were, Heron wheat, Prior barley and 2137 barley.

Three-day-old seedlings were grown in sterilised potting soil for 10 days to develop a healthy root system before replanting into uniformly infested field soil. After exposure to infection for 12 days, the plants were removed and the roots thoroughly washed before replanting each cultivar in batches of three, into each of several 5 inch pots containing sterilised potting soil. The pots were placed in a controlled environment growth cabinet at 20°C with a 14-hour-light period where conditions for growth were maintained as near optimum as possible. Soil was adjusted to field

capacity 2 to 3 times weekly throughout. At the commencement of egg laying, half the pots containing wheat or 2137 barley were moved to another growth cabinet where conditions were the same except the temperature was reduced to 12°C.

(1) Effect of environment on embryonic development and survival of eggs

As shown previously, eggs which had not developed to the second larval stage when removed from the feeding mother, failed to survive in water (page 69). Relatively few eggs were used, and so the experiment was repeated using larger numbers to ascertain whether eggs within the females could survive and complete embryonic development while the female was not feeding.

Prior barley was the only host used. From the start of egg laying, females were sampled at about weekly intervals for the first 3 weeks and then less frequently until the cysts had matured. At each sampling time, all females were hand-picked from the roots in one pot and divided into two equal batches. One batch was used to determine the average egg content per female using the dilution method, and hatching in soil was determined by the number of empty eggshells. Four replicates of about 100 of these eggs, (fewer in the first sample because of insufficient eggs), were examined to determine the proportion of normal and abnormal eggs. The same eggs were then incubated at 20°C for 2 months to record any changes. The remaining batch of cysts was incubated in water at 20°C for

2 months when hatching was assessed, and the cysts squashed to determine average egg content and the proportion of normal to abnormal eggs as before.

Results

All cysts were of similar size. After 2 months in water, abnormal eggs were easily distinguishable by their very dark and vacuolated appearance, and in some there were also signs of advanced disintegration of the egg and eggshell.

While the female was attached to the root and feeding, eggs produced, survived and developed to second-stage larvae (Table 20). When eggs at an early stage of development were removed from feeding females, as many as 77% failed to develop fully and survive after 2 months in water, but all eggs survived that had already reached the second larval stage within the feeding female (Table 20). Partially differentiated embryos survived better in females removed from roots than as free eggs.

Young females continued to produce eggs when removed from food, and most of these eggs developed fully and survived in females of the first two samples (Table 20). Older females gradually lost this ability so that by 36 days after commencement of egg production, few extra eggs were produced after feeding stopped. Whilst feeding, egg production continued for up to 76 days in a sigmoid fashion with

TABLE 20

Average egg content, embryonic development and survival of eggs within mothers feeding and removed from host roots, compared with embryonic development and survival of free eggs in water at 20°C.

Sampling Time (Days after commencement of egg production)	Eggs observed immediately after removal from host			Eggs observed after 2 months free in water			Eggs observed after 2 months in water as encysted eggs			
	% Partially differentiated	% Differentiated 1st & 2nd-stage larvae	Av. egg content/ female	% of normal eggs with 2nd-stage larvae	% hatch	% Abnormal eggs	% normal eggs with 2nd-stage larvae	% hatch	% Abnormal eggs	Average egg content / female
0	93	7	1.7	23	0	77	87	0	13	64
9	98	2	14	28	13	59	96	0	4	55
16	86	14	39	40	1	59	65	0	35	97
23	78	22	102	43	3	54	56	1	43	141
36	44	56	206	28	0	72	34	2	65	203
57	12	88	318	38	0	62	81	1	18	353
76	4	96	421	65	0	35	92	0	8	404
112 *	0	100	398	100	0	0	99	1	0	370

* All cysts brown with eggs containing second-stage larvae.

maximum rate of production occurring between 23 to 36 days after commencement (Table 20; Fig. 20A).

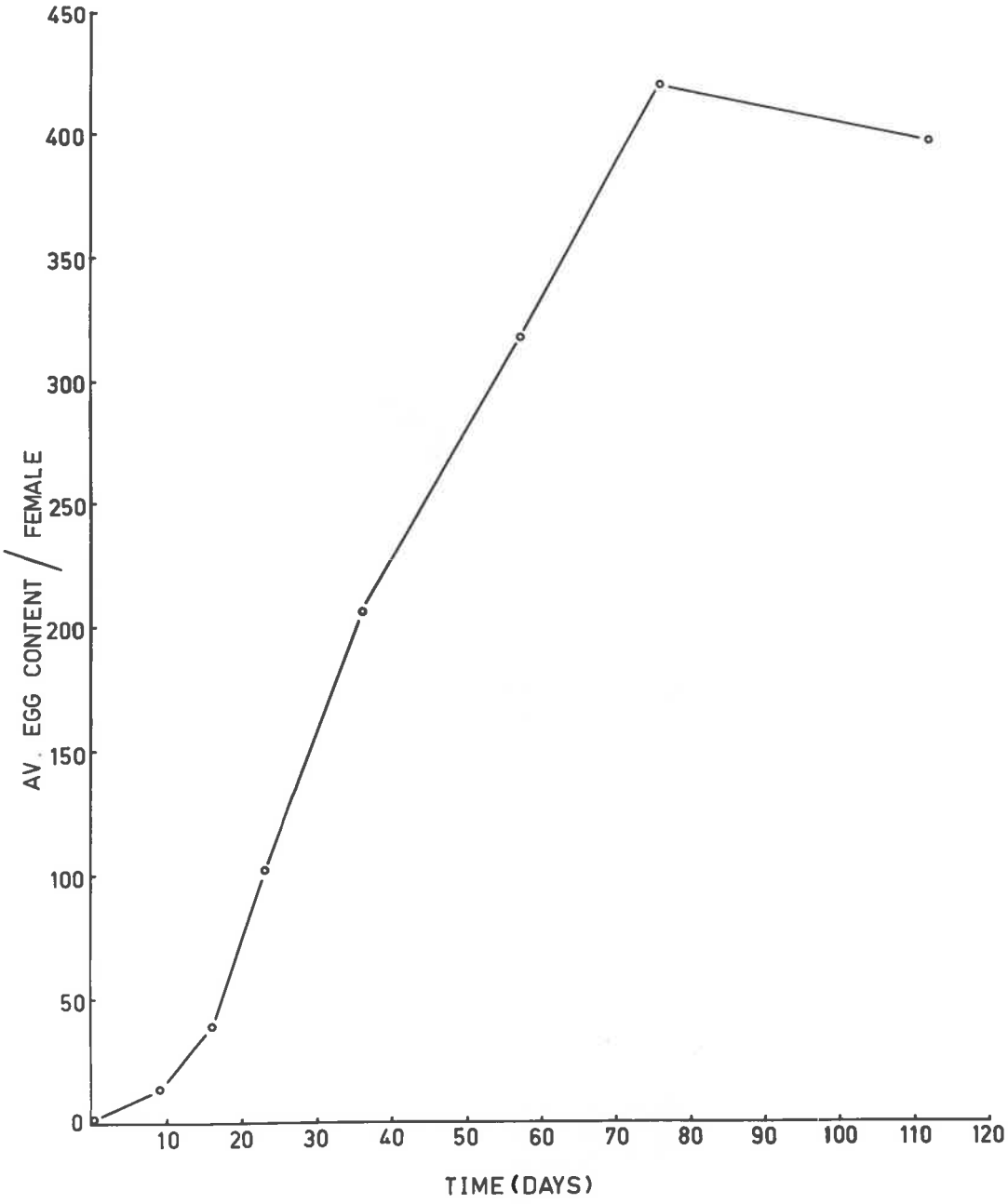
No hatching occurred in soil throughout the sampling period as no empty eggshells were found in immature or mature cysts, and negligible hatching occurred from free and encysted eggs in water after 2 months at 20°C (Table 20). Thus, hatching was negligible even after 170 days from when the first eggs were produced.

Discussion

An atypical environment may have been responsible for the failure of a large percentage of partially differentiated eggs to develop and survive outside the female, because survival was improved when these eggs were left within the female. Eggs removed from females may have been damaged, but this seemed unlikely considering the care that was taken to release them and the trends in percentages of eggs which deteriorated. Complete development of all eggs was only achieved while the female was feeding, suggesting that once the egg had been formed, possibly some substance or nutrients were supplied by the female which enabled embryogenesis to be completed. In early stages of egg production, females apparently contained sufficient reserves to allow complete development of the few eggs produced after removal from the host. Inability to produce more eggs in the absence of food occurred at

FIG. 20A

RATE OF EGG PRODUCTION ON PRIOR BARLEY AT 20°C.



the time of maximum rate of egg production. At this stage, older females probably had inadequate reserves to produce more eggs without feeding, and further feeding was necessary to acquire more of the necessary component to complete embryonic development of those eggs already produced. Such a component may be required for the first moult, as apparently it was not until development had proceeded beyond the first moult that survival over 2 months was assured.

The long time taken for hatching to commence will be discussed later in this section.

(2) Effect of host and temperature on length of life-cycle and fecundity

Effect of host and temperature on rate and extent of egg production and cyst maturation, were examined by sampling females which had developed for various times on 2137 barley plants growing at 12°C or 20°C, and on Prior barley growing at 20°C. For comparison, females and mature cysts from wheat growing at 12° and 20°C respectively, were sampled once, 237 days after infection. Egg production was based on 80 to 100 females.

Results

About the same number of mature cysts, (50 - 59 per plant) were produced on wheat and both barley cultivars at 20°C. The

average egg content per mature cyst produced on each of the three hosts was about the same at 20°C (Table 21). Maturation of cysts was earlier on the earlier maturing barley cultivar, 2137 (Table 21). Low temperature retarded maturation of barley and wheat, greatly extended the period over which eggs were produced and resulted in a significant increase in egg production per female ($P = 0.01$). Some females were probably still producing eggs at the low temperature 237 days after infection.

Discussion

Wheat and both barley cultivars supported about the same egg production at 20°C, but further work is required to determine whether they are equally efficient hosts.

Low temperature delayed maturation of the host which permitted feeding and egg production to continue for a much longer period. This more than compensated for the slower rate of egg production at 12°C, thus resulting in a greater number of eggs produced than at 20°C. Also, egg production appeared to commence earlier in the earlier maturing barley cultivar 2137, indicating that the life-cycle of the nematode was related to rate of maturation of the host. If maturation of the host is hastened by adverse environmental conditions, then a decreased number of eggs could be expected, as rate of maturation of the host has more influence than rate of egg production on the final number of eggs produced.

TABLE 21

Effect of host and temperature on cyst maturation, egg production and development

Days after infection	Host	Egg production at °C	Stage of host maturity	% Brown cysts	% differentiated eggs (1st and 2nd-stage larvae)	Av. egg content per female
85	Prior Barley	20°	Anthesis	0	50	206
	2137 "	20°	Late dough	10	70	365
	2137 "	12°	Jointing	0	5	62
125	Prior Barley	20°	Grain hardening	50	96	423
	2137 "	20°	Mature	100	100 *	361
	2137 "	12°	Ear emergence	0	20	158
161	Prior Barley	20°	Mature	100	100 *	398
	2137 "	20°	Mature	100	100 *	370
	2137 "	12°	Grain hardening	10	40	419
237	2137 Barley	12°	Grain hardened Plants alive	25	98	517
	Heron Wheat	20°	Mature	100	100 *	359
	Heron Wheat	12°	Grain hardened Plants alive	15	95	643

* All second-stage larvae

(3) Hatchability and survival of eggs

Because partially differentiated eggs from immature cysts failed to survive in water, only hatchability of fully differentiated eggs from mature cysts produced at 20°C was compared. However, to compare the hatchability of eggs produced at 12° and 20°C, on 2137 barley, it was necessary to use a proportion of white cysts from 12°C, as only 25% had matured at that temperature by the time of the final harvest. Most of these eggs, however, appeared to be fully differentiated.

Four replicates of about 200 eggs were sub-sampled from 80 to 100 cysts produced at 12°C on 2137 barley, and at 20°C on Prior and 2137 barley. "Total" percentage hatch, calculated after excluding abnormal eggs, was assumed to have occurred when hatching had practically ceased. Because the time taken to reach "total" hatch could not be precisely established, hatching was assessed on the time taken to reach 50% of the "total".

At the completion of incubation, the proportion of normal to abnormal eggs was determined. As the number of eggs in each block had been determined initially, those which had completely disintegrated by the end of incubation could be calculated, and were included in the abnormal egg count.

Results

Hatching was continuing in all treatment after 8 to 9

months, but at a slow rate.

Time taken to reach 50% of "total" hatch at 10°C was about half that taken at 20°C, and was independent of host and temperature at which the eggs were produced (Table 22). Eggs produced on 2137 barley at 20°C hatched faster than those produced on Prior. "Total" hatch from eggs produced at 20°C on both hosts was about the same at both hatching temperatures. "Total" hatch of eggs produced at 12°C was similar when hatched at 20°C, but much reduced when hatched at 10°C (Table 22).

There was a higher percentage of abnormal eggs after hatching at 20° than after hatching at 10°C (Table 23).

Discussion

The faster rate of hatching of eggs from the earlier maturing cultivar 2137 barley, indicated that these eggs were further advanced in their phase 1 development than those from Prior. This was further evidence that the life-cycle was related to maturity of the host. Hatching was not complete and was extended over a much greater time than was taken for egg production, regardless of the controlled environmental conditions at which they were produced. This suggested that time of hatching might be partly controlled genetically.

Of the eggs produced at 12°C, fewer hatched at 10°C than

TABLE 22

Effect of host and temperature during egg production, on rate and extent of hatching of eggs at 10° and 20°C.

"Total" percentage hatches, mean of 4 replicates.

Host	Temperature during egg production							
	20°C				12°C			
	"Total" % hatch at:		Time (days) taken to reach 50% of "total" hatch at:		"Total % hatch at:		Time (days) taken to reach 50% of "total" hatch at:	
	20°C	10°C	20°C	10°C	20°C	10°C	20°C	10°C
Prior barley	65	66	156	85	-	-	-	-
2137 barley	61	56	127	63	61	14	196	59

TABLE 23

Effect of host and temperature during egg production on the mean percentage of abnormal eggs after 8 to 9 months in water at 10° and 20°C.

Host	Temperature during egg production			
	20°C		12°C	
	% Abnormal eggs at:		% Abnormal eggs at:	
	20°C	10°C	20°C	10°C
Prior Barley	24	7	-	-
2137 Barley	28	14	32	15

at 20°C, but 50% of "total" hatch was reached sooner at 10°C, indicating that the eggs which did hatch, responded normally to low temperature. The reason why more of these eggs remained dormant at 10°C than at 20°C was not known, but the environment during egg production may have been involved. This aspect needs further investigation.

Eggs survived better at the lower temperature, suggesting that break-down may have been caused by micro-organisms, which presumably were more active at the higher temperature.

(4) Comparison of hatchability of cysts from controlled conditions and the field

In a previous experiment, negligible hatch was obtained from eggs produced under controlled conditions at 20°C, until after 170 days from the commencement of egg production (page 105). Cysts used in earlier hatching studies were sampled from the field usually no earlier than January, by which time hatching in water commenced immediately. To demonstrate this initial lack of hatching from younger cysts from the field, hatchability was examined from the white-cyst stage onwards (September to April) by incubating six replicates of 50 cysts for each sample at 20°C. Weekly counts were maintained throughout.

Results

Only the percentage hatches after 1 and 6 months

(Table 24) are presented as these are representative of the continuing differences.

Negligible hatching occurred from the immature cysts collected in September (Table 24), as most eggs failed to survive in water. By October, cysts had begun to turn brown and so breakdown of those eggs which had not fully differentiated would again have partly accounted for the poor hatchability at this time.

From November on, all cysts were brown and all eggs fully differentiated. However, hatchability assessed 1 month after incubation was negligible till the January sample, and increased thereafter indicating that eggs were slowly undergoing their phase 1 development. The period of negligible hatch was similar to that found in cysts produced at 20°C under controlled conditions, indicating that duration of phase 1 for eggs from both sources was similar.

TABLE 24

Change in hatchability (mean percentage hatch after 1 and 6 months) of cysts collected from the field from the white-cyst stage onward.

Sampling time	Maturity of wheat	% of Brown cysts	% hatch after 1 month	% hatch after 6 months
September	Ear emergence	0	0	< 1 *
October	Late dough stage	60	< 1	2
November	Mature	100	< 1	6
January	Mature	100	2	16
March	Mature	100	3	17
April	Mature	100	7	22

* Examined and found nearly all eggs had degenerated.

6. MECHANISMS CONTROLLING HATCH

Much of the work on the mechanism of hatching in the genus Heterodera has been concerned with isolating and identifying the active constituent of host-root diffusates that stimulate hatching (Shepherd, 1962). Diffusates may alter egg or larval permeability to permit the passage of water and of other molecules and ions (Dropkin, Martin and Johnson, 1958). Ellenby (1957) suggested a possible connection between hatching activity and ion transport. Studies with artificial hatching agents led Clark and Shepherd (1964) to suppose that irrespective of the means by which larvae were released from eggs, the mechanics of hatching were almost certainly accompanied by changes in the metabolism of the dormant larvae. They saw the crux of the whole question of hatching as the means by which energy was suddenly made available, advancing the idea that it was achieved by hatching agents which acted either directly or indirectly as electron acceptors.

Rogers (1962) has suggested that the hatching factor may be able to enter directly into a sequence of internal secretions, perhaps by replacing some missing component.

These suggested mechanisms involve larval activity and emergence from the eggshell following a series of stylet thrusts.

They pertain, therefore, to the relatively short term stage of eclosion, and presumably the stimulatory effect of root diffusates operates only when the larva is physiologically able to accept the stimulus for renewed activity. The low temperature effect on eggs of H.avenae involves an earlier and relatively long process (phase 1), and so an analogy cannot be made with that of root diffusates because the two effects involve different mechanisms. Viglierchio (1963), suggested that the hatching factor produced by the host may be a limiting component in one of a whole series of physiological reactions controlling hatch of H.schachtii. Part of the reaction could have occurred before the egg came into contact with the hatching factor, as his results suggested that stored cysts may have developed some constituent necessary for the stimulatory reaction of the external hatching factor. A similar hypothesis could partly explain the poor hatching response of young, mature cysts of H.rostochiensis, and the need to store them for about 12 months before obtaining maximum response to root diffusates. Similarly, eggs of H.avenae might need a period of low temperature to initiate the development of the missing constituent.

In some parasitic nematodes, hatching is passive after enzymic breakdown of the eggshell, e.g. Ascaris spp. (Rogers, 1958; Jaskoski and Colucci, 1964) whereas in others such as Trichostrongylus retortaeformis, the inner lipoid membrane of the

eggshell is emulsified by active movements of the unhatched larva, and the consequent uptake of water increases hydrostatic pressure until the eggshell bursts (Wilson, 1958). Wallace (1966) suggested that unhatched second-stage larvae of Meloidogyne javanica secreted enzymes which dissolved the impermeable inner vitelline membrane and so allowed the passage of water. The final stage of hatching was associated with increased larval activity, and was achieved by a series of stylet thrusts followed by escape from the egg by active locomotion.

Various suggestions have been put forward regarding the behaviour of second-stage Heterodera larvae that lead to emergence from the egg, and until recent years it was uncertain whether Heterodera eggs were hatched passively by hatching agents, or whether these substances stimulated unhatched larvae into activity that led to their emergence. Doncaster and Shepherd (1967) filmed hatching of H.rostochienses in a natural hatching stimulant and in a synthetic stimulant. They described larval activity just prior to hatching and the final escape from the egg after a series of stylet thrusts. To gain some understanding of the mechanism controlling eclosion in H.avenae, an attempt was made first to observe emergence of the larva from the egg, and then, differences in morphology and behaviour of unhatched and newly-hatched larvae were examined.

(1) Observations of eclosion

To observe the hatching process, about 100 eggs from cysts which had received 5 weeks incubation at 10°C were placed on the surface of tap-water agar on a slide and covered with a cover-slip. After each observation at 22° to 25°C, eggs were placed at 10°C until the following day.

Results

Slight movement was detected in a few eggs under low power (x 50), and appeared to alternate with periods of rest. Persistent movement was observed in one instance where the head of the larva moved slowly from side to side at one pole of the egg. Taking this as the commencement of hatching, the following sequence of events was observed at x 900 magnification:-

2 minutes after commencement

Five stylet thrusts at one thrust every 2 seconds, each in the same spot. Movement of the head at this time was forward, i.e. pushing forward in the direction of the pole rather than from side to side;

4 minutes after commencement

Three stylet thrusts about 2 seconds apart in the same spot, but in a different position from the earlier ones;

5 minutes after commencement

Two stylet thrusts 10 seconds apart;

7 minutes after commencement

Twenty nine consecutive stylet thrusts in the one spot, taking 48 seconds;

Between 8 and 9½ minutes after commencement

Four more stylet thrusts were made each at a different spot. After each thrust, the larva's head moved very slowly from one side of the pole to the other;

9½ minutes after commencement

The rate andnumber of stylet thrusts all at the same spot were increased to 46 in 55 seconds, and with no further thrusts the larva's head pushed slowly forward through the eggshell until it was clearly free. Motility outside the egg was continuous. Throughout the whole procedure the larva remained at the same pole of the egg, it's head moving slowly from side to side except when making a stylet thrust. At no time was vigorous body movement observed, and even when the eggshell had been clearly broken, the larva slowly pushed its way out and did not passively uncoil, like a spring suddenly being released from tension.

In a second egg where hatching was observed, the larva initially had periods of rest when no body movement was observed. Fifty five minutes after movement was first observed, 54 consecutive stylet thrusts were made in the one spot in 1 minute 38 seconds. No further probes were made during the next 5 minutes after which

time 40 consecutive stylet thrusts were made in the one spot in 2 minutes 40 seconds. Twenty seconds later, with no further stylet thrusts, the larva slowly pushed forward bursting its head through the eggshell at the pole.

Hatching of other eggs was also observed, and the time taken from the first observed head movement varied between individuals, and some failed to hatch, returning to a quiescent state. The following general pattern was common to those which hatched.

The first stage of hatching was positioning of the head at one pole of the egg. Movement was then mainly restricted to the region of the head which moved slowly from one side of the pole to the other except occasionally it would be pushed forward causing a noticeable bulge in the eggshell. This movement was broken by varying periods of rest. The first signs of stylet activity was a rapid backward and forward action without extending beyond the lips. After this, definite stylet thrusts were made while the head was stationary and pressed against the pole of the egg. Once stylet thrusting began, hatching always followed. The extent of each thrust was often clearly visible by the small sharp bulge in the eggshell. Such probes were made at various positions, around the polar hemisphere, initially at a slow rate. Subsequent consecutive probes were more numerous and made at a

faster rate until finally at a particular spot, stylet thrusts were continued at a most rapid rate until the eggshell had presumably been penetrated. The eggshell then appeared to be split apart from the weakened point by the force of the larva moving slowly forward. On no occasion was any vigorous body movement observed.

Each time eggs were moved from overnight storage at 10°C to room temperature, larval movement was observed in a small proportion, but all newly-hatched larvae treated similarly, became highly motile. In a few eggs, movement continued and hatching ensued, but the remainder became quiescent at the warm temperature after 30 minutes to one hour. Movement could be induced in a small percentage of eggs by an increase in temperature after 16 hours at 10°C, but in most cases the larvae eventually became quiescent once more.

Discussion

The events leading up to emergence of the larva were similar to those described for H.rostochiensis in response to a natural hatching stimulant (Doncaster and Shepherd, 1967) with a few noteable exceptions. After movement was detected, positioning of the larval head was at one pole of the egg only, and no circulatory movements within the egg were observed as described for H.rostochiensis. The pattern of stylet thrusting was also

different as it suggested that after a period of testing by spasmodic thrusts, at some particular point in the polar hemisphere, the eggshell was weakened and finally broken by sustained stylet thrusting. The eggshell thus weakened, was then split by the forward bodily thrust. In response to root diffusate, larvae of H.rostochiensis made a continuous, almost straight cut across the end of the egg by the tip of the stylet in order to emerge (Doncaster and Shepherd, 1967).

The best chance of observing the hatching process in H.avenae is in eggs pre-treated to a period of cold, when motility can be observed under high magnification immediately after moving to a higher temperature. If motility ceases, it can be resumed in some eggs after a further cold period. Relatively large numbers of eggs are also required as only a small percentage hatch at any one time.

Most unhatched larvae differed from newly-hatched in that they did not respond to a period of low temperature and remained immobile.

(2) Differences between unhatched and hatched larvae

(a) Motility

To examine the motility of dormant larvae, about 200 eggs from cysts collected in summer were placed in a small drop of water

on a slide, and unhatched larvae were freed from their eggshells by applying gentle pressure on a cover-slip. Motility of the released larvae was then compared with that of newly-hatched larvae from the same source of cysts at 22°C, before and after exposure to 10°C for 16 hours Larvae from eggs in the plateau phase were similarly released and motility also observed before and after the cold period.

Immediately after release, some of the dormant larvae moved sluggishly for a short time, but quickly became immobile and stayed this way for the remainder of a two-day observation period. Newly-hatched larvae alternated between a motile and an immobile state throughout the two days at 22°C. Fifty dormant or newly-hatched larvae were then incubated for 16 hours at 10°C, and their motility assessed immediately after moving to 22°C. All the newly-hatched larvae became highly motile within half an hour after removal from cold, whereas all the dormant larvae remained in a typically dormant position.
This was repeated with other similar batches of larvae with the same result. Larvae released from eggs that had been induced into the plateau phase also remained inactive after low temperature.

Discussion

Part of the processes leading to hatching must involve an activation of the dormant, immobile larva, because larvae must

be motile before they can hatch. During the last phase of hatching, motility of activated larvae can probably be stimulated by a rise in temperature following a cold period. There was some evidence for this in the previous experiment which suggested that the unhatched larvae that responded to a cold period, were those that had already been activated and were close to hatching. This probably explains why rate of hatching immediately increases in response to a rise in temperature following a cold period.

As the stimulating effect of a rise in temperature following a period of cold on motility was only temporary, the rate at which activated larvae could escape from the egg might be increased by a fluctuating temperature, provided the optimum degree of temperature rise and frequency of temperature change was applied.

Larvae from "plateau" eggs behaved similarly to inactivated larvae, suggesting that both types were in a similar condition.

(b) Morphology

Dormant and active larvae were examined to determine whether any anatomical characteristics were associated with the initiation of activation. Comparisons between about 100 newly-hatched larvae and larvae freed from eggs were first made using a dissecting microscope at X50 magnification.

Larvae immediately after hatching invariably appeared lighter in colour compared with unhatched dormant larvae released by squashing. In newly-hatched larvae, the body contents appeared less dense, particularly just below the cuticle and in the pharyngeal region. Occasionally an unhatched larva was found with a less intensely coloured pharyngeal region, and only infrequently was one found light-coloured. The intensity of colour was due to granules which were both abundant and dense in typically dormant larvae, but were almost absent, and far more diffuse in newly-hatched, active larvae. The relatively few unhatched larvae with a light-coloured pharynx were most active and presumably were those almost ready to hatch.

Morphology in more detail was examined using an ordinary light microscope (X900) after the following preparation. Larvae were killed in hot 0.5% acetic acid and fixed in F.A. 4:1 (Goodey, 1963). After 24 hours in fixative, the nematodes were processed in 0.0025% cotten blue, using the rapid lactophenol processing method (Goodey, 1963). As the concentration of cotten blue was insufficient to stain the nematodes satisfactorily, - 0.01% lactophenol cotten blue was tried, but it took more than an hour for the stain to be absorbed. Finally, 0.2% lactophenol cotten blue was found to be satisfactory for both types of larvae, but dormant larvae required a longer processing time.

Dormant larvae were in the second stage possessing a well developed stylet. Features of the anatomy generally were clearer in newly-hatched larvae, presumably because they contained fewer fat globules.

To achieve better resolution and contrast, larvae of both types were anaesthetised, mounted in 1% Beta phenoxyethanol in tap water and examined under phase contrast. Many of the features of hatched larvae were made much more distinct, but differentiation was not improved greatly in dormant larvae. Mounting larvae in 30% Bovine ox plasma in 1% Beta phenoxyethanol (Ellenby and Smith, 1964) did not offer any particular advantage.

Subventral pharyngeal glands of hatched larvae contained dense granules, but these were absent in pharyngeal glands of unhatched larvae, except in the few which possessed a light-coloured pharyngeal region when first released from the egg; these larvae, which presumably were ready to hatch, contained granules in the swollen distal ends of the subventral gland ducts near the entrance to the lumen of the pharynx (Plate 1). Other differences observed after examining about 100 larvae of each type are summarised in Table 25, which also includes behavioural differences.

PLATE 1

Active larva with clear pharyngeal region, photographed immediately after release from the egg to show granules (arrow) in swollen distal end of subventral pharyngeal glands.

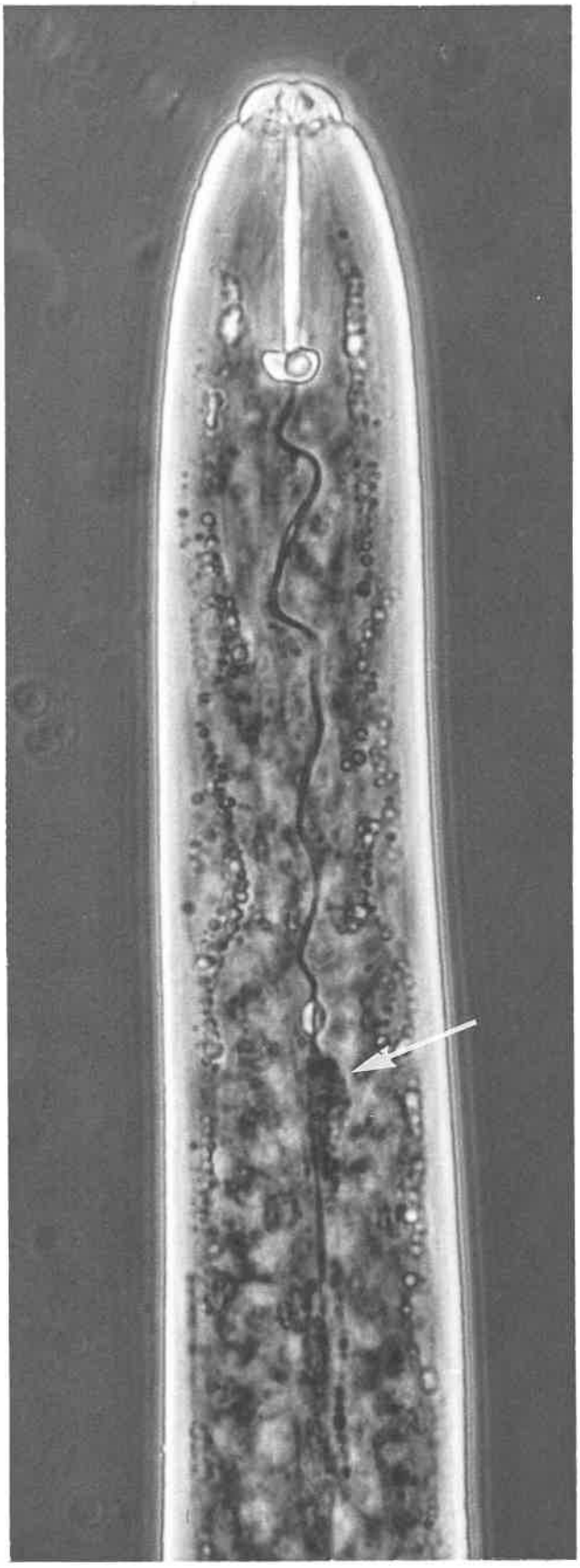

10 µ

TABLE 25

Behavioural and Morphological differences between newly-hatched and unhatched larvae.

Newly-hatched larvae	Unhatched larvae
Active	Inactive (until just before hatching)
Respond to a low temperature stimulus	Do not respond to a low temperature stimulus, unless just before hatching
Invariably light-coloured in pharyngeal region	Nearly always dark-coloured in pharyngeal region except in a few larvae presumably almost ready to hatch
Stains absorbed more readily	Stains absorbed less readily
Well developed pharyngeal glands with subventral gland full of granules	Pharyngeal glands less pronounced with no sign of granules, except in larvae presumably almost ready to hatch
The three gland nuclei prominent. The largest, (about twice the size of the other two) possessed a prominent nucleolus and was the most anterior suggesting it was in the dorsal gland	Gland nuclei difficult to see. No large nucleus observed except in larvae with a light coloured pharyngeal region
Annular rings more prominent	Annular rings less prominent

Discussion

Unhatched second-stage larvae of H.avenae differed from hatched larvae not only in morphology but also in behaviour. Thus, physiological and morphological changes take place before hatching occurrs. How quickly and when these changes occur before hatching is not known, except that motility and the appearance of granules in the subventral pharyngeal glands probably occur close to hatching. Whether these observed changes in morphology are associated with activation of the larva is also unknown. However, increase in pharyngeal gland activity just prior to hatching may be associated with enzymic secretions which denature the eggshell, thus aiding hatching. A similar mechanism has been suggested for eggs of Meloidogyne javanica in which protein granules were observed in the subventral pharyngeal glands immediately prior to hatching (Bird, 1968). Doncaster and Shepherd (1967), however, thought that activity of pharyngeal glands prior to hatching of eggs of H.rostochiensis, was unimportant in relation to hatch.

The significance of the change from a dark to a light-coloured pharyngeal region will be examined and discussed further in the following experiments.

(3) Changes in larvae associated with phase 1 of hatching

Introduction

Phase 1 might involve physiological processes leading to activation of the dormant larva and secretion and action of enzymes on the eggshell, while phase 2, which has an optimum temperature similar to that for other activities, is probably associated with subsequent morphological changes and motility of the larva just prior to hatching. Although the role suggested for phase 1 appeared likely, the possibility also existed that the effect of low temperature may be directly on the eggshell.

It was thought that if a larva, released from its eggshell, was able to "awaken" and become motile, it would be possible to gain direct evidence that phase 1 represented a period of larval development. First it was necessary to find a suitable means of deciding when a dormant larva, freed from its eggshell, had developed to the same stage as a newly-hatched larva. Larval motility was one obvious means, but another possibility was the change from a dark to a light-coloured pharyngeal region, and so the following experiment was carried out.

(a) Morphological changes in dormant larvae removed from eggs

Fifty eggs from a few new-season's cysts collected in summer were squashed using fine needles, and the freed larvae

incubated singly in water at 20°C.

Initially, all possessed a dark-coloured pharyngeal region, and were immobile (Plate 2). They were examined daily over the next 10 days, during which time, the pharyngeal region of six larvae gradually lightened, and the change from an immobile to a motile state accompanied this, i.e. the larvae were motile when their pharyngeal regions were light (Plate 2). The remainder did not undergo the same transition, and in time were found to be dead, probably as a result of injury when they were released from the eggshell.

Although the method of freeing larvae appeared unsatisfactory, the observation demonstrated it was possible for larvae to transform from an inactive to an active state outside the protective eggshell. This indicated it was valid to assume that larvae transformed from a dark to a clear pharyngeal region outside the egg, were at the same stage of development as newly-hatched larvae. Also, that it was valid to assume that larvae with clear pharyngeal regions immediately after removal from the egg were about to hatch.

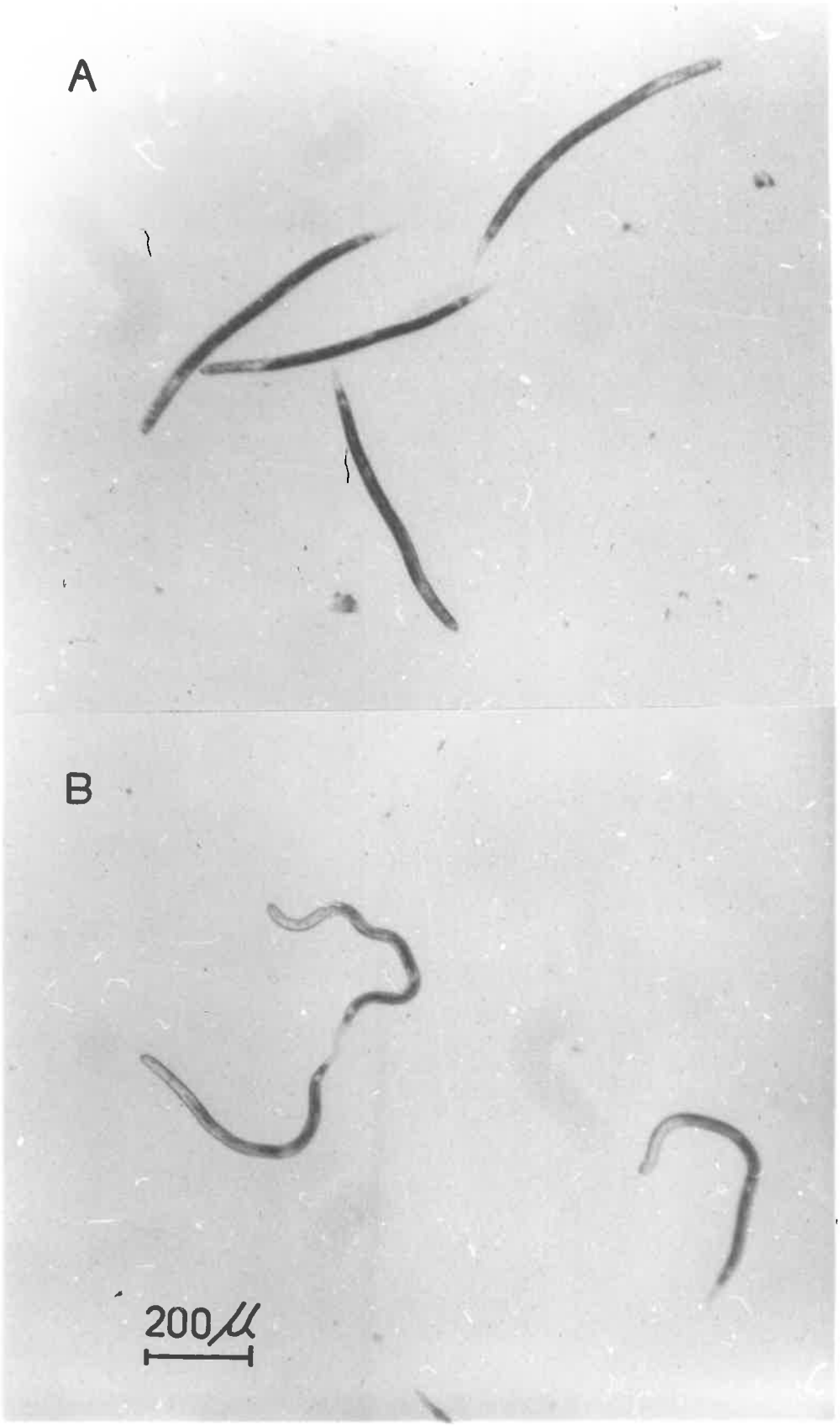
(b) Rate of transformation of dormant larvae to the active state in and outside the egg

Provided a suitable means of freeing dormant larvae from eggs was used, their transformation to the active state could be

PLATE 2

A. Immobile dormant larvae with dark pharyngeal regions when released from eggs.

B. The above larvae after transformation to the active state showing clear pharyngeal regions and motility.


A
B
200μ

recorded and compared with rate of hatching. If the rate of such a transformation paralleled hatching and if it proceeded more rapidly at 10° than at 20°C, this would indicate that phase 1 was primarily involved with changes within the larva. The following investigation was therefore carried out:-

Method

Several hundred eggs were released from 50 new-season's cysts taken from the field before the onset of low temperatures.

The first sub-sample of eggs was squashed in a small drop of water on a glass slide beneath a glass cover-slip, because the number of injured larvae was considerably lower using this method than when squashing by hand with fine needles. Freed larvae were placed singly in wells of haemoglutination plates containing distilled water. Each transparent perspex plate consisted of 80 separate wells of $\frac{5}{8}$ inch. diameter, $\frac{3}{8}$ inch. deep and $1\frac{1}{2}$ c.c. capacity. The plates were covered with a thin plastic sheet, and were incubated at 10°, 20°, 10°C for 4 weeks then moved to 20°C, or fluctuated daily between 10° and 20°C. Single eggs from the same batch were set up in similar plates and incubated at the same temperatures to determine hatching.

The second sub-sample of eggs from the initial batch released from cysts was divided and incubated in water at constant 10° or 20°C for four weeks. They were then squashed as before to

determine rate of transformation within eggs at both temperatures, and the freed larvae set up in haemoglutination plates at constant 20°C to observe further changes. Eighty single eggs were also incubated at both temperatures for controls.

Weekly observations were made at X50 magnification for the first 4 weeks then fortnightly for the next 3 months. The different categories used to describe stages in the transformation are shown in Table 26.

At the completion of the experiment a representative portion of each type of larva and a number of remaining eggs were squashed to examine for internal pressure. To enable a comparison with hatching, percentage transformation of freed larvae, excluding those injured in release from the egg, was calculated.

Results

Table 27 summarises observations made on released larvae from the first sub-sample of eggs and shows that:-

When first removed from eggs, about 90% of larvae were dormant ("D" and "H" larvae), but these gradually lightened so that after 4 weeks, 80% or more had transformed ("L" larvae). Temperature had little effect on this transformation.

Nearly all "D" larvae were immobile; a few in the intermediate classification "H" were motile, while most

TABLE 26

Classification of larvae freed from the egg

Designated Code	Type of larva	Description of distinguishing features
D	Dormant	Possessed a distinctly darkened pharyngeal region. Mostly immobile, banana shape.
H	Dormant	Possessed a lighter-coloured pharyngeal region than D, but not distinctly clear. Usually immobile.
L	Active. Transformed to apparently the same stage as a newly hatched larva	Possessed a distinctly clear, light-coloured pharyngeal region. Highly motile, but underwent periods of quiescence.
M	Motile larva	"L" larvae highly active.
I	Larva injured presumably during squashing	Larva dead - contained no internal pressure. Body contents very dark.
d	Larva formerly active (designated L) but died as a result of exhausted food reserves	Body very light coloured except for a darker head region. No internal pressure.

TABLE 27

Effect of temperature on the change from a dark to a light pharyngeal region in freed larvae compared with hatching over 16 weeks.

(For definition of D, H, L and d see Table 26)

Temp.°C	Time of incubation (weeks)	Condition of larvae				Nos. of motile larvae			Total no. of injured larvae	% of larvae transformed (%L) based on larvae not injured	% hatch
		D	H	L	d	D	H	L			
10°	0(Initial Obs.)	47	28	5		0	1	4		8	0
	1	20	27	32		1	13	24		52	2.5
	2	20	14	46		0	7	41		74	5
	3	19	12	49		0	7	48		79	10
	4	9	4	58		0	1	54		94	18.5
	16(Final Obs.)	3	0	53	6	0	-	3	18	95	80
20°	0	43	27	8		0	2	2		13	0
	1	25	25	30		0	2	16		49	6.3
	2	22	9	47		0	1	36		77	6.3
	3	21	9	47		0	1	26		77	11.3
	4	6	8	48		0	1	21		79	16.2
	16	3	0	9	49	0	-	2	19	95	31
10°-20° fluctuated daily	0	57	14	9		0	3	3		15	0
	1	34	20	25		1	11	23		47	7.5
	2	29	6	44		0	0	33		83	16.3
	3	29	5	45		0	2	36		85	24
	4	6	2	48		0	0	33		92	34
	16	2	1	7	43	0	-	7	27	94	70
10° for the first 4 weeks	0	51	19	8		0	0	2		14	0
	1	24	28	20		2	8	16		36	2.5
	2	19	23	31		2	8	27		56	2.5
	3	13	20	36		1	6	28		65	6.2
Then 20° ↓	4	5	11	46		1	4	36		84	15
	16	2	0	11	42	0	-	7	25	96	56

classified as "L" were motile, but the proportion of the latter at any one time was dependent on temperature. For example, they underwent alternate periods of rest and motility as did hatched larvae at 20°C, but most became highly motile during the brief temperature rise when removed from 10°C for observation. Degree of motility was also observed to increase as the larva lightened.

Percentage hatch at all times and temperatures was less than the corresponding percentage transformation. Percentage hatch after 1 week was less than the percentage of "L" larvae observed immediately after squashing. "Total" percentage hatch (80%) was reached after 16 weeks at 10°C only.

By 16 weeks, most larvae had transformed to the active state but some of these had died ("d" larvae). The average life of larvae assessed from when they first transformed at 20°C was 42 days, but at 10°C it was considerably lengthened and the majority were still living after 170 days. The average life of larvae which hatched showed little variation and was also 42 days at 20°C, while again at 10°C the majority were still living after 170 days.

Table 28 summarises observations made on larvae from the second sub-sample of eggs which was squashed after 4 weeks at 10° or 20°C and shows that:-

Nearly half the larvae possessed clear pharyngeal

TABLE 28

Percentage of transformed larvae after incubation as eggs at 10° and 20°C for 4 weeks, and after a further 4 weeks at 20°C compared with hatching.

	Eggs pre-incubated for 4 weeks at 20°C		Eggs pre-incubated for 4 weeks at 10°C	
	Observed immediately after releasing from eggs	Observed 4 weeks later	Observed immediately after releasing from eggs	Observed 4 weeks later
Percentage of "L" larvae (active state)	18	80	47	89
Percentage of eggs hatched	-	9	-	18

regions ("L" larvae) when first released from eggs which received 4 weeks cold, while only 18% were at the same stage initially from eggs pre-incubated for the 4 weeks at 20°C. After a further 4 weeks as released larvae at 10° and 20°C, 89 and 80% respectively were in the active state ("L" larvae) but the percentage of eggs hatched was even less than percentage of "L" larvae observed initially.

Discussion

Larvae with their eggshells removed became active, eventually appearing and behaving like larvae which hatched naturally. Accompanying this transformation was a gradual increase in motility suggesting that lightening of the pharyngeal region was due to a gradual consumption of food reserves as a consequence of an increasing rate of metabolism. As the rate of transformation of larvae outside the egg was very rapid and complete compared with hatching, this suggested that before release, the larvae were physiologically at about the same stage and ready to hatch. If this was true then the low temperature effect on hatching involved the eggshell, which must directly or indirectly inhibit hatch. However, larvae within the egg responded more to 10°C, because a larger proportion underwent the transformation to an active state than at 20°C. This demonstrated that the effect of low temperature was primarily involved with a change within the

larva. These two apparently contradictary results could be reconciled on the basis of an unconscious selection during squashing of those eggs closest to hatching. This would also explain why the proportion of hatched eggs after 1 to 4 weeks was less than the proportion of larvae designated initially as active immediately after squashing, i.e. those which would be expected to be in the process of eclosion. The possibility also existed that dormancy was broken by a shock to larvae when eggshells were removed, but this seemed less likely in view of the demonstrated low temperature effect on the larva within the egg.

If, as it appeared likely, larvae near the completion of their development were being unconsciously selected, then their eggshells must either be softer and therefore more easily broken, or the eggs might be more turgid due to imbibition of water, suggesting an increase in permeability. Because most larvae prior to any cold treatment were first observed to be dormant when squashed, any changes associated with the egg such as a denaturation of the eggshell, probably occurred either at the commencement of, or just before the activation process.

The active life of larvae, hatched either naturally or developed outside the egg, was about the same, being at least quadrupled at 10°C, compared with 20°C. This was further evidence that newly-transformed larvae had undergone normal changes outside

the egg, and were at about the same stage of development as newly-hatched larvae. There was a surprising lack of variability in active life between individuals, which suggested that dormancy was broken when food reserves were at about the same level.

(4) Changes within the larva associated with phase 1 development under field conditions

In the field, hatchability of new-season's eggs increased slowly from Spring on, but the rate could be hastened by exposure to low temperatures. To demonstrate that this increased hatchability was associated with changes within the larva, and that these changes occurred more rapidly at 10°C than at 20°C, the following experiment was conducted:-

Eggs from new-season's cysts collected from the field in January and again in May were squashed, and a sub-sample of 80 freed larvae were incubated singly as before at 10° or 20°C. Their rates of transformation were then compared.

Any difference in pressure needed to release the required number of larvae at each sampling time, should be reflected in the different rates of transformation between the two samples. Such a difference would also reflect the amount of development that had occurred in the field between the two times.

Results

Rate of transformation of larvae collected in May was faster than that of larvae collected in January, particularly at 20°C (Fig. 21).

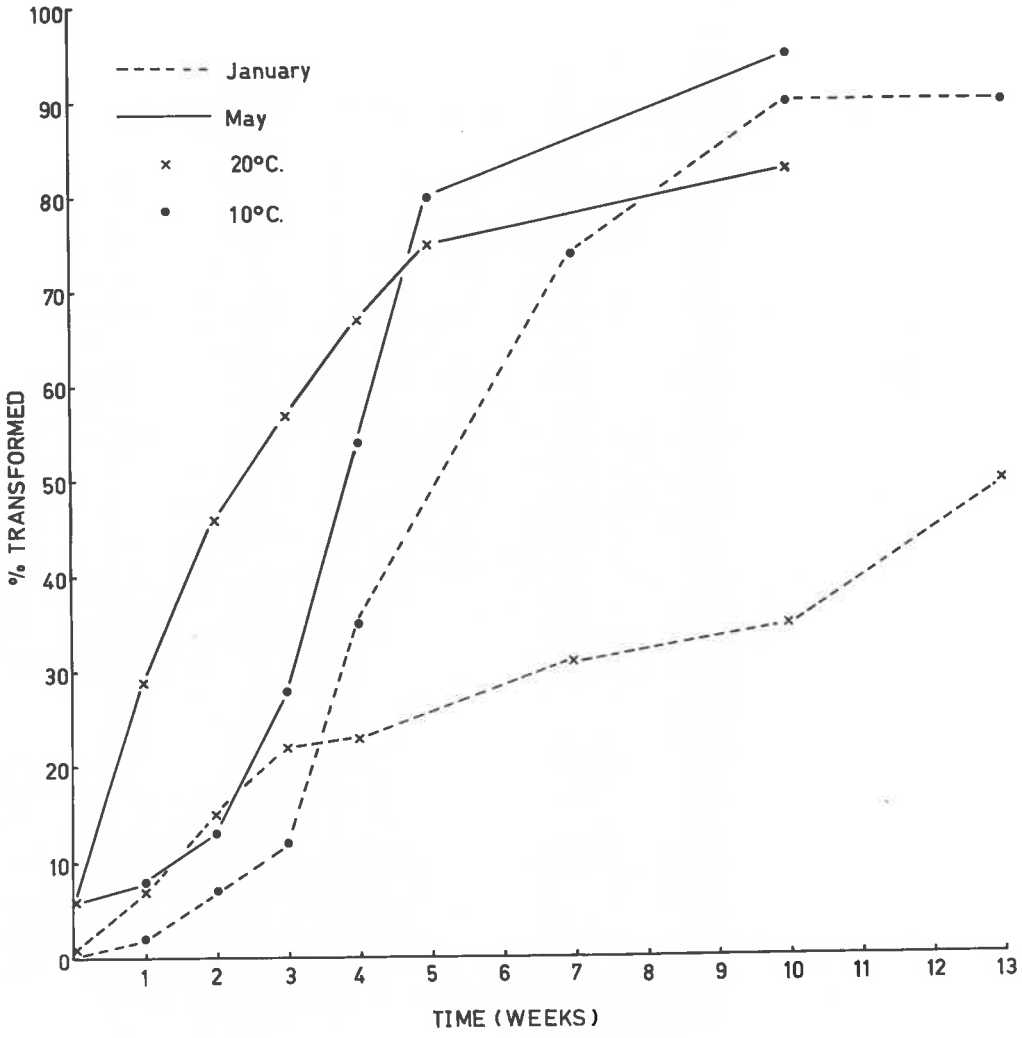
Initially, rate of transformation was faster at 20°C for each sample, but was overtaken after 3 to 5 weeks by the rate at 10°C where "total" transformation was reached sooner.

Discussion

The faster rate of activation of freed larvae collected in May showed that they had undergone some development in the field. This change was hastened by low temperature in the laboratory, confirming that phase 1 of hatching was primarily associated with changes within the larva. The faster rate of transformation initially at 20°C, indicated that some larvae had completed phase 1 and were undergoing phase 2. Thus, increased hatchability of new-season's eggs in the field was due to completion of phase 1 development, i.e. primarily a physiological change had taken place within the larva and this proceeded best at a low temperature of about 10°C.

FIG. 21

RATES OF TRANSFORMATION AT 10°C. AND 20°C. OF LARVAE FREED FROM EGGS COLLECTED FROM THE FIELD IN JANUARY AND MAY



IV. DISCUSSION

Relation to Diapause

Diapause, a type of dormancy occurring chiefly among insects, enables them to withstand extremes of environment and so is regarded as a survival mechanism (Lees, 1955; Howe, 1967). Defined as "a stage in the development of certain animals during which morphological growth and development is suspended or greatly retarded", diapause is a stage in physiogenesis which must be completed before resumption of morphogenesis (Andrewartha, 1952). The onset of diapause usually coincides with seasonal conditions unfavourable for normal growth and development. Before normal activities can be resumed, there is a delay while the individual undergoes the necessary physiological processes, and so diapause differs from quiescence, in which there is no delay in resumption of normal growth once conditions become favourable.

Duration of diapause provides a measure of its intensity, which varies between individuals. The physiological processes associated with diapause development usually have a different optimum temperature and range of temperatures than those required for morphological development. In species, where the temperature ranges do not overlap, diapause is most obvious.

Amongst nematodes the effect of low temperature on hatch of Nematodirus battus, a parasite of sheep, has been interpreted in

terms of diapause (Christie, 1962), and the seasonal variation in hatch of H.rostochiensis has been explained in terms of facultative diapause (Shepherd and Cox, 1967). Diapause has also been suggested for eggs of H.avenae (Shepherd and Cox, 1967; Rogers and Sommerville, 1968), but this was based on a minimum period of 8 weeks of low temperature being required to stimulate hatch (Fushtey and Johnson, 1966) which on the basis of my results is questionable, unless a different species or biotype is involved. A number of aspects of dormancy in eggs of the local population of H.avenae, appeared similar to those described for diapause in insects. These were:-

1. Hatching of eggs was retarded when unfavourable conditions prevailed; throughout summer they did not hatch spontaneously when exposed to favourable conditions in the laboratory.

2. Dormancy was partially arrested with the onset of low temperatures in the field, and this happened most rapidly at about 10°C, the temperature most commonly found to be optimum for diapause development in insects.

3. The division of hatching into two phases with different optimum temperatures fitted the functional definition of diapause.

4. The optimum temperature for phase 1 was abnormally low for morphogenesis i.e. embryonic development, and motility.

However, there were differences from classical diapause in insects.

For example, in many insects where diapause is broken by a period of low temperature, the temperature range for diapause development is markedly different from that for morphological development and little overlap in the ranges occurs, but in eggs of H.avenae the temperature ranges for both phases were about the same. The length of time needed at low temperature for the completion of diapause development in insects is generally specific and the subsequent response to temperatures normal for morphological development is rapid and complete. A complete hatch of eggs of H.avenae did not occur in any experiment, and has not been demonstrated elsewhere.

The different phases for hatching of H.avenae described in this thesis may represent only different processes having different optimum temperatures, which may not necessarily be those regulating physiogenesis and morphogenesis associated with diapause and its arrest. Physiological evidence is therefore needed before diapause can be definitely established.

Mechanisms controlling hatch

The process accelerated by 10°C (phase 1) is known to be associated primarily with activation of the larva and denaturing of the eggshell. This may be purely a long maturation period of variable duration amongst individuals, probably beginning immediately after the first moult in the egg. Subsequent development thereon

probably proceeds towards the formation and secretion of enzymes which may soften or denature the lipoid layer of the eggshell. This could render the eggshell more permeable, thus allowing the passage of water which might be required for the second phase of hatching involving larval motility and eclosion from the egg. The inactivation or release of inhibitors from within the larva, leading to its activation is an alternative hypothesis, although appearing less likely, needs examination. The active life of larvae, whether hatched naturally or transformed from a dormant to an active state outside the egg, showed little variation. This suggested that there was a threshold level of food reserves above which hatching could not ensue, i.e. eggs taking longest to hatch would have the greatest amount of food reserves. The electron microscope, solutions of different osmotic pressure and histochemistry might all be useful in substantiating the hypothesis for hatching.

The failure of eggs to hatch spontaneously once they became fully differentiated appeared to be obligatory and hence probably genetically controlled, because the same order of variation in hatching time was found for eggs produced under controlled conditions as for those collected from the field. That time of hatching is controlled genetically might be demonstrated by selecting over a number of generations, only those larvae which hatch first; this might produce a population that becomes

increasingly hatchable. Further information is required, however, on the effect of different environmental conditions on the mother before commencement of egg laying, and on the developing eggs in order to establish whether subsequent hatching can be modified.

Another type of dormancy (the "plateau phase") can be induced in both free and encysted eggs in water and encysted eggs in soil, by conditions involving a rise in temperature. In these eggs, activation of the larva is delayed but whether this is due to retardation of development or to a reversal of the processes leading to hatching is not known. Results suggested that this dormancy might vary in intensity in individuals depending on the actual rise in temperature and the physiological stage of development when exposed to the temperature increases. Further studies are required to establish this, particularly those concerned with defining optimum conditions for breaking the dormancy. The plateau phase was most easily induced in eggs which were well advanced in their development. Detailed morphological comparisons of larvae undergoing uninterrupted development with those which have been induced into the plateau phase may reveal more precisely when the most responsive stage to a rise in temperature occurs. The influence of the plateau phase probably modified all hatching responses to increasing temperatures and for this reason it is doubtful whether an 80% hatch or greater could ever be obtained in 2 to 3 weeks after initially cooling cysts in soil dry enough to stop

hatching, particularly if the plateau phase can be induced by desiccation. Fluctuating temperatures overcome the onset of the plateau phase in water, and so the greatest subsequent hatch obtainable in 2 to 3 weeks might be possible by applying optimum fluctuating temperatures to cysts in soil at an optimum level of moisture. Hatching in some experiments was allowed to continue for long periods (up to 2 years) in an attempt to achieve a complete hatch, but this was never attained. Induction of the plateau phase was often responsible for a low final percentage hatch, but even at constant temperatures there always remained up to about 25% of unhatchable, viable eggs. It was not known whether these eggs were in the plateau phase or inherently different. Presumably these eggs with a low hatchability eventually respond to low temperature in a similar way to other eggs. This might best be substantiated by testing the hatching responses of encysted eggs older than one year from the field.

Survival

The induction of the plateau phase is a survival mechanism, and in these experiments its controlling influence on hatching in the field was demonstrated in spring when the cereal crop was rapidly maturing. Diurnal fluctuation of temperature might be involved but rising soil temperatures in spring are probably mainly responsible for its onset. Drying soil conditions may be another factor involved with its onset and possibly its arrest,

and this aspect needs further examination. Because the plateau phase is environmentally controlled its extent must vary greatly from year to year by restricting field hatching to a greater or lesser extent thus allowing more or less individuals to carry over from one year to the next. Apart from this important mechanism, other reasons why H.avenae is very well adapted to survive in our cereal regions, and therefore, why it is probably the most important single disease of cereals in South Australia, are:-

(1) The soils are largely lighter textured and are thus suitable for the nematode's activity.

(2) The life-cycle of the nematode is related to maturity of the host. Thus, as there is a wide distribution of naturally occurring, early-maturing grass hosts, in very short growing seasons (drought years), or in situations where there is a wide cropping rotation, some reproduction of the nematode is assurred each year.

(3) Even under our hot, dry soil conditions over summer, there is negligible loss of viability of new-season's encysted eggs, because during this time the majority of eggs are in phase 1 in which they are thought to be more resistant to dasiccation than eggs in phase 2.

(4) Losses of infective larvae through hatching from new-season's cysts in summer in the absence of a host, are negligible because at that time most eggs are still undergoing

their first phase of development and are not hatchable.

Application of results to possible means of control

Phase 1 development was slowest during the hot summer but was most rapid when soil temperatures during late autumn and throughout winter dropped to about 10°C. This occurred even when the soil was at the wilting point for plants which inhibited phase 2 (eclosion). Thus hatching responses to temperature and equivalent osmotic potentials were similar to those obtained in the laboratory. Rate of phase 1 development was not influenced by any inherent seasonal rhythm and so was under environmental control particularly temperature. If it could be substantiated that the mean hatching time, i.e. the time taken for 50% of the population to complete phase 1 and phase 2 is about the same for each new generation it might be possible to predict each year from meteorological data the extent of completed development, and thus when a substantial hatch might be expected to occur in the field. However, further information on the rate of phase 1 development under field conditions and particularly the influence of soil suction would also be required. A more direct but laborious method would be to sample cysts at intervals from late summer onward from various centres each year, and so determine the extent of completed development from hatching tests. With knowledge of the infective life of larvae in soil it might then be possible to advise farmers on the best time to sow to avoid severe

attack, and more objective use of trap crops might also be possible. Such information could also be useful for the better timing of applications of suitable nematicides which might eventually be developed. Ploughing between July and August in preparation for the next season's cereal crop would prevent the development of a new generation thereby reducing the size of the population, but results in this thesis suggest that under certain conditions, a carry over of a substantial proportion of the previous generation might be sufficient to cause serious damage to the subsequent host crop.

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