DIAPAUSE IN THE NEMATODE *MELOIDOGYNE NAASI* (Franklin, 1965)

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A thesis submitted for the degree of Doctor of Philosophy
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August, 1983

ABSTRACT

Factors influencing egg diapause in six isolates of *M. naasi* from Europe (Belgium, France, Germany, Wales), New Zealand and USA (California) have been investigated.

Spontaneous hatch at temperatures from 0° to 40° C was very low in all populations (1 - 3%) with the exception of the Belgian isolate (29% at 20° C).

In all populations chilling was necessary before many fully embryonated eggs could hatch at 20° C. The most favourable chilling temperature and incubation time varied with population: Belgian 10° C for 19 weeks (72% maximum hatch); Californian 10° C for 15 weeks (80% maximum hatch); French 10° C for 17 weeks (73% maximum hatch); German 5° C for 13 or 17 weeks (45% maximum hatch); New Zealand 10° C for 16 weeks (89% maximum hatch); Welsh 10° C for 17 weeks (73% maximum hatch).

Increased hatch at 20° C also followed incubation at "warm" temperatures (above 20° C, up to 35° C) in all populations, but was a weaker and more variable response than to chilling.

Chilling or warm treatments, slowed or stopped embryonation in the eggs, which was resumed normally as soon as they were transferred to 20° C.

Chilling interrupted by short periods of warmth (up to 30 minutes) was found to stimulate hatch, while longer periods abolished the chilling effect, and hatch was inhibited.

A hypothesis (similar to that proposed by Banyer and Fisher (1971) regarding hatch in *H. avenae*) is suggested for egg hatch in *M. naasi* The process is divided into three phases, each with a different optimum temperature: (i) embryonic development (20°); (ii) chilling requirement (5° to 10° C); (iii) eclosion (20° C).

Treatment before hatch and stresses on the parent population, were found to influence the disposition of the produced eggs to enter diapause.

These results are interpreted with reference to similar work, considered when presenting a literature review on the wider subject of arrested development of plant parasitic and free living nematodes.

ACKNOWLEDGEMENTS

I wish to acknowledge the encouragement and support of Dr. A.A.F. Evans throughout this work, for which I am grateful.

In addition, I should like to thank Dr. S. Young and Brenda Flavell for their advice with statistics, computing and graphics, and Professor T. Lees for very enlightening discussions and for reading the manuscript. Thanks are also due to Dr. W.M. Hominick for advice and to Dr. Susan Jepson for her measurements on *M. naasi* ; to B. Briscoe and F. Wright for printing the photographs, and the rest of the staff at Silwood Park for all their co-operation and encouragement.

I also wish to thank Christos Kourouniotis for his encouragement throughout the 3 years of study, and all my friends for their support in every way, especially by making these years enjoyable.

Finally, I wish to thank all the people, too many to mention by name, who were involved in supplying the different isolates of *M. naasi*.

This work was financially supported for two years by the University of London (The Gwynne-Vaughan Studentship and a University Postgraduate Studentship), which I gratefully acknowledge. Also, I owe a lot to my parents for their support, financial and otherwise.

CONTENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

A. General Introduction

Because of their small size, nematodes remained mainly undetected until the last century, and only lately was their economic importance taken seriously. By 1967 about 12,000 nematode species were described, of which 1250 are known or suspected to be plant parasites (Oostenbrink, 1970).

Among the most important plant parasitic nematodes are those belonging to the genus *Meloidogyne*. They are "among the top five major plant pathogens affecting the world's food supply" (Sasser, 1979).

Approximately 37 species of *Meloidogyne* have been described so far, but five probably cause most damage. These are, in the order of importance: *M. incognita*, *M. javanica*, *M. hapla*, *M. arenaria* and *M. naasi* (Sasser, 1979).

M. incognita, *M. javanica* and *M. arenaria* are strictly tropical species with a vast host range. *M. hapla* is found beyond 35° latitude mainly in the northern hemisphere. It is a temperate nematode but can reproduce satisfactorily under tropical temperatures (Dao, 1970). It attacks many vegetables, ornamental plants, shrubs and fruit trees and is probably indigenous in north-western Europe (Franklin, 1979).

In temperate regions, *M. naasi* is the most important *Meloidogyne* species found apart from *M. hapla*. *M. artiellia*, *M. graminis*, *M. arderensis* occur sporadically; many other species attack a small number of plants, so are considered of little economic importance.

M. naasi is widespread (see Table 1) with most reports concerning damage in cereals, but sugar beet and onion are also attacked. This species may be indigenous in both north-west Europe and north-western USA (Franklin, 1979).

M. artiellia was found in England, France, Spain and Greece feeding on brassicas, clovers and cereals, but no serious damage has been reported (Franklin, 1979). *M. graminis* has only been reported in southern USA attacking cereals, turfgrasses and alfalfa (Lamberti, 1979). *M. arderensis* was reported in England attacking woody plants (Taylor and Sasser, 1978) and Scotland, surviving at temperatures as low as -5° C (Stephan and Trudgill, 1982).

Other temperate *Meloidogyne* species known to attack Graminaceae other than *M. naasi*, are: *M. microtyla*, reported from Canada attacking oats, barley, wheat, rye and some grasses (Taylor and Sasser, 1978); *M. ottersoni* (previously known as *Hypsoperine ottersoni*), reported from England and USA attacking cabbage, brussels sprouts, swede, pea, bean, clover and alfalfa (Taylor and Sasser, 1978).

M. naasi is therefore the most important *Meloidogyne* species of the strictly temperate regions, causing serious damage to economically important crops. In common with many parasites, *M. naasi* has to synchronize its life cycle with that of its hosts which, in temperate climates, follow seasonal cycles. "The essence of parasitism is punctuality and the most important effective aid to punctuality is the ability to mark time" (Michel, 1974). To achieve this punctuality, plant parasitic nematodes focus their active life around that of their hosts and become dormant during the time the host is absent or the climatic conditions are unfavourable (see section 1.C).

Dormancy in plant parasitic nematodes may be conveniently divided into two types: Quiescence (a spontaneous response to unfavourable conditions in the environment); and Diapause (an inbuilt mechanism which synchronizes activity of parasite to that of its host) (see section 1.C, page 17)

In synchrony with the host's regular seasonal cycle, *M. naasi* exhibits the latter, more specialized type of dormancy. Most of its eggs hatch in springtime and it appears that warm temperatures (10°-20° C) after a chilling period (temperatures below 10° C) is the signal for terminating diapause in nature (Watson and Lownsbery, 1970; Ogunfowora and Evans, 1977a).

Heterodera avenae, another example of a plant parasitic nematode exhibiting diapause, may depend on host root exudates as well as temperature for terminating dormancy (Kerry and Jenkinson, 1976; Williams and Beane, 1979; Banyer and Fisher, 1980).

M. naasi therefore provides a good model for studies on diapause in plant parasitic nematodes, and the wide geographical range from which isolates have been recovered, also provided an interesting variety of material for comparative studies.

In studing the embryonic development of *M. naasi*, Siddiqui and Taylor (1970) reported that the J_2 stage is reached after two weeks at 22° to 26° C. However, in the field as well as in the laboratory, the J_2 inside the eggs stay dormant during winter and hatch in the following spring (Watson and Lownsbery, 1970; Ogunfowora and Evans, 1977;

Gooris and D' Herde, 1977). If secretions from the host roots do not stimulate hatch (Watson and Lownsbery, 1970), then the environment, the host, the female or the J_2 itself are responsible for the induction, duration and termination of this dormancy. Knowledge of the factors involved would help us understand the epidemiology of such parasites and aid in their control.

To understand dormancy in plant parasitic nematodes, to relate this phenomenon to their survival strategies in general and to the way *M. naasi* in particular adapted its life cycle to that of its host and temperate climate, the literature on arrested development in plant parasitic and free living nematodes was reviewed (see section 1 C).

M. naasi, like other plant parasitic nematodes may become quiescent after the termination of diapause, in unfavourable enviromental conditions. Understanding both types of dormancy (quiescence and diapause), was therefore considered very important for general understanding of the theoretical aspects of this work, as well as the problems that were likely to be encountered while working with this nematode.

In this study, factors responsible for the induction and termination of diapause in *M. naasi* were looked for and investigated. The survival and hatching behaviour of eggs under constant, alternating and interrupted incubations at various temperatures were studied extensively as the main environmental factors concerned with the termination of diapause.

Populations from Europe (Belgium, France, Germany, Wales), USA (California) and New Zealand were cultured together in the laboratory to observe differences in their hatching behaviour. To characterize the populations, details of various biological factors were studied and the different populations were compared morphologically.

Stress was exerted on the eggs, on the female via the host or directly, and on the J_2 before invasion, to examine possible effects of such factors on hatching behaviour.

However, by setting up cultures in the laboratory under standardized conditions, it is possible to introduce factors in the experimental system which might differ from those under natural conditions. The biggest problem is the possibility of introduction of bias, by selecting individuals best suited to the conditions offered in the laboratory and the loss of others due to their lack of competitiveness. To allow

for such problems, the hatching behaviour of the original material sent from the field was studied before culturing started, and compared to that of the later generations reared in the laboratory.

During the project, an account of the fate of all eggs per eggmass is given.

B. *Meloidogyne naasi*: Bionomics

M. naasi, the "cereal root-knot nemadode", "barley root-knot nematode" or "gramineae root-knot nematode" as it is known in UK, USA and Belgium respectively, is an obligate endoparasite of grasses, cereals, sugar beet and onion. It is reported to attack 125 hosts belonging to 25 different families (Vovlas and Inserra, 1979).

In California, *M. naasi* was reported to cause up to 75% crop losses on barley (Allen, *et al.*, 1970) and in southern Chile over 70% on wheat (Kilpatric, *et al.*, 1976).

M. naasi galls on cereal roots are cylindrical, spindle-shaped, hooked, ring-shaped or club-shaped (Franklin, 1973). A single gall may contain many females, which lay eggs in a gelatinous matrix. The eggs require 15 to 17 days at 22°to 26° C to develop into the J_2 stage and 39 to 51 days, to complete their life cycle in the laboratory on wheat (Siddiqui and Taylor, 1970). In UK on spring barley, the life cycle takes 12 to 14 weeks in the field, and required 222 day degrees above 10° C or 137 above 12° C from invasion to egg formation (Franklin, *et al.*, 1971).

A small proportion of the eggs produced at the end of summer (about 6%) hatched spontaneously soon after development was completed (Ogunfowora and Evans, 1977a). Such J_2 if they are successful in invading a host root, are usually unable to complete their life cycle because of the falling environmental temperatures and the senescing host roots (Franklin, *et al.*, 1971). Gooris and D'Herde (1976) reported a relatively high percentage hatch in the Belgian population at the end of the growing season.

All reports on *M. naasi* from all over the world, confirm that there is only one generation per year on all known hosts. The eggs stay protected in the gelatinous matrix in the J_2 stage until the following spring, when temperatures rise above 10° C and host roots are in abundance (see Fig. 1). There is however a very small proportion of eggs that hatch throughout the year even at low temperatures. Such J_2's

die of starvation since they are unable to find hosts or to invade roots at temperatures below 10° C (Siddiqui, 1971). A small percentage of eggs does not hatch in the following spring and require a second winter period before they can hatch in the second spring. This over-wintering of the eggs in the soil, until environmental conditions become favourable, represents dormancy. However, the concept of dormancy in plant parasitic nematodes is relatively new, and there are still many ideas needing clarification, especially on diapause. Most nomenclature and definitions were borrowed from entomology, and their application on nematodes is not yet satisfactory since we still lack a lot of biochemical information concerning nematode dormancy.

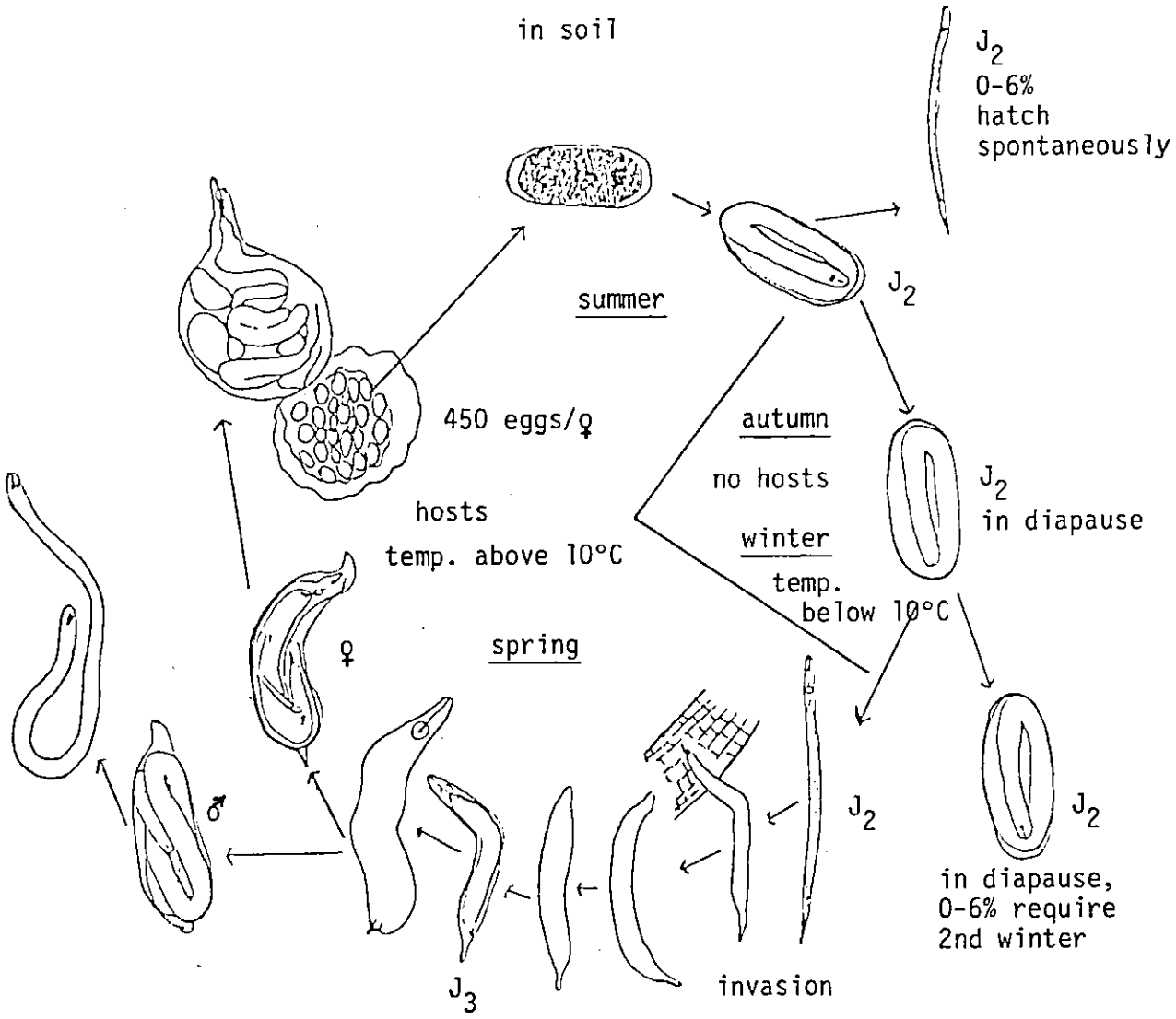
Some workers, regard *M. naasi* as the best example of nematode diapause (Evans and Perry, 1976; Ogunfowora and Evans, 1977a), while for others, it is just another example of quiescence (Gooris and D'Herde, 1977).

It was necessary before starting this project to understand the difference between the two forms of dormancy, how they operate, and how they apply in nematodes, from the work already done on the different species. It proved very useful in understanding nematode strategies for survival and how *M. naasi* fits into the picture.

Reproduction in *M. naasi* is by meiotic parthenogenesis although occasional reproduction by cross-fertilization is suspected in cultures with abundant males (Triantaphyllou, 1969). The haploid chromosome number is 18 with no variation (Triantaphyllou, 1969).

Table 1. Geographical distribution of *M. naasi*

country	latitude °	race	main hosts	eggs produced in the field in:	population decrease in fallow	best recorded chilling conditions	best recorded hatching conditions	bibliography
Belgium	50.5(N)		wheat, onion, sugar beet	July	90% 1st year 96% 2nd year			108
Chile	41(S)		wheat					147
France	48(N)		cereals, sugar beet					33
Germany	53(N)		cereal, sugar beet					244
Italy	41(N)		durum wheat	May				157
Iran								150
Malta	36(N)							134
Netherlands	52(N)		cereals, sugar beet					155
New Zealand	41(S)		wheat, barley					230
UK England	51.5(N)	1	barley, wheat	May or September			20° C	94
Wales	52.5(N)		barley, wheat	August	93% 1st year	5°-10° C 7-30 weeks	20° C	94
USA California	35(N)	2	barley, wheat			6°-9° C 7 weeks	21° C 5 days	2
Illinois	40(N)	3	cereals, sugar beet					103
Kansas	37(N)	5	barley					8
Kentucky	37(N)	4						249
Michingan	42(N)							103
N.Carolina	35(N)					4° C 6 weeks		103
Oregon	42(N)		barley, oats, grasses					140
USSR								108
Yugoslavia	43(N)		cereals, sugar-beet, mangolds					112



one generation per year

Fig. 1 : The life cycle of *M. naasi*.

C. Arrested development in Plant Parasitic and Free Living Nematodes

A literature review

Contents

1. Introduction

Under optimal environmental conditions, most organisms are born, develop to maturity, senesce and die in a continous process, with no interruptions. But "when the physical conditions pass beyond the developmental limits for the species, death, quiescence or diapause must ensue" (Howe, 1962).

Organisms with no adaptations to sudden environmental changes perish, while the ones that have evolved ways to overcome them are able to survive and colonize such unstable niches.

Michel (1978) believes that nematodes of all, or nearly all animal parasitic species, have an innate capacity to interrupt their development at an early parasitic stage, and extend their survival while metabolism is depressed. Demeure *et al.* (1979) considered that the ability to enter dormancy at any stage in the life cycle due to unfavourable conditions, "is a wide-spread phenomenon in soil nematodes".

The literature is full of terms, with a variety of definitions, describing different states of life.

dead:	lifeless and not viable	(Preyer, 1891)
	abiotic	(Schmid, 1948)
anabiotic:	lifeless and viable	(Preyer, 1891)
	in ametabolism (latent life)	(Bernard, 1878)

	hypobiotic		(Monterosso, 1934)
	cryptobiotic		(Keilin, 1959)
	in hypometabolism		(Keilin, 1959)
	dormant which can be:	hibernation	(Keilin, 1959)
		aestivation	(Keilin, 1959)
		diapause	(Keilin, 1959)
		quiescence	(Keilin, 1959)
		cryobiosis	(Keilin, 1959)
		anoxybiosis	(Keilin, 1959)
		osmobiosis	(Keilin, 1959)
		anhydrobiosis	(Giard, 1894)
active life:	normal metabolism		(Keilin, 1959)

The terms anabiosis (ἀνα+βίος -greek- meaning "to revive") hypobiosis (ὑπο+βίος -greek- meaning "diminished life") ametabolism (ἀ-μεταβολισμος -greek- meaning "lack of metabolism"); hypometabolism (ὑπο-μεταβολισμος -greek- meaning "diminished metabolism"); latent life (latin, meaning "unseen life"), all describe diminished or absent life or metabolism. They have been used by many students of this subject to describe different states in the life of an organism. Their quantitative implications make it difficult to describe a general state of suppression of normal activity.

Keilin (1959) defined cryptobiosis (κρυπτο+βιος -greek- meaning "hidden life") as "the state of an organism when it shows no visible signs of life, and its metabolic activity becomes hardly measurable, or comes reversibly to a standstill". This state of life, we recognize in animals which have lost too much water and become dormant. Cryptobiosis as used by Keilin in 1959, implied all forms of dormancy. Today, we know of animals, or just organs (eg. gonads) of otherwise active animals, in a dormant state, showing few signs of activity. Their development has been arrested but not necessarily their whole metabolism. To clarify and simplify the terminology, the adoption is suggested here of terms which proved both more useful and more relevant to the definitions.

dead:	when lifeless and not viable	(Preyer, 1891)
dormant:	when normal development is suppressed	
active:	when metabolism and development are normal	

Because there are many types of dormancy described so far, it is

useful to create subdivisions.

Laudien (1973) was followed by Evans and Perry (1976) in using the cause of the arrest in development as the criterion to separate dormancy in nematodes into 2 main categories: Quiescence and Diapause. Evans and Perry (1976) further subdivided these into Facultative and Obligate Quiescence/Diapause(see Table 2).

2. Quiescence

"A sudden, unanticipated, non-cyclic and usually short duration deviation of one or more environmental factors from optimum, produces this type of dormancy. The reaction of the individuals is spontaneous and results in growth retardation only, within their natural tolerance limits. It can occur at any time of the year, and may be experienced by any, or all of the stages in the life cycle". (Mansingh, 1971).

2.A. Facultative Quiescence

This type of dormancy is believed to be common among soil nematodes (Evans and Perry, 1976). It can be induced by environmental factors acting on their own or in combination with each other. Such factors are: loss of water (anhydrobiosis), osmotic stress (osmobiosis), lack of oxygen (anoxybiosis), unfavourably low temperature (cryobiosis) or unfavourably high temperature (thermobiosis).

a. anhydrobiosis: Nearly all above factors are interrelated and may induce anhydrobiosis (Demeure, *et al.*, 1979). The same authors considered anhydrobiosis as a broad category, appearing in all forms of dormancy in varying degrees. Evidence suggests that most nematodes, if dried slowly, are able to withstand dessication. Freckman, *et al.*, (1977), developed a technique (the anhydrobiotic technique), for extracting nematodes in the anhydrobiotic state. This new study, revealed many nematodes in the anhydrobiotic state in the soil (see Table 3).

(1) Behavioural adaptations to survive desiccation

(1) i. coiling: Coiled anhydrobiotic nematodes were found in various life stages, trophic groups, genera (Demeure and Freckman, 1981)(see Table 3).

Anguina tritici J_2 assume a characteristic coiled posture "Tönnchen

Table. 2: A classification of types of dormancy in nematodes. From Evans and Perry(1976).

	DORMANCY (a suppresion of normal activity)			
	QUIESCENCE		DIAPAUSE	
controlled by:	unfavourable environmental conditions		endogenous factors	
	FACULTATIVE QUIESCENCE	OBLIGATE QUIESCENCE	FACULTATIVE DIAPAUSE	OBLIGATE DIAPAUSE
induced by:	unfavourable environmental conditions	unfavourable environmental conditions	unfavourable environmental conditions	endogenous factors
stage in the life cycle	all stages	only one stage	only one stage	only one stage
process	readily reversible	readily reversible	not readily reversible	not readily reversible
terminated by:	return of favourable environmental conditions	return of favourable environmental conditions	spontaneous endogenous factors, acting after a minimum period of time	environmental conditions acting on the diapaused individual for a minimum period of time
examples	anhydrobiosis (Giard,1895) osmobiosis (Keilin,1959) anoxybiosis (Keilin,1959) cryobiosis (Keilin,1959) thermobiosis	dauer larvae	animal parasitic nematodes	plant parasitic nematodes

form" when dehydrated (Crowe, 1971). Perry (1977) found that all stages of *Ditylenchus dipsaci* exhibit coiling if dried slowly. Cayrol (1970) reported that individuals of *D. myceliophagus* coil tightly when dehydrated, and Womersley (1978) found that coiled *D. myceliophagus* control drying much better than straight individuals.

Aphelenchus avenae larvae and adults coil when exposed to desiccation (Mankau and Mankau, 1963; Townshend, 1964). Other fungivorous and microbivorous nematodes were reported to coil on dried agar by Madin and Crowe (1975). Demeure, *et al.*, (1978) suggested that the force of water films in the soil may influence initiation of coiling of *A. avenae* and *S. brachyurum* more than relative humitidy. Demeure, *et al.*, (1979a) in experiments with *A. avenae* , *H. dihystera*, *S. brachyurum* and *Acrobeloides sp.* in the laboratory, found that dehydration of all 4 species induced 91-98% coiling. Demeure (1980) reported similar results for *S. cavenessi* and he found that 97% of the nematodes revived after 48 hours in water. Demeure, *et al.*, (1979a) suggested that survival of desiccation was not correlated with coiling, but with duration of dehydration. Coiling they concluded was a short term physical response to dehydration, while anhydrobiosis was a longer term physiological response to slow dehydration.

Rössner (1973) found that *R. robustus* shrinks when desiccated, forming an open ring or remaining straight. He observed that the body undergoes a considerable decrease in length, while the width remains constant. He described it as a reversible process, which lead to a controlled narrowing of the grooves between the transverse annulations. Rössner and Perry (1975) found that there was an initial rapid loss of water in this nematode, then the rate was reduced at the time when the surface changed and remained almost constant thereafter.

Rapid dehydration usually does not result in coiling or shrinking, but death (Demeure and Freckman, 1981). In nature, dehydration takes place slowly in the soil, and a coiled or shrunk body is possibly better adapted to reduce water loss. It might be useful also in avoiding predators and wounding of the body while otherwise unable to protect itself. Dehydrated (shrunken) muscles might bring about those body changes.

(1) ii. clumping and sticking together: Many nematodes survive by clumping and sticking together, thus reducing loss of water. While single J_2 of *Anguina tritici* survived for 7 days in a desiccator, clumped J_2 survived up to 32 years (Limber, 1973).

Table 3 : Nematode species known to be able to withstand desiccation

nematode species	resistant stages	found in	longest survival reported	coiling	aggregations	bibliography
Acrobeles sp.		desert soil		yes		99
Acrobeloides sp.		dry soils		yes		67
Actinolaimus hintoni		dry mud	10 years			160
Anguina feruiae	J_3	dry galls or soil				138
A. plantaginis	J_3	dry galls or soil	several months			259
A. tritici	J_2	wheat galls	32 years	yes	clumps	164
Aphelenchoides besseyi	all stages, mainly adults	dry rice grains	3 years		aggregates	1,131
A. ritzemabosi	adults and J_4	dry leaves	3 years			154,267
Aphelenchus avenae	J_4	dry agar or soil	1 year	yes	clumps	67
Chiloplactus sp.		desert soil		yes		99
Ditylenchus augustus		rice panicles	overwinters			52
D. dipsaci	all stages, mainly J_4	stems, bulbs	23 years	yes	"eelworm wool"	195
D. myceliophagus	all stages, mainly J_3, J_4	dry soil	3-4 years	yes	"curds"	36
Dorylaimus keilini		dry mud	10 years			160
Globodera rostochiensis	eggs in cysts	dry air at 5°C	8 years			295
Helicotylenchus dihystera		laboratory expts.		yes		64
H. nanus		dry soil	12 months			233
Hemicycliophora typica					swarming	177
Heterodera avenae	eggs and J_2 in cysts	dry soil	13 months			233
H. glycines	eggs in cysts	dry soil	9 years			133
Hirschmanniella spinicaudata						11
Hoplolaimus columbus	preadults and adults	dry soil	over 1 year			88
Meloidodera charis	eggs inside body	soil and roots				63
Meloidogyne arenaria	eggs					114
M. hapla	eggs					114
M. incognita	eggs and J_2	dry soil	6 weeks			113
M. javanica	eggs and J_2	dry soil	51 months	yes		171
Nacobbus aberans			8 months			139
Nothotylenchus acris	J_4, immature female	dry strawberry buds	410 days			149
Panagrellus redivivus						153
Paraphelenchus sp.		desert soil		yes		99
Paratylenchus projectus	J_4	dry soil	2 weeks			233
Pratylenchus penetrans		lab. expts, dry soil	13 weeks 11 months(?)	yes		154,63, 216
P. minyus		dry soil	13 months			233
Rotylenchulus reniformis	all stages, mainly J_4	dry soil	25 months			214,233
Rotylenchus robustus	J_4, J_3, , , J_2	dry soil	3 weeks	'shrink'	clumps	217,233
Scutellonema brachyurum		dry soil, laboratory		yes		64,65,67
S. cavenessi	all stages	desert soil, lab.	8 months	yes		60
Tylenchorhynchus dubius		dry soil	3 weeks			233
Tylenchus polymorphus		dry herbarium specm.	39 years			84
Xiphinema sp.		laboratory		yes		63
X. mediterraneum		dry soil	summer			57

Ultrastructural studies showed fusion of the outermost lipid layer of the cuticles of *D. dipsaci* (Perry, in Evans and Perry, 1976), forming a barrier to water loss. The same phenomenon has been suggested by Bird and Buttrose (1974) regarding *A. tritici* . *D. dipsaci* aggregations are found on the host tissue forming "eelworm wool" under dry weather conditions, and the viability of the nematodes in these aggregations declines with time (Ellenby, 1968 b). If stored at 2° to 4° C, they remain viable for 7 years (Bosher, 1960). Death occurs first in the periphery of the aggregations; the larger the aggregation fragments, the longer their survival (Ellenby, 1968 b). He found that as the periphery dries, the rate of drying in the central region is slowed.

T. martini swarmers adhere to each other along transverse and lateral ridges (Ibrahim and Hollis, 1973).

(2) Morphological adaptations

Bird and Buttrose (1974) found differences between the anhydrobiotic and the active J_2 of *A. tritici*, in the composition of the external cortical layer of the cuticle; the spatial arrangement of muscle filaments and their nuclear density; the morphology of the lipid droplets in the cuticle and in the body. They found no difference in width or cross sectional area between the two forms.

Perry (in Perry and Evans, 1976), reported a reduction in thickness of the various layers of the cuticle of the desiccated J_4 of *D. dipsaci* compared to the hydrated larvae, which made it more compact. The cuticular ultrastructure of *T. martini* was the same in both swarming and non-swarming individuals (Bird and Buttrose, 1974).

(3) Aids to reducing water loss

Rotylenchus reniformis J_4 were found better adapted to survive adverse conditions than other stages, as they were protected by the previous larval cuticles which were not shed (Sivrakumar and Seshadri, 1979). Godfrey and Morita (1929), and Godfrey and Hoshino (1933) found that at 0% R.H., larvae of *Meloidogyne* species survived only a few minutes, free eggs almost 1 hour and eggmasses for nearly 2 hours. The gelatinous matrix and the egg shell aid in survival, since all stages within either of them are relatively resistant to desiccation. Wallace (1968a) suggested that *M. javanica* egg sacs had no water retention properties. He believes that on drying, the gelatinous matrix exerts

a mechanical pressure on the enclosed eggs and this factor may be responsible for inhibiting uptake of water by the eggs, and thus preventing hatching when the soil is too dry for movement.

Endo (1962) reported that *H. glycines* eggs and free larvae survive only for days and minutes respectively at low humidities, while cysts remain viable for months. *M. charis* develops an extracuticular subcrystalline layer during desiccation according to Demeure and Freckman (1981).

(4) Metabolic adaptations

Kostůk* found no evidence for metabolism of lipid, protein or glycogen in anhydrobiotic *A. tritici* J_2. He concluded that fermentation of simple sugars takes place in these forms, and no respiration. Anhydrobiotic *A. tritici* and *D. dipsaci* stored trehalose in preference to glycogen and only small amounts of glucose were detected in them (Womersley, *et al*, 1982). They also did an analysis of lipid composition in anhydrobiotic *A. tritici* and *A. avenae* and found no indication of any specific metabolic adaptations to desiccation survival.

A. avenae was found to utilize lipid and glycogen stores during the induction of anhydrobiosis (Madin and Crowe, 1975). When reviving it synthesised glycogen and used up trehalose, glycerol and lipids (Crowe, *et al.*, 1977). Madin and Crowe (1975) reported a strong correlation between survival of this species in dry air and glycerol and trehalose contents.

Incubation in 5% inositol solution, improved the survival of *A. tritici*, *D. dipsaci* and *D. myceliophagus* during desiccation and revival (Womersley, 1978). *A. tritici* lost its small amount of glycerol when desiccated, but had far more bound and total inositol than other species (Womersley, 1978). Inositol is believed to replace bound water in the molecules in the desiccated cells very effectively (Webb, 1965). *A. tritici* and *D. dipsaci* may obtain much inositol through feeding on plant tissue and/or produce it themselves during adverse conditions (Evans and Womersley, 1980).

Limber (1973) suggested that low humidity or low temperature induced reduced respiration and metabolism which results in the long life of *A. tritici*.

(5) Effect of dehydration on development

Under moisture stress, embryonic development is completed up to

* (1965)

the point of hatching, but the J_2 then becomes quiescent until conditions are favourable for emergence. This phenomenon has so far been described by Bird (1968) for *M. javanica*, by de Guiran (1979) for *M. incognita*, *M. arenaria*, *M. hapla* and *M. javanica*, by Linford (1941) for *M. arenaria* and *M. hapla* and by Ross (1963) for *H. glycines*.

High pF values in the soil induced water loss and the shrinking of the eggs in *M. javanica* (Baxter and Blake, 1969).

(6) Advantages of a dehydrated body

Nematodes in the desiccated form are known to be more resistant to extremes of many environmental factors, such as heat, cold, nematicides, low oxygen concentrations etc (Demeure and Freckman, 1981).

A. tritici J_2 contained less water than other genera examined. It can maintain viability in the anhydrobiotic state over a range of -190°to 105° C for short periods of time, and can survive in liquid nitrogen in this state (Bird and Buttrose, 1974).

D. dipsaci in "eelworm wool" survived freezing for 5 years at -80° C, while individual J_4 in "eelworm wool" survived freezing for 20 minutes at -80° C (Bosher and Mckeen, 1954). At room temperature *D. dipsaci* is known to survive for 3 to 4 days in a desiccator (Ellenby, 1968b ; Webster, 1964). At 50% R.H. J_4 survived for at least 1 month (Wallace, 1962). O'Dell and Crowe (1979) reported that anhydrobiotic forms of *A. avenae* are relatively unaffected by exposure to -196° C for 15 minutes, and that revival gave 91% survival.

(7) Rehydration

Freckman (1978) found that the time for resumption of activity following rehydration varies between species, from 30 minutes to several hours, and that metabolic activity is resumed at 2.7% soil moisture.

A. tritici J_2 removed from dry wheat galls and allowed to remain at 95% R.H. for 24 hours., took up 1.6 µl oxygen (mg dry weight)$^{-1}$ hr^{-1} (Bhatt and Rohde, 1970). The same authors reported that the longer *A. tritici* had been desiccated, the more time was required to resume maximum oxygen consumption.

When rehydrated *A. avenae* took up water and oxygen rapidly (Crowe, *et al.*, 1977). In the desiccated state, larvae had 50% more ATP than when active. Willet *et al* (1978) found that

anhydrobiotic *A.avenae* had ATP levels of 4.95 $\pm$ 0.78 µg/mg dry weight, and hydrated forms 2.23 $\pm$ 0.33 µ g/mg dry weight.

It was found that inorganic ions and primary amines leaked from anhydrobiotic and quick dried *A.avenae* when they were placed in water from dry air. The leakage was diminished in both types when they were moisturised slowly. The rate of leakage was inversely correlated with the glycerol contents of the worms at the time they were placed in water. Anhydrobiotic *A.avenae* leaked about $\frac{1}{3}$ as fast as dried ones (Crowe *et al.*, 1979).

(8) Losing the ability to withstand desiccation

It was found that repeated dehydration/rehydration cycles reduced the revival rate and the percentage of the individuals revived. Womersley (1980) reported this for *A.tritici* .

Repeated drying reviving treatments in *D.dipsaci* , resulted in a more rapid water loss every time, and a smaller number of individuals reviving Perry (in Evans and Perry, 1976). found no delay in rehydration in the J_4 of *D.dipsaci* . The rate of water uptake was the same, irrespective of the length of time the nematodes had been desiccated, but the longer the nematodes were kept dry, the smaller the proportion of infective larvae revived. Demeure and Freckman (1981) also reported that repeated desiccation rehydration for *A. avenae* could be lethal. The rate of water loss was found to increase with every treatment.

Perry (1977a) reported that dead *D. dipsaci* lost their ability to dry slowly. Their cuticles were completely permeable to water in both directions. He went on to suggest that this mechanism was controlled by the live organism. Rössner and Perry (1975) reported a similar phenomenon for *R. robustus*. They as well as Rössner and Porstendörfer (1973), reported that anaesthetised animals lose water at the same rate as active ones when dried under the same conditions, but the dead ones dry up very fast. Rössner (1973) also noted that dead and anaesthetized nematodes differ from active specimens in the type of shrinkage.

(9) Theories concerning desiccation survival

Perry (1977a) believes that is the cuticle that is instrumental in controlling water loss, and that this ability may be dependent largely on physical factors altering the permeability of the drying cuticle. He suggested that the initial desiccation of previously undesiccated individuals may cause an irreversible alteration to one

or more of the peripheral layers, which either changes the permeability of the cuticle, or alters the ability to change so as to reduce water loss. The cuticle stucture then remains unaffected by subsequent desiccation treatment.

Perry (1977$_b$) concluded from studies with *D. dipsaci* that the adults and J_2 were dependent upon environmental conditions to reduce the rate at which they lose water. J_4 and to a lesser extend J_3, showed evidence of an intrinsic ability to control water loss. He went on to suggest that the rate at which water was removed from the cuticle may be controlled by some active process of the nematode body. He divided the drying curve of the J_4 into 3 main phases: (i) rapid loss of water (ii) certain stability of the water content irrespective of increase in period of desiccation (iii) an increased rate of water loss until almost all the water has been removed from the organism. During the second phase, he found that the nematode has about 30 to 40% water content and less than 10% after the third phase.

b. osmobiosis: In the soil environment, osmotic potentials depend on water availability, the type of soil, the presence and activity of other organisms. Saprophytic nematodes from decaying organic materials are able to tolerate widely fluctuating osmotic potentials (Stephenson, 1942).

High osmotic pressures result in dehydration of the body. In the soil, most processes take place gradually, so there is a period of time available to allow adjustment. Wallace (1971) suggested that soil nematodes may experience high osmotic pressures in drying soils after application of fertilizers, which he suggested induced quiescence.

(1) Tolerance to osmotic stress and recovery

Rhabditis terrestris, *D. dipsaci*, *Tylenchorhynchus icarus*, *Xiphinema index* were reported to show tolerance and good recovery in osmotic treatments (Blake, 1961; Wallace and Greet, 1964; Roggen, 1966; Viglierchio, *et al*, 1969). In the J_2 of *M. javanica*, 0.3 M NaCl induced quiescence. The food reserves were consumed 7.5 times slower than in controls. Motility and infectivity did not change after 10 weeks treatment. In the concentrated medium, J_2 were straight and motionless. They always recovered on return to dilute medium (Reversat, 1981).

H. schachtii J_2 became quiescent in concentrations higher than

0.5 M of sucrose and inositol (Perry, *et al.*, 1980). Clarke, *et al.* (1978) stored *G. rostochiensis* J_2 in the laboratory in a quiescent state in solutions which caused stress, for long periods of time.

Simons (1973) reported that eggs of *H. glycines* in cysts were not affected by soaking the cysts in a 5% sucrose solution for 96 hours. The same author reported that *Pratylenchus penetrans* eggs survived high osmotic pressure (up to 25 bar) for 12 days.

Rössner and Porstendörfer (1973) reported that *R. robustus* specimens placed in a solution of 1 M glucose, shrunk in the same way as the slowly dried specimens.

(2) Effect of osmotic stress on development

High osmotic pressure was reported in many cases to suspend embryonic development and/or hatch in many *Meloidogyne* species (Wallace, 1966; Dropkin, *et al.* 1958). For example, 1 M sucrose and 1 M urea inhibited hatch significantly in *M. incognita* (Jones and Nirula, 1963). These solutions correspond to an osmotic pressure of about 22.4 atm. or pF 4.35. Similar results were obtained with *M. arenaria*, *G. rostochiensis*, by Dropkin, *et al.*, (1958) using NaCl, KCl, $CaCl_2$ and dextrose, and Blake (1961) with *D. dipsaci*.

Yousif and Badra (1981) reported that hatching of *M. javanica* was reduced by several organic and inorganic amendments, like sheep dung, rice straw, pigeon droppings, urea and ammonium sulphate. The process was reversible at low concentrations, after placing the eggs in water. Sucrose solution (0.6 M) and inositol in 2 mM picric acid completely inhibited hatch in *H. schachtii*, which otherwise hatched readily in water (Perry, *et al.*, 1980). The egg fluid of this nematode has a lower osmotic pressure than other *Heterodera* species. Wallace (1956; 1966) found that osmotic inhibition of hatching was reversible for solutions up to 1 M.

Reversat (1975) reported that hypertonic test solutions of NaCl or KBr temporarily inhibited hatch in *H. oryzae*. The rate of hatch increased with the duration of the inhibition and decreased with the higher the concentration of the test solution. Also he found that Rubidium chloride, urea, Lithium chloride and Barium chloride inhibited hatch.

c. anoxybiosis: Low levels or lack of oxygen in the soil environment is known to result in quiescence in some nematodes. Flooding may

result in such a condition in the soil.

(1) Survival under anaerobic conditions

Cooper *et al.* (1970) found that survival of *A. avenae* after 20 days under anaerobic conditions, was more than 80%. During short periods of anaerobiosis (12 to 16 hours), glycogen catabolism yielded lactic acid and during longer periods, ethanol was produced in this nematode (Cooper and Van Gundy, 1971). De Guiran and Demeure (1978) reported that hatched J_2 of *M. incognita* became quiescent if oxygen was lacking.

(2) Effect of anoxybiosis on development

There are many reports on the induction of quiescence due to reduced availability of oxygen in the egg stage. In many *Meloidogyne* species for example, low oxygen concentrations reduced hatching and stopped embryonic development. Baxter and Blake (1969) reported that few eggs of *M. javanica* hatched in an atmosphere of nitrogen.

Only complete absence of oxygen inhibited hatch in *M. hapla* . Maximum hatch occured at 21% O_2 : 0.03% CO_2. As the oxygen concentration decreased, hatch decreased (Wong and Mai, 1973).

Sprau (1970) reported that increasing concentrations of oxygen, increased the hatching rate of *G. rostochiensis* and that carbon dioxide had a strong inhibitory effect on hatch. He found that hatch in this nematode could occur in an atmosphere of pure nitrogen.

The egg-shell is semipermeable, and so the shell is unlikely to be a barrier to the diffusion of oxygen (Baxter and Blake, 1969). "The mechanism for using oxygen is probably the same for both the development of the embryo and hatch of the egg. The quantity of oxygen required is similar for both processes in *M. javanica*" (Baxter and Blake, 1969). Collis* and Wallace (1968) found that one egg of *M. javanica* consumes 2.9×10^{-5} g/sec of oxygen.

d. cryobiosis: Cryobiosis is a state at which water freezes, so it is not available in chemical reactions in the cell. If a nematode is already adapted to do without water, then in theory it is able to survive freezing for at least short periods of time.

A. tritici, *D. dipsaci*, *A. avenae* nematodes well adapted to anhydrobiosis are known to survive freezing well. Lozina-Lozinski and Namatov (1970) found that larvae of *A. tritici* and *Rhabditis sp.*

* Collis George

can be cooled to -20° and -30° C.

O'Dell and Crowe (1979) reported that *A. avenae* could be rehydrated from the anhydrobiotic state without being affected by exposure to liquid nitrogen while its water content was below 0.26 mg H_2O/mg dry weight (21% H_2O).

Aphelenchoides besseyi was insensitive to chilling (Huang and Chiang, 1975), and *D. dipsaci* survived 18 months at -150° C (Sayre and Hwang, 1975). Øyduin and Hammeraas (1974) reported *2 Grostochiensis* pathotypes to hatch after storage in temperatures as low as -35° C. *M. incognita* eggs survived at -15° C for 10 minutes (16%), at 0° C for 14 days (48%), and *M. hapla* eggs survived for 10 days at -4° C (90%) (Vrain, 1978). *Caenorhabditis briggsae* J_2 and J_3 survived in liquid nitrogen at -190° C for more than 100 days (Haight *et al.*, 1975).

Hogger and Estey (1977) believed that *Xiphinema americanum* can withstand frozen soil conditions if temperature falls gradually.

e. thermobiosis: I introduce a new term to describe survival at unfavourably high temperatures (θερμός :-greek- meaning hot).

Temperature directly affects movement, growth rate, sex determination, hatch in nematodes; and indirectly regulates the environment by determining water, oxygen and food availability.

Bird (1974) found that brief heat treatment (46° C for 10 minutes) supressed embryogenesis and hatch of *M. javanica*. He found that treatment in the earlier stages of development retarded egg development for short periods of time, but normal development followed these periods of suppression. He reported a delay in egg hatch, and that heat treated eggs gave maximum hatch compared to 20 - 30% mortality in the controls. Bird (1979) observed that the closer to the completion of embryogenesis and first molt the heat treatment was applied, the longer the delay in hatching in *M. javanica*.

Bird and McClure (1976) found that egg-shell permeability at different temperatures changed, due to changes in the properties of lipoprotein membranes in the lipid layer. They suggested that this property of the shell might be responsible for the delay in hatch but not for the delay of embryogenesis. Some other changes in the embryo must take place to induce thermobiosis.

Demeure (1978) reported that at 50° C all active forms of *Scutellonema cavenessi* were killed, but 100%, 58% and 5% of the anhydrobiotic forms survived 60°, 70°, 80° C respectively. *Ditylenchus myceliophagus* embryos older than 4 days, survived temperatures higher than 40° C,

Table 4 : Nematode species known to exhibit quiescence.

nematode species	osmo-biosis	anoxy-biosis	cryo-biosis	thermo-biosis	bibliography
Anguina tritici			*		166
Aphelenchoides besseyi			*		130
Aphelenchus avenae		*	*		85,183
Caenorhabditis briggsae			*		120
Ditylenchus dipsaci	*		*		27,222
D. myceliophagus				*	35
Globodera rostochiensis	*	*	*		69,238, 194
Heterodera avenae				*	14
H. oryzae	*				208
H. schachtii	*				200
Meloidogyne arenaria	*				69
M. hapla		*	*		289,263
M. incognita	*	*	*		263,142, 116
M. javanica	*	*		*	14,17, 209
Nacobbus aberrans			*		139
Rhabditis sp.			*		166
R. terrestris	*				242
many saprophytic species	*				242
Scutellonema cavenessi				*	61
Tylenchorhynchus icarus	*				27
Xiphinema americanum			*		127
X. index	*				214

which killed younger embryos, and that excessively high or low temperatures slowed down the development of embryos in this nematode (Cayrol, 1969).

2. B. Obligate Quiescence

Quiescence which can be induced only at a specific stage of the life cycle of the nematode, by unfavourable environmental conditions.

dauer larvae: Some free living nematodes which may depend on insects for dispersal, have evolved a stage in their life cycle able to tolerate starvation and desiccation. During reproduction, such species (mainly *Rhabditidae*) produce a proportion of the larvae which are able to become dauers when environmental conditions became unfavourable. Normal development is resumed shortly after the return of favourable conditions (see Table 5).

(1) Induction of the Dauer condition

It has been suggested that unfavourable culture conditions in the laboratory can induce dauer formations (Evans*, 1980), for example, gradual starvation in *C. elegans* (Cassada and Russell, 1975). Yarwood and Hansen (1968) proposed that in *C. briggsae*, dauer larvae occured as a response to crowding or accumulating metabolic products. Cliff *et al.*, (1978) suggested the same factor for *Pelodera strongyloides*. *Acrobeloides nanus* was found to produce dauers in declining cultures (Sohlenius, 1973a).

Evans*(1980) suggested moisture stress as the factor inducing dauer formation in *R. dubia* and *Neoaplectana bibionis*, and Lees (1953) found slow drying to induce dauers in *Panagrellus silusiae*. Riddle *et al.*, (1981) suggested that dauer formation in *C. elegans* depended on a genetic pathway corresponding to a neural pathway involved in the detection of environmental signals.

(2) Physiological and behavioural adaptations in Dauer larvae

Bovien (1937) reported that the dauer larvae of *Diplogaster stercorarius* appeared to leave minute oil drops behind them while moving. Grootaert (1976) observed the same phenomenon in *Mesodiplogaster cheritieri*. The dauer larvae of *Acrobeloides nanus* were found to be shorter than the normal larvae (Sohlenius, 1973).

The larvae of *Turbatrix sp.* and other viviparous nematodes, survive

* and Womersley

Table 5 : Some Nematode species known to form dauer larvae

nematode spp.	stage in the life cycle	dauer stage tolerant to:	longest survival in this condition	bibliography
Acrobeloides nanus				236
Caenorhabditis briggsae	J_2, J_3	cryobiosis		3,146
C. elegans	J_2, J_3	cryobiosis thermobiosis anoxybiosis chemicals	70 days without food	32,3, 146
Diplogaster stercocarius		thermobiosis desiccation	6 hours	30
Mesodiplogaster cheritieri				111
Neoaplectana bibionis				30
Panagrellus silusiae		desiccation	2 months	162
Pelodera acarambates				204
P. strongyloides		thermobiosis		291
Rhabditis dubia		thermobiosis		30
R. maupasi		thermobiosis		235
R. reciproca				245
Tubatrix sp.	larvae inside mother	desiccation		77

desiccation inside the body of their drying mother (Ellenby, 1969).

R. maupasi dauer larvae are usually found in the tissues of earthworms, where they remain until the host dies. They then quickly mature and breed in the decaying carcass ; 33 - 37 % of the larvae of this nematode are able to become dauers (Otter, 1933).

3. Diapause

The word diapause (παυσις -greek- meaning rest, interruption of work) is used to describe an inhibition, or a pause in development. It is the most highly evolved system of dormancy, for overcoming cyclic, long term, extreme environmental conditions.

In this category of dormancy, can be placed those examples with the following characteristics:

(a) endogenous factors play a primary role in receiving the right signals from the environment or from within the organism to induce or terminate the phenomenon.

(b) these endogenous factors (special physiological mechanisms) can become functional only in certain stages of the life cycle of the nematode.

(c) a diapausing nematode is not immediately reactivated by favourable conditions. It is necessary for the signal to act for a minimum period of time on the receptor mechanism of the nematode before diapause is broken.

(d) this dormancy is induced well before the adversity.

Obligate Diapause

An obligate diapause effectively converts the life cycle to an annual one. The best known environmental signals triggering the termination of obligate diapause, are low temperature and host root diffusates.

The environmental factors inducing quiescence are part of every day life and can deviate from the optimum any time of the year, thus acting for a short time on any stage in the life cycle of the nematode. In obligate diapause however, the low temperature and host root diffusates are markers of seasons which help the nematode time itself in order to synchronize with its host.

Table 6 : Plant Parasitic Nematodes known to exhibit Obligate Diapause.

nematode species	stage	environmental factors influencing diapause	followed by quies/ce	% in diapause	bibliography
Ditylenchus dipsaci	J_4	chilling (5°C)	yes		277
Globodera pallida	egg	root diffusates	yes	43%	91
G. rostochiensis	egg	low temperature	yes		80, 128,229
	egg	root diffusates	yes		81,282
Heterodera avenae	egg	root diffusates	yes		146,280
	egg	chilling(10°C,8 weeks)Europe	yes		12,79,212
	egg	drought, Australia, S.Italy	yes		174,108
H. carotae	egg	root diffusates at 10°C 2 months after being laid			109
H. cruciferae					282,229
H. galeopsidis					229,282
H. glycines	egg	root diffusates			187
H. goettingiana	egg	root diffusates		15%	201
H. humuli					229,282
H. oryzae	egg	root diffusates			176
	egg	cyst extracts			
H. punctata					84
H. schachtii	egg	root diffusates			200
Hypsoperine ottersoni	egg	root diffusates			276
Meloidogyne ardenensis	egg	chilling			241
M. arenaria	egg	strain on female		7-9%	119
M. hapla	egg	strain on female		12%	119
M. incognita	egg	strain on female		21-43%	113,119
M. javanica	egg	strain on female		10-18%	119
M. naasi	egg	chilling (10°C, 7 weeks)	yes	94%	97,274, 186

The Nematodes exhibiting Obligate Diapause

The best studied nematodes exhibiting diapause are found in temperate environments. They parasitise plant hosts which follow seasonal growth. To synchronize effectively their life cycle to that of their hosts, inbuilt mechanisms interrupt their development and keep it switched off for long enough to ensure the best timing. Very specific environmental factors acting on the animal directly or indirectly, for a minimum period of time, bring about the reactivation of the organism at precisely the time the host is ready for invasion (see Table 6).

(1) Temperature

Work so far has been done mainly on important crop parasites, *Globodera*, *Heterodera* and *Meloidogyne sp.* which exibit diapause in the egg stage. The eggs are produced by the females at the end of the host growing season and stay protected in the cysts or eggmasses until the next season.

The bionomics of *M. naasi* are fully outlined in section 1.B. Here it suffices to note that *M. naasi* eggs overwinter in the gelatinous matrix in the soil. They require a period of chilling to enable the hatching process to be completed (Watson and Lownsbery, 1970; Franklin *et al.*, 1971; Evans, 1974; Ogunfowora and Evans, 1977). *M. naasi* eggs enclose the fully mature J_2 which stay motionless in the egg until at least 7 weeks of chilling (5° C) is experienced and hatch at temperatures around 20° C (Ogunfowora and Evans, 1977). Some of the eggs (about 6%), require 2 or more such treatments before they hatch (Ogunfowora and Evans, 1977a;1977b).Depending on the origin of the population and therefore the climatological conditions experienced by it, *M. naasi* eggs show a small variability as to the best chilling and hatching temperature requirements (Gooris and D'Herde, 1977; Watson and Lownsbery, 1970; Ogunfowora and Evans, 1977).

H. avenae, the cereal cyst nematode, overwinters in Europe and oversummers in Australia, hatching at approximately the same months of the year throughout the world. It seems that a drought resistant strain has evolved in Australia for synchronization with its host life cycle. In Australia, *H. avenae* is found only in regions which experience a cold wet winter and a hot dry summer (Meagher, 1970). In Europe, *H. avenae* cysts require a period of low

temperature before appreciable hatch will occur . Hatch was found greater when eggs were subjected to diurnal fluctuations of 10° - 20° C (Williams and Beane, 1972).

Banyer and Fisher (1976) suggested an interaction between the temperature at which the eggs were produced and the temperature at which they were hatched, on the extent of dormancy. Cotten (1962) proposed that hatching occurs as a result of a rise in temperature after a period of low temperature, and Williams and Beane (1979) suggested that within limits, the greater the temperature change, the greater the stimulus to hatch in *H. avenae*.

In France, Rivoal (1979) found 2 distinct geographical races of *H. avenae* with different temperature requirements for hatching. Fr_1 race exhibits an "obligatory aestival diapause" and is found in mediterranean climate. Fr_4 race is septentrional and exhibits a facultative diapause during summer and autumn. Fr_4' s hatching behaviour is like that of other septentrional (oceanic temperate climate), European or American populations. Fr_1 is like the Australian race. Aestival diapause is induced or suppressed by rising and falling of temperature; a similar phenomenon is reported for the Italian population of *H. avenae* by Greco (1981).

Ellenby and Smith (1975) and Hominick (1979) reported different responses to low temperatures by populations of *G. rostochiensis* from Newcastle and Ayrshire in UK. The Ayrshire population was found to be adapted to lower temperatures for hatching than the Newcastle population.

Maximum numbers of *D. dipsaci* were extracted from soil in summer after the soil was chilled for 80 days at 5° C (Saigusa and Yamamoto, 1972). Also, Webster (1964) found that infectivity of J_4 in the soil was minimal in autumn and maximal in winter, when the bulbs began growing. Similarly, *Punctodera punctata* hatches in the field at the begining of January when the host starts growing (Evans and Perry, 1976).

H. glycines gave a delayed hatch in distilled water when the eggs were first chilled at -10° C for 0 - 90 days before they were set to hatch at 25° C (Ishibashi *et al.*, 1973).

H. carotae was found to give up to 94% hatch at 10° C without host root diffusates. High summer temperatures inhibited hatch in the Italian population, and hatch resumed in autumn (Greco, 1981).

H. trifoli at temperatures above 15° C resulted over 70% hatch in the absence of host plants (Yeates and Risk, 1976).

(2) Host root exudates as the diapause terminating factor

Species of cyst nematodes vary in their dependence on root exudates to stimulate hatching (Perry, 1978). The potato cyst nematode, *G. rostochiensis* overwinters in the J_2 stage in the egg inside the cyst. In the absence of host plants, cysts contain viable eggs for at least 8 years (Fraklin, 1937). About 2% of the J_2 hatch without any host root stimulation, but in the presence of host roots nearly all J_2 are stimulated to hatch. About 0.3% require a second stimulation to ensure a carry over to the next year (Shepherd and Cox, 1967). Eggs within cysts appear to be unaffected by drying, and after rewetting and stimulation with root diffusate, they hatch normally (Ellis,1966)

Doncaster and Shepherd (1967) showed that the response to the stimulus takes 3 days to show effects. Oxygen consumption of a single cyst increases within 24 hours of the addition of diffusate (Atkinson and Ballantyne, 1977). The water content of the unhatched J_2 increases when stimulated by root diffusate, reaching maximum after 48 hours (Ellenby and Perry, 1976). During this period, there is also a significant fall in the adenylate energy charge (Atkinson and Ballantyne, 1977 a). Doncaster and Shepherd (1967) observed that the 3 oesophageal glands became active soon after stimulation, but emission of gland secretions was not seen.

Tsutsumi (1978) reported that root leachates continued to stimulate hatching throughout the growing season, and leachate from the previous year, remaining in the soil, also stimulated hatching. Leachates from non-host plants gave low hatching ratios, but of potato varieties resistant to *G. rostochiensis* stimulated hatching. Forrest and Perry (1980) found that a 5 minute exposure to diffusate each week for 4 weeks, induced a 43% hatch in *G. pallida*. *G. rostochiensis* usually produces only one generation per year in the field, although healthy host roots are present when the first generation completes its development (Evans, 1982). This suggests that the eggs are in diapause and host root diffusates can only be effective when the diapause is terminated.

Ishibashi *et al.*,(1973) reported that *H.glycines* produces 2 "types" of eggs, according to the needs of the population. Eggs in a gelatinous

matrix, which hatch more readily in water and are more susceptible to unfavourable environmental conditions; and eggs retained in the female body, like in *Globodera spp* . The female body becomes a brown cyst and the darker it becomes, the more resistant it is to unfavourable conditions. The production of gelatinous matrix in *H. glycines* seems to be a seasonal variation as well as a geographical one. When environmental conditions are favourable, numerous gelatinous matrices are produced. They also noted that *H. glycines* survived 9 years in the field without host. Okada (1971) reported that soybean and kidney bean root diffusates stimulated hatch in *H. glycines* at various concentrations, and retained their activity 6 - 15 months at 5° C. The hatching factor responsible for breaking the diapause of *H. glycines* has been isolated by Masamune *et al.*,(1982). Glycinoeclepin A at concentrations 10^{-11} - 10^{-12} g/ml in water at 25° C was found to stimulate hatch *in vitro* in this species.

H. schachtii hatches more readily in water than *G. rostochiensis* but there seems to be an additional hatch induced by root diffusates (Perry and Clarke, 1981). Tefft *et al.*, (1982) reported that zinc chloride induced hatch in *H. glycines* (see Table 7). Lehman *et al.*, 1971, found that pH 3.5 gave the greatest hatch in this nematode and pH 5.5 reduced hatch.

Aqueous extracts from the cyst contents of *H. oryzae* strongly stimulated hatching of its eggs (Okada, 1972). Merny (1972) also reported a stimulating effect of the rice root diffusate on *H. oryzae* hatch, several weeks after application.

Winslow (1955) found that *H. goettingiana* cysts removed from soil in which peas were growing gave 15% hatch in water, compared to 1% from cysts from fallow soil. Also, Jones and Moriarty (1956);

Shepherd (1963) and Perry *et al.*, (1980a) showed that *in vivo*, *H. goettingiana* is stimulated to hatch by host root diffusates. The last authors suggested that the period of active root diffusate production by the pea plant is short and varies according to the time of planting and rate of growth of the plant, and that inhibitors seem to be absent from these diffusates.

There is evidence that root exudates from wheat, oats and barley, including resistant varieties, stimulate hatch in *H. avenae* , at soil temperatures 5° - 15° C in England and Australia (Kerry and Jenkinson, 1976; Williams and Beane, 1979; Banyer and Fisher, 1980). In India, Swarup and Singh (1961) found that barley root exudates stimulated hatch

at 22° - 23° C. Swarup and Gill (1972) found that 1 week old barley seedlings significantly increased hatch of the Indian population, but not older seedlings.

Greco (1981) reported that *H. carotae* eggs in newly formed cysts hatched no sooner than 2 months after being laid, at 10° C with or without stimulation by carrot root diffusates. High summer temperatures inhibited hatch in this nematode which was resumed in autumn. Constant 10° C alone was reported by the same author to stimulate up to 94% hatch in the laboratory, but fluctuating temperatures in the field gave very little hatch. Winslow (1955) suggested that *H. carotae* requires both a suitable temperature and root diffusate to hatch, and that the 2 factors produce a separate effect on the organism.

Webber and Baker (1968) found that emanations from young canary-grass roots (host plant) increased the larval emergence of *Hypsoperine ottersoni* (*M. ottersoni*).

(3) Factors inducing diapause

From the literature it appears that nematode species exhibiting diapause in a small proportion of the population under normal conditions, would produce a bigger proportion of progeny in diapause if the mother experienced stress during egg production.

Most *Meloidogyne* species are reported to exhibit diapause in all strains, *M. arenaria, M. hapla, M. incognita* and *M. javanica* in particular (de Guiran and Villemin, 1978; 1980). They also suggested that the mean percentage of diapause within eggmasses depended on the host plant and on the strain of the parasite. After experiments with *M. incognita* grown under different nutrient deficiencies, they concluded that "diapause has no need of any constraint before it appears, and seems to be a biological constant in this species and its close relatives". They reported that eggs in diapause (at an early embryonic stage) appeared from the begining of egg laying, and their percentage remained constant throughout egglaying, unless stresses were applied to the female during this period. The percentage of the eggs in diapause (about 10%) increased with the age of the female, and at the end of egg production, the majority of the eggs produced were in diapause. Ishibashi

(1969) and de Guiran (1979a, 1980) suggested that lack of nutrition in the plant gave an increased percentage diapause in *M. incognita* (up to 80%). De Guiran (1980) found that moisture availability in the soil was responsible for the type of eggmasses produced by the females and not nutritional stress. He (1979) also suggested that the frequency distribution of diapaused eggs follows a log-normal distribution, which is transmitted from one generation to the next, irrespective of the percentage in the original eggmass.

Meagher (1974) reported that dry storage of *H.avenae* cysts appeared to induce dormancy, so that when hatched at 15° C, there was no emergence for a period of 24 weeks. Hatching then recommenced. This, he concluded, fits very well with the hot dry summer conditions the nematode has to go through in Australia.

Franco and Evans (1979) reported that hatching of *G. rostochiensis* and *G. pallida* was affected by the daylength the host was growing at. Cysts produced in a 16 hour-day, gave higher hatching rates than those produced in a 12 hour-day. Short photoperiods decreased hatching from Katahdin potato variety, but increased hatching from Superior and Sebago varieties (Evans, 1982). *G. rostochiensis* eggs from potato plants grown in total darkness, hatched in the same fashion throughout the year, unlike eggs grown on plants under normal conditions (Hominick, personal communication).

Bird *et al.*,(1980) found that over a 40 hour period, no *M. javanica* J_2 hatched from roots of tomato plants exposed to R (red-enriched) illumination, whereas a mean of 5,252 J_2/plant hatched from roots of plants exposed to FR (far red) illumination.

(4) Terminating diapause in the laboratory

It is possible to mimic the environmental factors terminating diapause in the laboratory and so reactivate the nematode earlier than it would in nature.

M. naasi experiences low temperatures in the soil throughout the winter. In nature these temperatures fluctuate and at the depths the nematode eggs are found, dont rise above 15° C. In the laboratory, at constant 10° or 5° C, hatching rose significantly after 8 weeks (Watson and Lownsbery, 1970, Ogunfowora and Evans, 1977a). The same authors used NaOCl to artificially hatch *M. naasi*. Mechanically hatched J_2 of this nematode, were found to be infective (Gooris and D'Herde, 1977).

Many chemicals have been found in the laboratory which mimic the potato root diffusates and induce hatching in *Globodera* species (see Table 7).

Significantly more *H. avenae* juveniles hatched from cysts exposed to white light than from cysts kept in the dark (Koshy and Swarup, 1971).

Electric shock was found to induce hatch in *M. incognita acrita* eggs by Caveness and Caveness (1969).

(5) Differences in diapausing and non-diapausing individuals

Watson and Lownsbery (1970) found no morphological differences between cold-warm treated eggs and controls of *M. naasi* eggs under the electron or light microscopes.

Banyer and Fisher (1972) observed behavioural differences between mechanically hatched J_2 and normally hatched ones, of *H. avenae*. The former were sluggish and became inactive compared to the active latter ones. In the diapausing J_2, the pharyngeal glands showed no activity or contents.

(6) Diapause followed by quiescence

Cooper and Van Gundy (1971), Evans and Perry (1976), Ogunfowora and Evans (1977a) and other workers, reported *M. naasi* J_2 inside the egg surviving for some months after termination of diapause if conditions were unfavourable, due to a quiescence period following diapause.

Osmotic stress and anaerobiosis inhibited hatch in *M. naasi*, but activity was regained within 30 minutes when aerated at room temperature (Ogunfowora and Evans, 1977a; Ogunfowora, 1979). Desiccated eggmasses would not hatch, but moistening the soil produced emergence of J_2 (Fraklin *et al.*, 1971).

Similar results were obtained for *H. avenae* by Hesling (1956) who reported drying to inhibit hatch in the European population. In Australia, Banyer and Fisher (1971) found that alternating temperatures and osmotic stress inhibited normal hatch in this nematode.

Wallace (1955) found that fluctuating oxygen concentrations reduced hatch in *H. schachtii*. The higher the concentration, the higher the rate of hatch in beet diffusate.

(7) Theories on diapause

There are many hypotheses trying to explain the events leading to

the induction and termination of diapause. Some propose the active participation of the J_2 in receiving and utilizing a stimulus from the environment, some believe that the eggshell is responsible for stopping the J_2 from escaping when it is ready to do so. Others suggest the presence of a hatching inhibitor in the gelatinous matrix or cyst.

It is not easy to come to definite conclusions from the work done so far. No enzymes or inhibitors have been isolated from within the eggmass or cyst system. Chemicals that aid in breaking diapause in the laboratory, may cause a completely different effect on the egg than the factors involved under natural conditions.

Evans (1974) suggested that a single enzyme or systems of enzymes having their temperature optima around 10° C might be responsible in breaking diapause in *M. naasi*. He goes on to say that such enzymes or neurohormones as in insects might be produced by the nematode at the time required due to the chilling treatment. Watson and Lownsbery (1970) proposed that the temperature treatment produces a chemical rather than a structural effect, perhaps inactivating a hatching inhibitor in *M. naasi*. Ogunfowora and Evans (1977) found that free eggs and eggs in the gelatinous matrix of this nematode, produced the same percentage hatch, so the matrix did not appear to contain a hatching inhibitor, as Gooris and D'Herde (1972) had proposed. It is however possible, that if an inhibitor exists, it could be sited in the eggshell, and that NaOCl treatment or ascorbic acid, inactivated it (Ogunfowora and Evans, 1977).

Banyer and Fisher (1971) divided egg hatch in *H. avenae* into 2 phases: (i) period of J_2 development, with optimum temperature 10° C; (ii) eclosion, with optimum temperature 20° C. They believed that these optima are independent of the temperature at which the other phase underwent. They proposed that the rate of hatching at constant temperature is the resultant of the different times taken to complete each phase. Alternating temperatures failed to hatch or speed up phase (i). Fisher's (1981) evidence supports this theory. In Australia he found that females raised at 20° C were brown and contained eggs at the J_2 stage, but the ones raised at 10° C were white and contained eggs at all stages of development. The latter eggs needed a period of time at 20° C before they could hatch at 10° C, but the former ones hatched normally.

Banyer and Fisher (1976) found that 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.

Similar theories were proposed by de Guiran (1979a) and Ishibashi (1969) regarding diapause in *M. incognita* and other *Meloidogyne* species.

Rivoal (1978) suggested that diapause is a hereditary character. Working with the Fr_1 and Fr_4 races of *H. avenae* in France, he reared both populations under the same climatic conditions and found that the differences between them regarding their temperature requirements for hatching, remained stable. El-Shatoury (1978) suggested that diapause in *G. rostochiensis* is genetically controlled. In experiments in Ireland, early hatched parents gave a progeny which hatched early. Late hatched parents gave a progeny which exhibited dormancy, the extent of which was in most instances determined by the parent's time of hatching. The same author (1977) reported that mass matings of *G. rostochiensis* using larvae hatched readily in water, gave a significantly high proportion of sterile matings. Evans (1980) suggested that genotypes of *G. rostochiensis* exist which are able to hatch in water, while others require a hatching stimulus.

Banyer and Fisher (1980) working with *H. avenae* found that diapaused J_2 which were hatched mechanically, were not mobile and had granules in the pharyngeal region. Low temperature treatment of these J_2 "transformed" them into active J_2. They went on to suggest that the J_2 is affected by the low temperature stimulus and not the egg-shell.

There are theories regarding the effect of the host root diffusate on the eggs of *Globodera* species. Ellenby and Perry (1976) suggested that the hatching factor from the host roots either softens the egg-shell so that the J_2 is less constricted inside it, or produces some changes in the J_2 which then takes up water thus exerting greater pressure on the egg-shell.

Ellenby (1974) showed that the J_2 and the egg-shell of *G. rostochiensis* are freely permeable to water in both directions. He believes that the J_2 is physically constricted by the egg-shell, in which it fits completely, so restricting its movement and water content. Thus, softening of the egg-shell would allow movement of the J_2 which would exert pressure to break it. The moment the J_2 is liberated from the egg, it takes up water and increases its length.

Clarke and Perry (1977) proposed that a change in permeability of the egg-shell due to root diffusate stimulation, allowed the diffusion of egg fluid solutes out of the egg. This removed, they suggested, the osmotic stress on the J_2, which then took up water, which induced the behavioural sequence leading to hatching. They suggested that trehalose

might be the solute in question. Clarke and Hennessy (1976) found that trehalose is retained within the egg. The internal osmotic pressure of *G. rostochiensis* J_2 inside the egg was found to be equivalent to 0.4 M trehalose (Clarke *et al.*, 1978).

Clarke and Perry (1977) suggested that the hatching factor might interfere with the close packing of the molecules of the lipid layer, causing osmoregulation changes. However, the extreme dilutions at which the hatching factor can act (10^{-14} g/ml) suggests that they do not operate directly (Masamune *et al.*, 1982)

Ellenby (1946;1956) observed that hatching occurs sooner in half or punctured cysts and isolated eggs than in whole cysts. He attributed this to a possible accumulation of solutes from hatched and hatching eggs, which temporarily delayed hatching until the concentration is reduced by diffusion. Also, Ellenby and Perry (1976) reported different rates of water uptake in J_2 inside eggs from whole and halved cysts in root diffusate. Okada (1972a) concluded that oxygen deficiency as well as increased acidity of the hatching medium resulting from the activity of the J_2 hatched, inhibited further hatch in *G. rostochiensis* . Similar reports for *H. schachtii* exist (Wallace, 1955).

Kaul (1962) isolated a catechin substance from cyst walls of *G. rostochiensis* which he found present in the cyst in considerably larger amounts during autumn and winter than in the following spring. He believes that this substance is responsible for the inhibition of hatching during autumn and winter.

Hague (1958) suggested the presence of inhibitors in the root diffusates of potato plants which interact with the stimulants to give the final hatching effect. Okada (1972a) supported the presence of both a hatching stimulant and a hatching inhibitor inside the egg-shell of *H. glycines*. He proposed that the balance of the activity of the two, plays a significant role in hatching.

Perry *et al.* (1980) working with *H. schachtii*, showed that the egg fluid may not provide as great an osmotic stress as that found in *G. rostochiensis* eggs. Root diffusates, they suggested, may induce a slight dilution of the egg fluid, which may be important in either decreasing the low osmotic pressure, or removing possible inhibitors in the egg fluid. Perry (1978) found no significant change in water content of *H. schachtii* J_2 before hatching, whether they had been immersed in root diffusate or water.

Steel (1969) proposed that environmental factors may be responsible

for producing a diurnal rhythm in hatching of *H. schachtii* eggs. He found that during periods of maximum activity, accumulated daily hatch was proportional to time.

Atkinson and Taylor (1977) and Atkinson and Ballantyne (1979), suggested that the hatching mechanism of *G. rostochiensis*, is a calcium-mediated process. They believe that at the inner surface of the egg-shell of this nematode, a membrane contains a calcium-binding protein (a glycoprotein). They used ruthenium red and lanthanum chloride (which are specific inhibitors of calcium-mediated processes) to study their effect on hatching of *G. rostochiensis* eggs which were stimulated by potato root diffusate. They found that, at very low concentrations, these chemicals caused a 90% inhibition of hatch in *G. rostochiensis* but had no effect on *H. schachtii* eggs, which hatch readily in water. They concluded that these chemicals inhibited the hatching mechanism by competing with calcium for its binding site, and thus blocking calcium tranport. They also found that calcium ionophores (which initiate calcium-mediated processes), stimulated hatch in *G. rostochiensis*. The findings of Atkinson *et al.*,(1980) supported this hypothesis. They reported calcium uptake in eggs exposed to a hatching stimulus, so they concluded that the active factor in potato root diffusate may initiate hatching through a calcium-mediated process. However, Clarke and Hennessy (1981) obtained substantial hatch in root difusate, from which Ca^{2+} had been removed.

Wallace (1966, 1968a) among others, believes that hatch in *M. javanica* and other species, takes place after the secretion of enzymes by the J_2 inside the egg. He suggested that the hemizonid (see Goodey, 1951) may function as a receptor for hatching stimuli and trigger enzyme synthesis, a model similar to that of the insects, where in the "brain" a centre exists for receiving such stimuli from the environment (Lees, 1955).

In *Artemia salina* (a brine shrimp) the post ribosomal supernatant of the dormant embryos, contains a large quantity of a 19S protein complex. The quantity of this complex decreases during further development to nauplius larvae to only 15% (de Herdt *et al.*, 1981). It is possible that such chemicals exist in nematodes and act as a clock (clepsydra) in diapausing organisms.

Raison (1973) considers the effects of temperature (high and low) on phase changes in membrane lipids and how this affects metabolism. He concludes that changes in the physical properties of membranes induced by lowering temperature, can affect the binding of enzymes and the

Table 7 : Chemical stimulation of hatching.

nematode species	host root diffusates	non-host root diff.	cyst contents of own sp.	cyst contents of other sp.	egg contents of own sp.	female contents	fungi exudates	some nematicides	some herbicides	digestive enzymes	sodium hypochloride	flavianic acid	picric acid	anhydrotetronic acid	picrolonic acid	tetronic acid derivatives	some fatty acids	some aminoacids	citric acid	fumaric acid	zinc salts	cadmium chloride	potassium permanganate	some pyrazolone compounds	sodium metavanadate	calcium sulphate	lanthan um chloride	ruthenium red	thiamine hydrochloride	some mineral salts	oxalic acid	chlorogenic acid	caffeic acid
Globodera pallida												+110			+110						+110	+110			+110								
G. rostochiensis	+168	+168	+190	+190			-173			+188	+39	+46	-43	+45	+110	-45			+262	+262	+45	+191	+45	-45	+188		-5	o188			-262	-262	-262
Heterodera amarantbi	+243											+110																					
H. averae	+246								+156												-44									-156			
H. cajani	+151																																
H. carotae										+92	+46										+44												
H. cruciferae											+46			-45	-45						+44												
H. glycines	+191	+190	+188	+191	+191	+191				+92					+45	+248					+45				+163								
H. goettingiana	+46	-46																			+44												
H. oryzae	+182			+190																													
H. oryzicola	+207											+207																+207					
Osborne cyst nematode										+92																							
H. sacchari	-209	+121																					+207										
H. schachtii	+122						-261	-240	+143	+92	+46		+45				-261				+44	+44											
H. solanacearum											+110				+110						+110	+110			+110								
H. tabacum											+110				+110						+110	+110			+110								
H. trifoli											+46										+49												
H. virginiae											+110				+110						+110	+110			+110								
H. weissi										+92																							
Meloidogyne hapla	+31																																
M. incognita	o114						-10			o114																							

hatching effect: + stimulation
- inhibition
o no effect

numbers refere to bibliography.

permeability of membranes.

4. Conclusions.

As an approach to experimentation with *M. naasi*, several important points have emerged from this review.

Dormancy is an economical and often "safe" (risk free) way for an organism to spend long periods of time during which survival would otherwise be impossible. In this case, the "fittest" members of the population are the ones that would be able to alter their metabolism (enter quiescence), and/or their development (enter diapause) successfully, when conditions demand, and resume normal activity when favourable conditions return. Dormancy therefore results in conservation of the species, by extending the population's life span and by carrying it through unfavourable environmental conditions (see fig. 2).

In quiescent organisms, it appears that the whole of the body is adapted to cope with an environmental stress, and to undergo metabolic and other changes for survival. In diapaused individuals however, special mechanisms are involved, which are not yet understood in nematodes.

The incidence and intensity of diapause may be due to genetic variation in the population. In insects, diapause has been shown to depend on several genes (Lees, 1955). Some experimental evidence suggests that this might also be the case in nematodes.

Nematode bodies, especially those of plant parasitic and free living species are very small, and biochemical studies involving small changes during the induction or termination of diapause are difficult to follow. Studies using gel electrophoresis however, might soon give us clues to answer some of the questions.

It is desirable that all the hormonal and biochemical implications associated with a given example of dormancy should be understood before it is classified as quiescence or diapause.

At the moment, the case of nematode dormancy in the egg stage is thought to be diapause. In such cases, although we might know which environmental factors are the "right signals" for terminating diapause we do not know whether they act directly on the organism inside the egg, or indirectly by altering the egg-shell or the egg-membranes, thus allowing a signal (oxygen, water, chemical) to reach the nematode inside the shell.

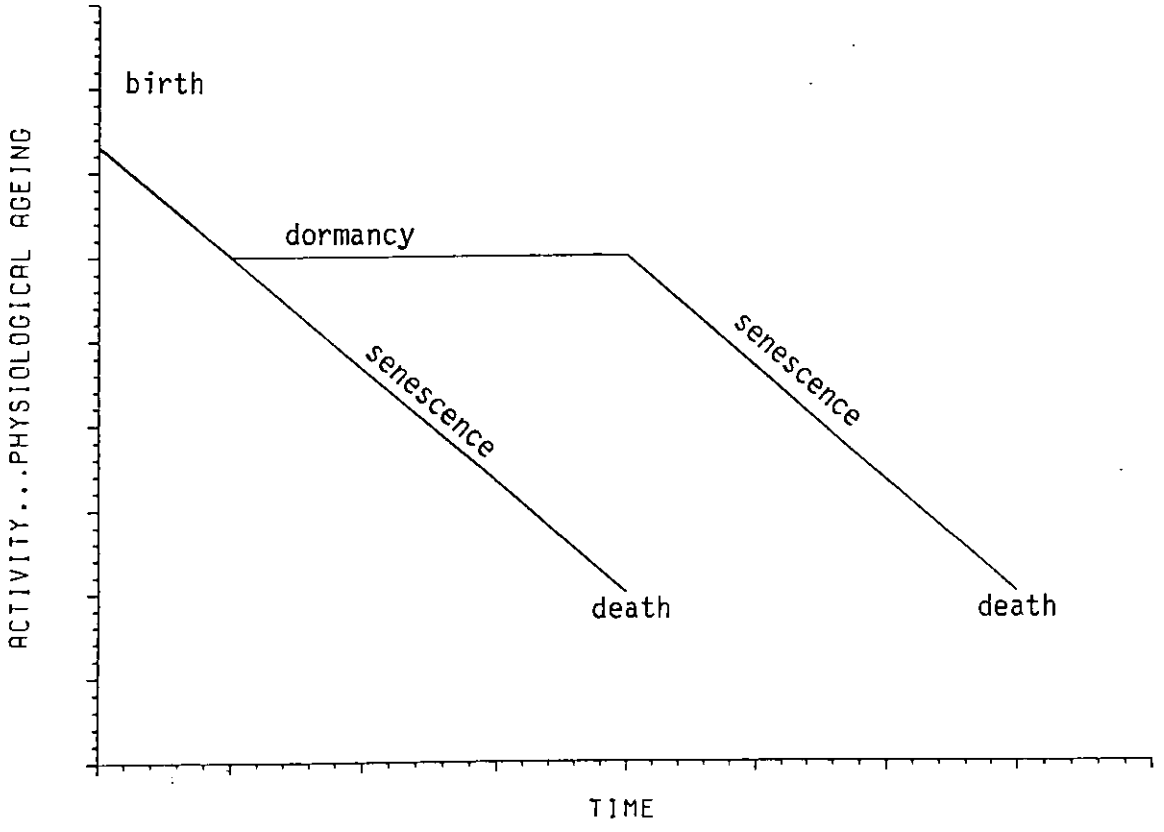


Fig. 2 : A diagrammatic representation of the life span of a population (from Cooper and Van Gundy, 1971).

Many classical examples of diapause from the arthropods (from where we borrowed our terminology), include cases where chemical, mechanical or thermal shock treatments, are effective in ending diapause prematurely (Lees, 1955).

In nature it is relatively easy to estimate the minimum time required for the signals to act on the nematode receptor system, using seasonal clues, but the possibilities of premature termination of diapause in the laboratory confuse the matter. Another problem is that in nematodes, as in many insects, 100% diapause in a population is rare. This is a good strategy for spreading the population in time, to take advantage of any suitable conditions for development. However, as the number of the individuals varies from low to high (depending on the species, climatological conditions and probably generation), workers in the field may overlook the diapausing members of the population, or describe the phenomenon as quiescence. Furthermore, not all diapausing individuals require the same "amount of stimulus" for reactivation, and diapause may be followed by quiescence if conditions at the time of the termination of diapause are unfavourable. Often it is difficult to determine the end of one process and the begining of the other.

Full understanding of the phenomenon quiescence and diapause, might help us interfere with the life cycle of economically damaging pests in the field, for control purposes.

Thus, as presently understood from the limited evidence on a few populations, *M. naasi* overwinters as a diapausing and/or quiescent juvenile in the egg, and temperature changes signal conditions leading to hatch. However, the exact limits of temperature changes are unknown so a detailed study of the effects of a wide range of constant, fluctuating and interrupted temperature treatments on a range of isolates was made, to improve our understanding of the phenomenon (see sections 3. A,B,C; 4.G).

As quiescence may follow diapause in this nematode, the effects of osmotic stress, desiccation and effects of the presence of the female on the hatchability of the eggs were investigated (see sections 3.D; 2.II.A; 4.E).

Studies were made on genetic influences (see section 4.F), maternal effects, eg stress on the egg laying female (see sections 3.E,F,G,H; 4.A,B,C,D).

The ability of some mechanically hatched J_2 to infect host, does not allow the conclusion that *M. naasi* eggs are in quiescence and not

in diapause. For further information, the percentage infectivity of such J_2 was compared with that of J_2 that hatched spontaneously or after a chilling treatment (see section 4.H).

Variability within and between populations of *M. naasi* was studied and related to their place of origin. Morphological as well as behavioural and physiological differences would suggest the presence of more than one race or climatic type of *M. naasi* as has been found in *H. avenae*.

Since *M. naasi* has a world wide distribution, differences in their diapause requirements were sought in relation to local climatic characteristics.

CHAPTER 2

MATERIALS AND METHODS

2.I. General materials and methods

A.(i) populations used

In response to written requests, infested soil, roots and eggmasses of *M. naasi* were obtained from the countries shown in Table 8 and cultured in constant temperature rooms.

The history of these populations and the climatic conditions of the country of origin, were noted (see Table 9).

Notes on the culturing history of each population were kept to ensure all treatments which the particular eggmasses had experienced, were known. An example of these notes is given in appendix 1 for the Belgian population.

In the various experiments reported, the populations used were chosen mainly for their availability, unless otherwise stated.

the New Zealand population: Four populations were obtained from New Zealand, arbitrarily labelled A - D, and cultured in their original soil in the laboratory, as described in section 2.I.B.

Infected roots from each culture were stored in their soil at 5° C for 9 weeks, then cut into small (1 cm long) pieces and set to hatch at 20° C, in small Whitehead trays (see section 2.I.C(i)). Hatched J_2 were counted daily for 2 weeks and then once every week.

Population B produced a steady number of hatched J_2 every day (see Fig. 3), and was therefore chosen for use in all the experiments described subsequently.

(ii) morphology of the populations used

To date, no important morphological differences have been recorded between populations of *M. naasi*. The populations used in this study, were characterized morphologically to find out whether differences in hatching behaviour had any morphological correlates, suggesting that they may not all belong to the same species.

The most important taxonomic characters were studied for all populations of *M. naasi* used in this work. Eggmasses were obtained from cultures (see section 2.I.B) 12 weeks after invasion, and females were

Table 8 : Source of material used to set up cultures in the laboratory.

country of origin	code used	material received	received in
Belgium	BEL	infested soil roots	late summer 1980
France	FRA	infested soil	autumn 1980
Germany	GER	infested soil roots	late summer 1980
Italy	ITY	infested soil eggmasses	summer 1980 autumn 1981
New Zealand	NZL	infested soil roots	autumn 1981
USA, California	CAL	infested soil	winter 1981
Wales	WAL(1)	infested soil	1979
	WAL(2)	infested soil	late summer 1980
	WAL(3)	infested soil	1971

Table 9 : Populations of *M.naasi* used, and their history

population from:	code used	latitude	soil types	climate	rainfall per year inches	main hosts
Belgium	BEL	50.5(N)	gray-brown podzolic	maritime	20-40	spring wheat
France	FRA	48(N)	gray-brown podzolic	maritime	20-40	winter spring wheat
Germany	GER	53(N)	podzolic	maritime	20-40	winter spring wheat
Italy	ITY	41(N)	gray-brown podzolic	mediterranean	20-40	winter durum wheat
New Zealand	NZL	40(S)	gray-brown podzolic	mediterranean	20-40	winter wheat
USA California	CAL	35(N)		mediterranean	10-20	winter barley wheat
UK: Wales	WAL	52.5(N)	podzolic	maritime	20-25	spring winter barley wheat

The information on soil types, climate and rainfall was obtained from Peterson, 1965.

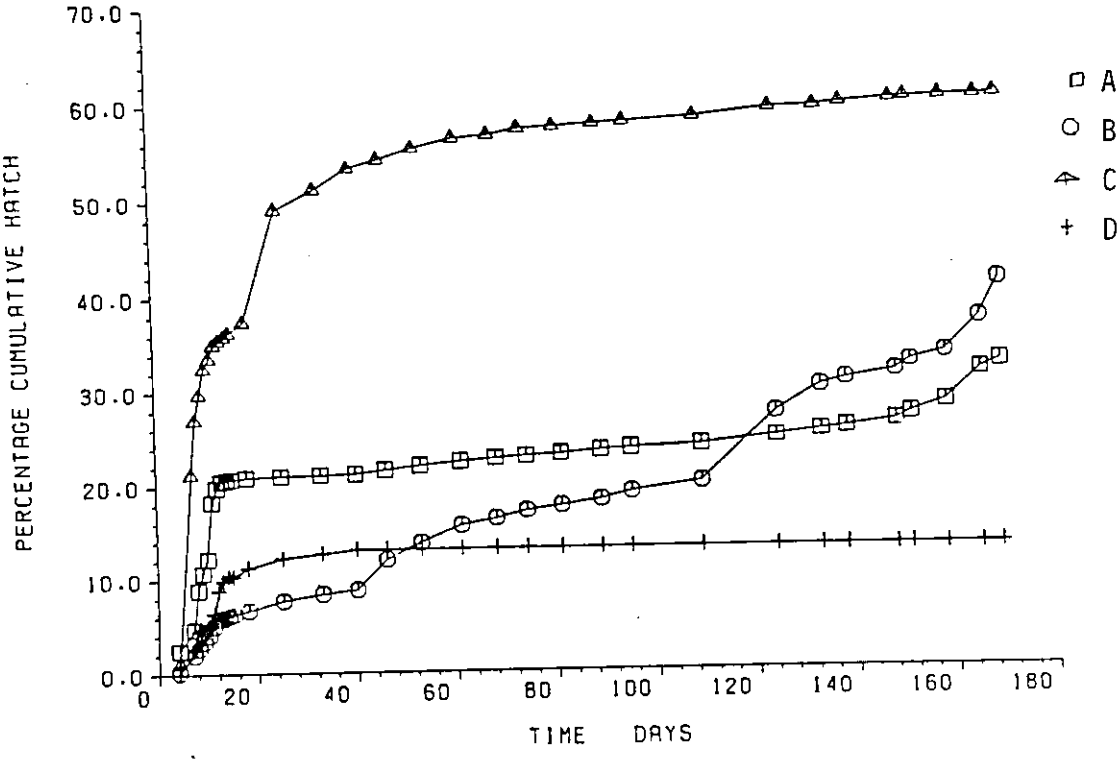


Fig. 3: The hatching behaviour of the 4 New Zealand populations of *M. naasi* after 9 weeks chilling at 5° C.

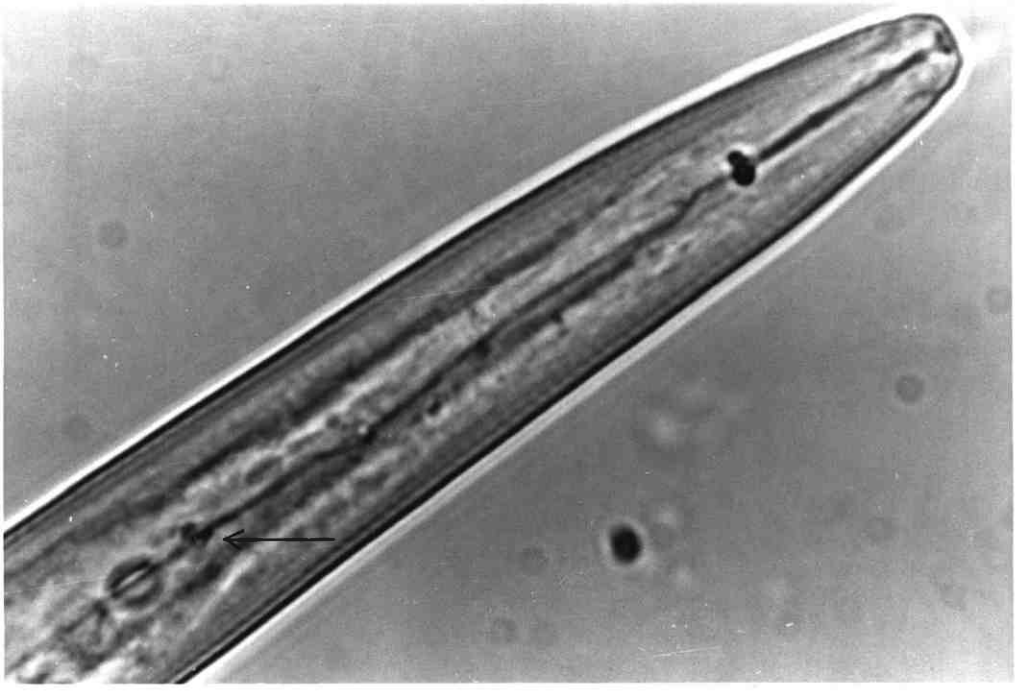


Fig. 4: Vesicle structures in the J_2 stage of *M. naasi*.
mag. x 2530

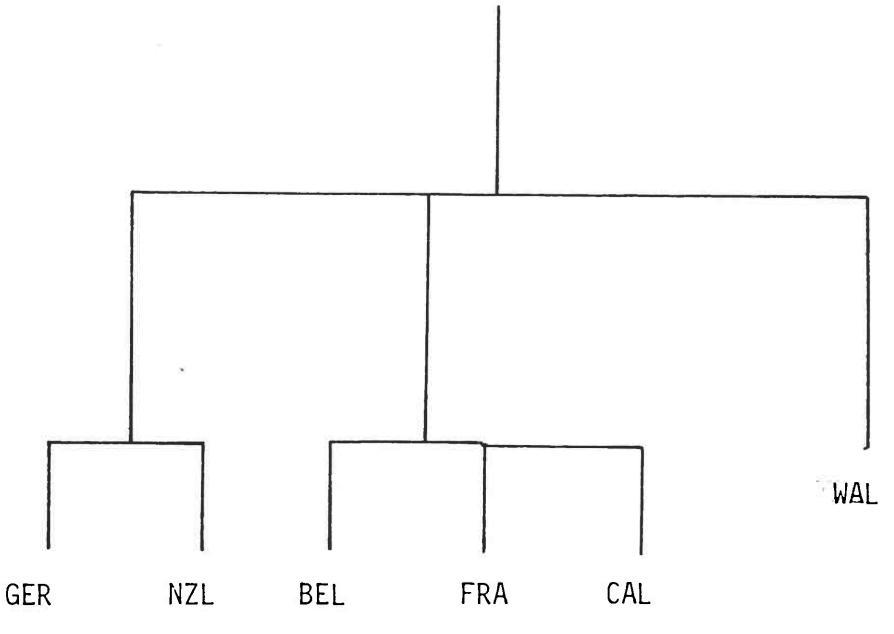


Fig. 5: Dendrogram of hierarchical cluster analysis, using 15 characters to study taxonomical differences between the 6 populations of *M. naasi* (see Table 10).

Table 10: Morphometrics of the 6 *M.naasi* populations.

character		BEL	FRA	GER	NZL	CAL	WAL
Taxonomy							
♀ stylet length	*	13.9µ (6)[2]	14.4µ (5)[2]	14.4µ (2)[2]	14.8µ(20)[4]	13.5µ (1)[2]	13µ (25)[1]
♀ stylet cone length		7.65µ (6)[2]	8.1µ (5)[2]	7.65µ (2)[2]		8.1µ (1)[2]	
♂ body length		1006µ (8)[2]					1148µ(25)[1]
♂ stylet length		18.4µ (6)[2]					18µ (25)[1]
J_2 body length	*	444µ [3] 416µ (20)[2]	435µ (20)[2]	463µ (20)[4]	471µ (20)[4]	435µ (15)[2]	435µ (25)[1] 435µ (25)[2]
J_2 stylet length	*	18µ [3] 10.8µ (5)[2]	10.8µ(20)[2]	13.8µ(20)[4]	13.6µ(20)[4]	10.8µ (1)[2]	14µ (25)[1] 12.9µ(21)[2]
J_2 stylet knob width	*	2.5µ (20)[2]	2.6µ (20)[2]	2.5µ (20)[4]	2.5µ (20)[4]	2.3µ (15)[2]	1.9µ (17)[2]
J_2 stylet knob length	*	1.4µ (20)[2]	1.4µ (20)[2]	1.2µ (20)[4]	1.4µ (20)[4]	1.7µ (15)[2]	1.4µ (17)[2]
J_2 DGO from stylet base	*	2.9µ (20)[2]	2.7µ (20)[2]	3.1µ (20)[4]	3.1µ (20)[4]	3.0µ (13)[2]	3.2µ (3)[2]
J_2 tail length	*	72µ [3] 71.4µ(15)[2]	74.3µ(20)[2]	74.8µ(20)[4]	75.1µ(20)[4]	73.2µ(12)[2]	70µ (25)[1] 71.5µ(12)[2]
median bulb valve length	*	3.6µ (20)[2]	3.6µ (20)[2]	5.1µ (20)[4]	5.3µ (20)[4]	4.0µ (15)[2]	4.3µ (25)[2]
median bulb valve width	*	2.9µ (20)[2]	2.9µ (20)[2]	3.0µ (20)[4]	3.1µ (20)[4]	2.9µ (15)[2]	3.0µ (25)[2]
excretory pore to anter.end	*	71.2 µ (15)[2]	72.2µ(18)[2]	75.9µ(20)[4]	74.5µ(20)[4]	77.2µ(11)[2]	72.6µ(13)[2]
hyaline tail end	*	23.1µ(19)[2]	23.4µ(20)[2]	24.7µ(20)[4]	24.4µ(20)[4]	23.4µ(15)[2]	20.7µ(25)[2]
stylet base to anterior end	*	15.3µ(20)[2]	15.3µ(20)[2]	15.4µ(20)[4]	15.1µ(20)[4]	15.3µ(14)[2]	16.0µ(25)[2]
Biology							
average number of eggs per female: in field		495 spring wheat 149 winter wheat					500 spring wheat
in laboratory: in soil ⊙	*	245	296	229	272	252	231
in water culture ⊙⊙	*	182	239	144	185	160	182
male to female ratio	*	23 : 311	0 : 88	6 : 312	5 : 160	19 : 201	0 : 69
vesicles in egg, J_2, male		+, +, +	+, +, +	+, +, +	+, +, +	+, +, +	+, +, +

* Characters used in hierarchical cluster analysis.

[1] M. Franklin, 1965.

[2] S. Jepsen, personal communication.

[3] Gooris and D'Herde, 1977.

[4] Own measurements.

( ) Sample size.

Added in proof: In reviewing the literature, Hewlett and Tarjan (1983) found that reported J_2 body length varied from 418 to 465 µ and stylet length from 13 to 15 µ. Female stylet length they found to vary in reports from 11 to 15 µ and male stylet length from 16 to 19 µ.

⊙ after 12 weeks of culturing.

⊙⊙ after 10 weeks of culturing.

dissected out of the eggmasses for observations. Eggs were obtained directly from eggmasses, while J_2 were collected after hatching (see section 2.I.C). Males were obtained from the Hydroponic cultures (see section 2.I.B), 5 to 10 weeks after inoculation.

The nematodes were heat relaxed in tap water on a glass slide,a coverslip placed on top and secured with petroleum jelly, and measured under high power.

The number of eggs per female was recorded as described in section 2.I.D(iii), without the staining process.

Measurements of the Belgian, French, Californian and Welsh populations were made by Dr. Susan Jepson at Rothamsted Experimental station after rearing material, sent by me, under similar conditions on the same host, for one generation. Due to her unsuccessful culturing of the German and New Zealand populations, I measured those isolates at Silwood Park.

RESULTS

All six populations studied had characteristics consistent with *M. naasi* Franklin, 1965. This was confirmed by Jepson (personal communication). There were no obvious morphological differences between the isolates (see Table 10). One way analysis of variance on larval tail and stylet lengths however, showed significant differences between the populations ($p < 0.001$). To study the taxonomical differences between the six populations in more detail, a hierarchical cluster analysis (using a Genstat package on the ICCC computer), using 15 different characters was performed (see Table 10). The dendrogram produced, showed the Welsh population differing taxonomically from the other 5, while German and New Zealand populations had similarities. The Belgian, French and Californian populations also showed similarities (see Fig. 5).

B. Setting up cultures

(i) materials used

Cultures were set up by infecting 1 week old plants of a spring wheat *Triticum aestivum* cv Sappo with either naturally infested soil, infected roots, free eggmasses or hatched J_2 (hatched spontaneously, after a chilling treatment, or mechanically).

Apart from naturally infested soil, the following soil mixtures were used: 50% river sand, 50% loam; 100% river sand; 50%river sand, 50% coarse sand; 100% loam.

Material containing nematodes was chilled for various periods of time at 5° C before planting. Roots were cut into about 0.5 cm pieces before use. Eggmasses were introduced either free, or in miniature muslin bags so they could be recovered easily.

Eggmasses were hatched as described in a later section (2.C) and a known number of J_2 in tap water suspension was pipetted into the appropriate containers, containing wheat seedlings at various ages, according to the experiment.

To liberate J_2 mechanically for infectivity experiments, large numbers of eggs were separated from the gelatinous matrix of the egg-masses using fine forceps and hair loops. Such eggmasses were previously set at 20° C, with no previous chilling for 2 weeks to allow spontaneously hatched larvae to escape (see section 2.I.C). The freed eggs were placed in 50 ml of water with 1 cm^3 of fine river sand in an "Ultra Turax" colloid mill for 5 minutes, as described by Gooris and D'Herde (1977). Juveniles released by this treatment were separated from the mixture, using 90 μ sieves lined with one layer of kleenex tissue. Many larvae were lost this way because not all eggs were successfully made to release their larvae, and many J_2 that did hatch were damaged. Likewise, not all J_2s hatched successfully became mobile and able to pass through the kleenex tissue. Only a very small proportion ($<$ 1%) of the original number of eggs yielded healthy, active J_2s.

Before planting, the wheat seed was germinated by soaking it in tap water for 48 hours, changing the water every 24 hours. Only germinated seed was used. If well established seedlings were required, they were transplanted from 100% fine river sand to the chosen soil mixture when needed.

(ii) culturing methods used

Several types of containers were tested for suitability both in producing heavily infected hosts, and in allowing easy recovery of egg-masses from roots.

a) a range of "traditional" plastic pots.

b) plastic transparent pots (for root observation)

c) plastic transparent 4-chamber pots with a large surface area for observation of roots (see Fig. 6)

All transparent pots were covered with black polythene to keep the root systems in the dark.

d) petri-dishes, modified from Crump and Kerry (1977). Plastic petri-

dishes (9 cm diameter) were cut to give a maximum depth of 1.5 cm from the circumference. The lid and base of the petri-dish were held together by an elastic band. One wheat seedling was planted in soil mixture in each petri-dish. Eight to 12 such dishes were stood together in a plastic rest made from a 9 cm diameter black plastic pipe, cut longitudinally into 2 equal parts. The pipe was open at the bottom to allow excess water to drain from the petri-dishes. It was stood on 2 "legs". The petri-dishes were shielded with black polythene, allowing only the top cut side of the dishes and the plants to have light (see Fig. 7). This method resulted in very good observation areas and saved space.

e) hydroponics. Several columns (as shown in Fig. 8) were set up in order to rear *M. naasi* infected wheat seedlings, in nutrient solution.

Five-day old wheat seedlings (stem length 3 cms; roots 7 cms) were replanted in small (7.5 cm tall) canisters, in sterile river sand and 1,000, one-day old J_2 inoculated as described above. Three replicates for each population were set up. After 3 days at 20° C (16hours light, 8 hours dark), the seedlings were uprooted and the roots washed. When completely free of sand, a seedling was placed in the nutrient solution in the apparatus and secured with aluminium foil, until harvesting, 10 weeks later. The system was aerated continuously using an electric aquarium air pump. The nutrient solution (see appendix 2) was changed weekly, and tap water was added daily to make up for losses.

f) closed canister technique. Foot's (1977) technique was followed, using small (7.5 cm tall, 7x7 cm top cross section) transparent plastic canisters. The canisters were surface sterilized with 2% NaOCl, and filled to within 1.5 cm of the top with a steam sterilized mixture of 50% river sand, 50% coarse sand, containing 5% moisture. Wheat seeds were soaked for 24 hours, then surface sterilized for 15 minutes in 0.26% NaOCl and rinsed 5 times in sterile tap water. Six seeds were placed just under the sand surface of each canister. The canisters were closed with air-tight lids which were perforated with minute holes to allow oxygen to reach the plants.

After 3 days incubation at 20° C in the dark, 600 J_2 hatched over the previous 24 to 48 hours, were added in a 2 ml sterile tap water suspension. Three ml of full strength sterile nutrient solution were also added. The canisters were left open in a sterile cabinet at 20° C for 24 hours to allow evaporation of excess water. In such cultures, *M. naasi* was kept monoxenically in the dark, under controlled temperature and moisture conditions.

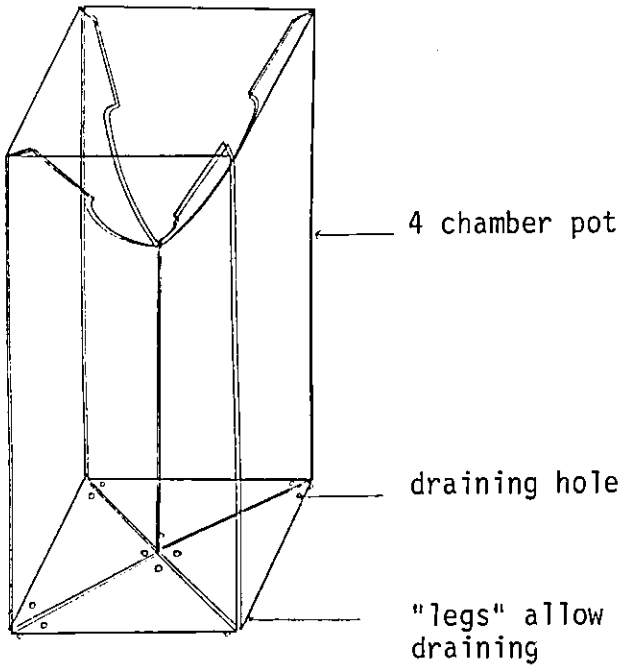


Fig. 6: Four-chamber pot (8 fl. oz. capacity).

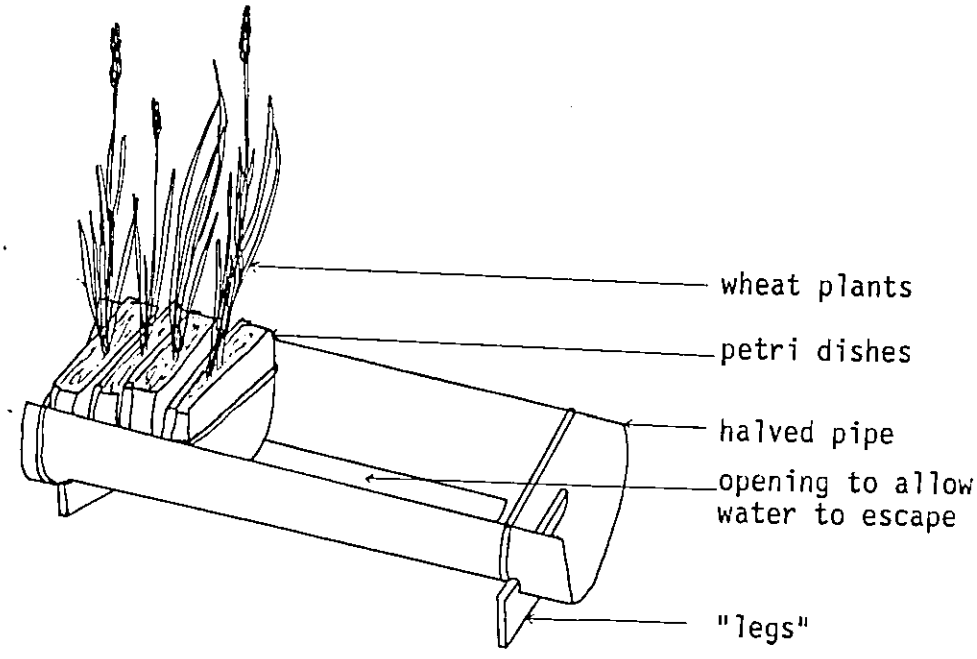


Fig. 7: The petri-dish culturing technique. Modified from Crump and Kerry, 1977.

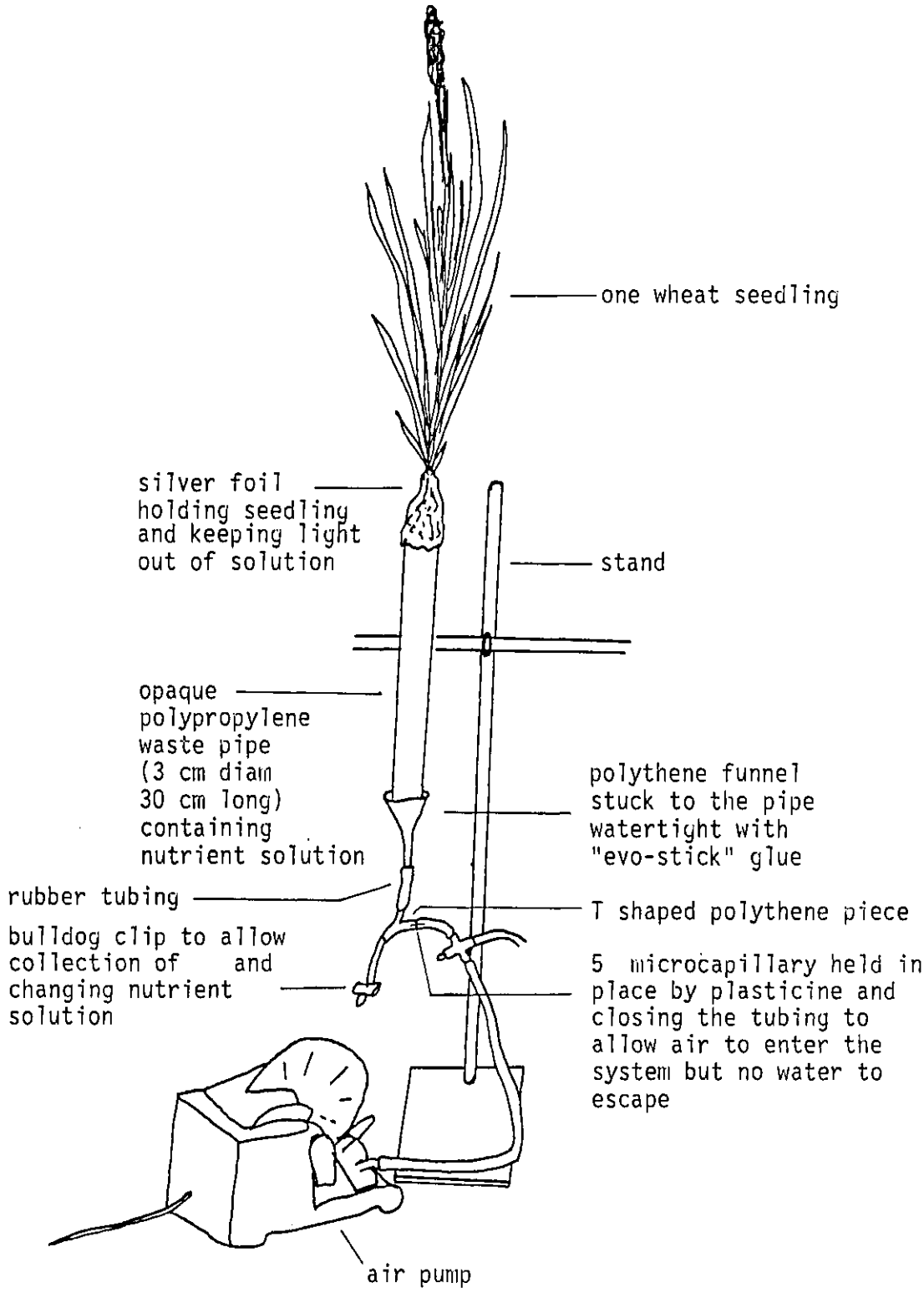


Fig. 8: Set up for hydroponic cultures.

The experiment was repeated using the same culture medium, but different moisture regimes (2 ml, 2.5 ml, 3 ml, 4 ml of tap water added to 42 g dry sand mixture, adding 2 ml of water suspension containing the inoculum). The same experiment was repeated using 50% loam, 25% river sand, 25% coarse sand. For these cultures, French and Welsh populations were used.

g) plot culture. A small plot at Silwood Park, grown with wheat or barley since 1971, sustains a population of *M. naasi* introduced from Wales. The plot was planted with spring wheat or barley every April. In April 1981, some Aberysthwyth, old soil was added to the plot to increase the population levels.

During 1980 to 1982 the plot culture failed to build up its population, either due to the degeneration of the original population, or to the build-up of populations of antagonistic soil organisms which killed off the eggs.

(iii) growing conditions of infected plants

Laboratory-grown cultures were usually kept at 20° C, 16 hours light and 8 hours dark, in a constant temperature room, watered daily and given full strength phostrogen solution (0.5 g / litre of tap water see appendix 2), once every week.

In each pot, unless stated otherwise, several seedlings were planted about 1 cm apart from each other. In these conditions, females produced mature eggmasses full of embryonated eggs, 12 weeks after inoculation.

(iv) uprooting cultures

The pots were not watered 1 to 2 days before uprooting, to allow the soil to dry. The contents of the pots were carefully emptied into individual big plastic bags. By holding the tops of the wheat plants and the opening of the plastic bag together, the soil and roots were tapped gently against the side of the bench, until most of the soil fell from around the roots into the bag (see Fig. 9). The remaining soil was shaken from the roots and a relatively clean root system was recoverd. The roots were washed under water (see later section 2.I.B.(v)).

Eggmasses were picked individually from the roots under the stereoscopic microscope in water. If galls were formed by more than one female, they were dissected out and the females were counted to estimate the number of eggmasses produced per plant. Females with

intact undamaged eggmasses were used in hatching experiments. Very young galls were ignored.

(v) washing roots "under water"

The recovered roots were placed on a coarse sieve over a bigger container and tap water was run over the roots as they were agitated in the sieve (see Fig. 10). The root system and detached roots were collected.

A summary chart of the materials and methods described to this point is presented in Fig. 11.

C. Setting up experiments

(i) hatching experiments

Individual eggmasses picked from the roots of freshly uprooted wheat seedlings, were the units assessed for hatching experiments. Each eggmass was placed on a small (7 mm diameter) 45 μ nylon sieve, kept inside the well of a multi-well plate (Linbro tissue culture) (see Figures 12 and 13). Enough tap water (or solution, the chemical specified in the experiment), was added to wet the bottom of the sieve (about 2 ml). The plates were covered to reduce water loss by evaporation and stored in the dark, in controlled temperature incubators.

Counts of J_2 hatched were made daily at first, or every 3 days, later every week until the completion of the experiment. To do the counts, the sieves were removed from the wells, and each well checked under the stereoscopic microscope. Tap water or chemical solution was renewed and the hatched larvae were discarded.

At the completion of the experiment, the eggmasses were stained in New Blue R (NBR) stain (0.05%) (Shepherd, 1962), and the remaining live and dead eggs counted (see later section 2.I.D(iii)).

Eggmasses for "heat treatment" were picked from freshly uprooted roots, placed in small glass vials with 2 to 4 ml of sterile tap water and incubated at the desired temperature. Later they were set to hatch at 20° C as described above. For incubation at 0° C, the glass vials were stored in vacuum flasks in an ice-water mixture inside a 5° C incubator.

To obtain a large number of J_2 for inoculations, infectivity

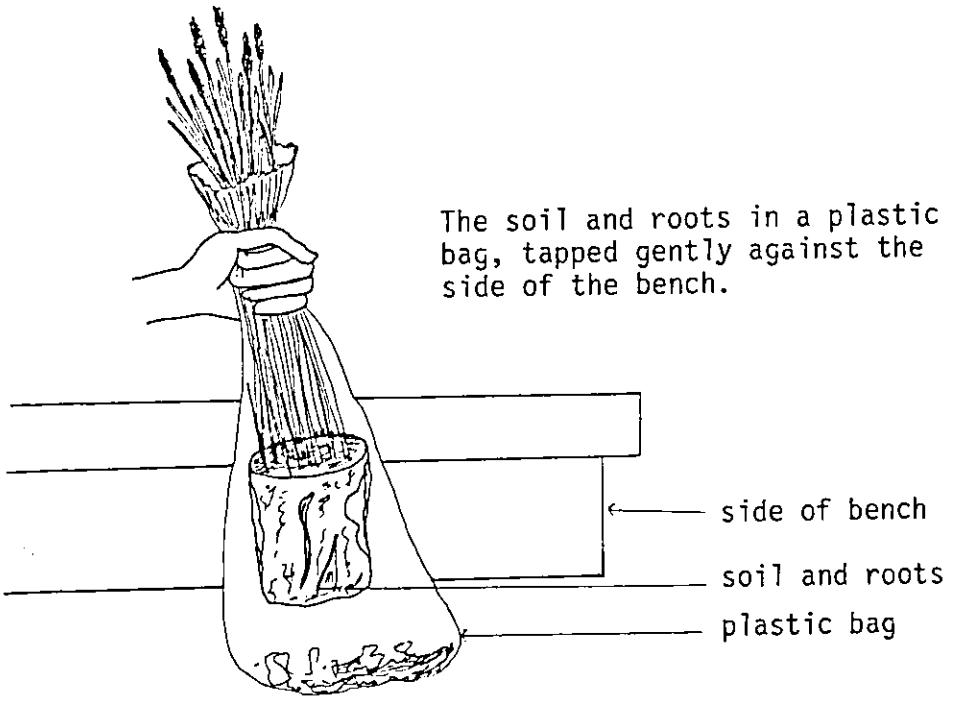


Fig. 9 : Uprooting cultures

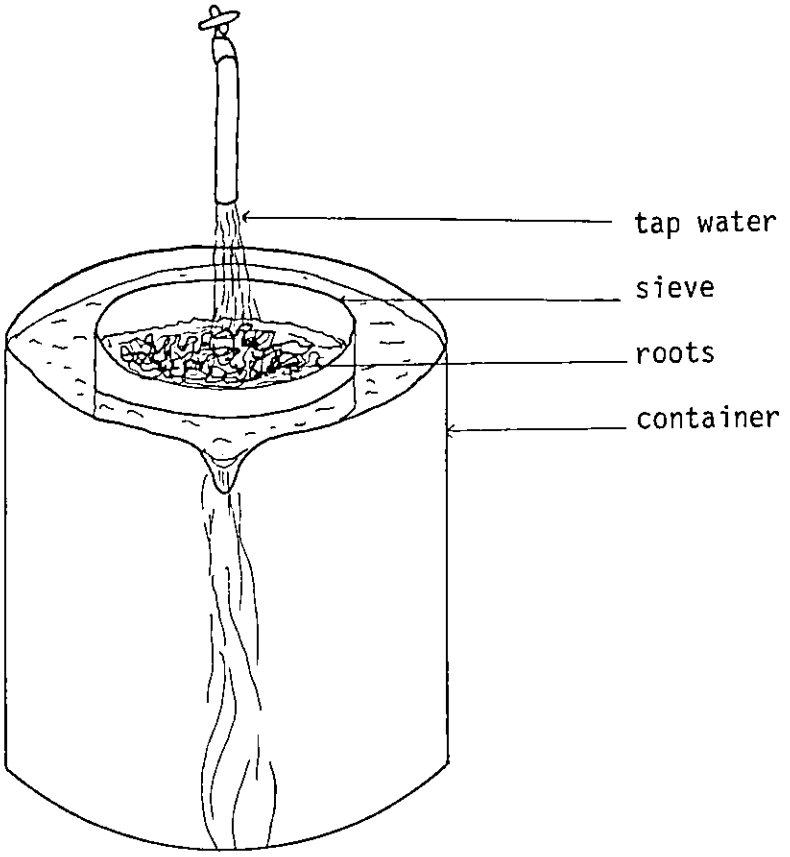
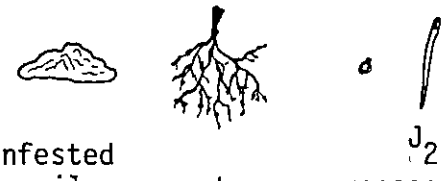


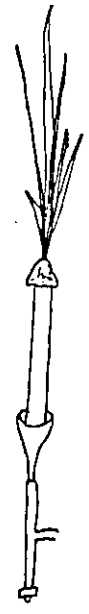
Fig. 10 Washing roots "under water".

A. Materials used



infested soil | roots | J_2 eggmasses | seedlings | seed (germinated)

SAPPO spring wheat



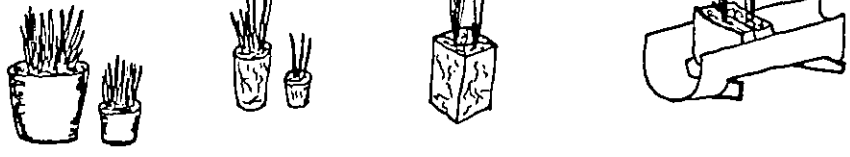
hydroponics

Origin of materials

United Kingdom, Belgium, Germany, France, Italy, USA, New Zealand.

B. Culturing techniques

i)



plastic pots | plastic transparent pots | 4-chamber transparent pots | petri-dishes

at 20°C, 16 hrs dark, 8 hrs light.

ii)

closed canisters, 20°C, 24 hrs dark, axenic, constant moisture.

iii) plot in open field.

Fig. 11 : Materials and methods: summary chart.

experiments or morphological studies, either eggmasses, chopped infested roots or infested soil were set up in Whitehead trays (Southey, 1970), of a suitable size.

In preliminary experiments, the percentage hatch at 20° C (before and after a chilling treatment) of eggmasses set to hatch: in groups of 100 to 200 in Whitehead trays; as chopped infested roots in Whitehead trays; as individual eggmasses in miniature 45 μ sieves in individual hatching dishes (using 6 replicates), was studied daily for 2 weeks.

The trends in percentage hatch between the 3 hatching methods were the same. Graphs similar to those in Figs. 21 and 23 were obtained in all 3 cases.

For a detailed study of hatching behaviour, individual eggmasses were used. Six replicates per experiment were considered a representative sample (about 1525 eggs, since each eggmass contained 254 $\pm$ 50 eggs). Based on previous studies, Watson and Lownsbery (1970), used 10 egg-masses of *M. naasi* for each hatching experiment, while Ogunfowora and Evans (1977) used 3 replicates containing 3 to 5 eggmasses each. However, these numbers are smaller than replications used in hatching experiments involving cyst nematodes, where greater variability in the number of eggs per cyst is more likely.

(ii) sterilizing eggs for hatching experiments

a) using NaOCl: Eggs were separated from eggmasses in tap water using hair loops. They were then placed in a 5 μ nylon sieve and transfered to 0.26% NaOCl in a suitable dish to cover all eggs in the sieve for 30 seconds. The NaOCl solution was removed using vacuum suction and the eggs, still in the sieve were irrigated about 5 times with sterile tap water under vacuum suction.

The eggs were then placed in sterile tap water ready to use.

b) using prolonged washing: The separated (as above) eggs were centrifuged at 3,000 rpm for 5 minutes, in sterile tap water. After 5 minutes, the water was changed with clean sterile tap water to dilute away any contaminating fungal spores or bacteria, and centrifuged again. Triton X-100 detergent was added during the second run (2 drops in 10 ml of sterile tap water) to help the process. Fifteen water changes and centrifugations were done before the eggs were set to hatch under sterile conditions.

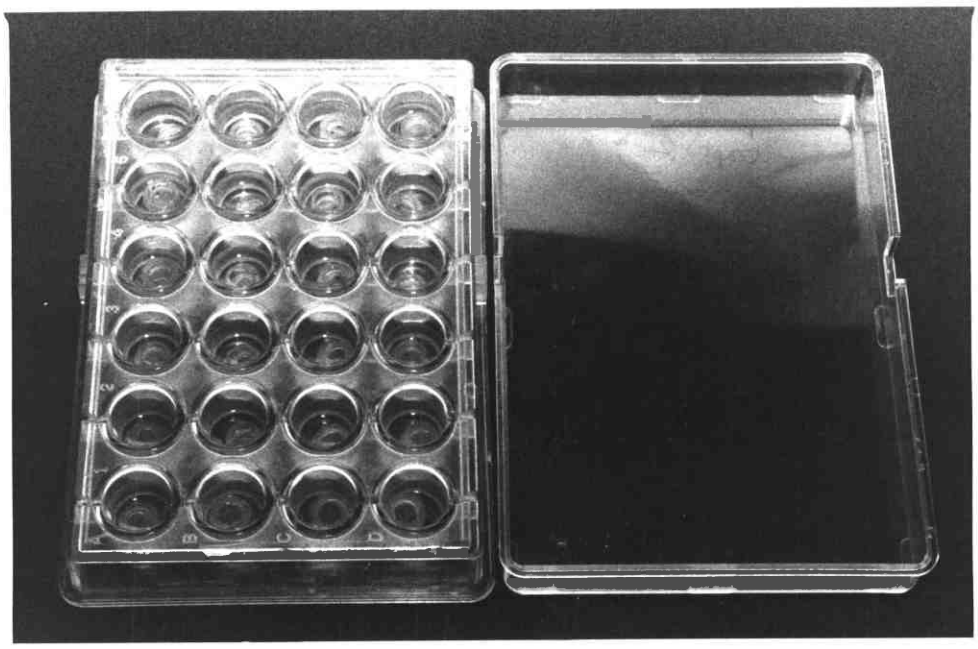


Fig. 12 : Linbro Tissue culture multi-well plate used in hatching experiments.

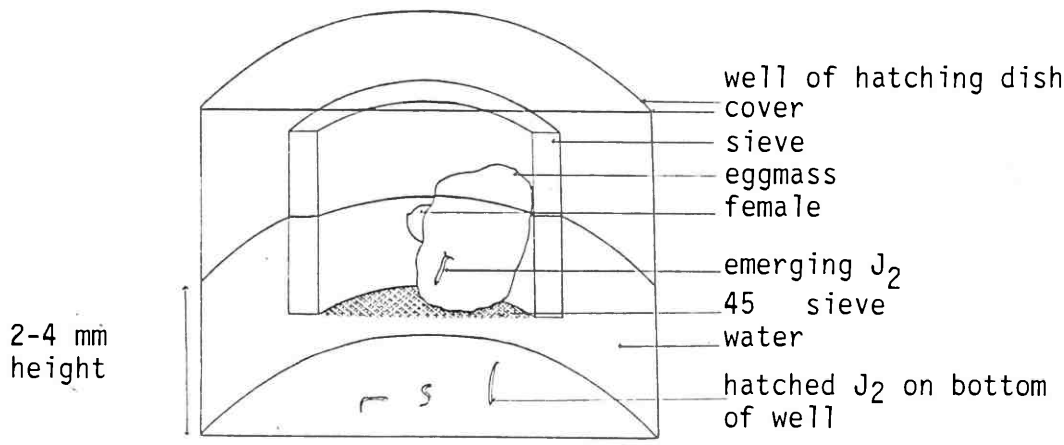


Fig.13 : A diagrammatic longitudinal section through a hatching well containing a sieve with egg mass.
Actual size of well: 1.5 cm diam., 2 cm height
sieve: 7 mm diam., 3 mm height

(iii) females set to produce their eggs away from the host

Wheat seeds strerilized in 2% NaOCl were germinated and placed onto sterile tap water agar in small petri-dishes (one seed per petri-dish), and allowed to grow for 1 week. The young seedlingswere planted in sand in petri-dishes (see section 2.I.B(ii)), under sterile conditions and inoculated with about 1,000 one-day old J_2.

When egg production was about to start (9 weeks after inoculation), females which were visible on the sides of the petridishes were removed before they laid any eggs, placed singly in a watch glass containing physiological salt solution (Ishibashi *et al.*, 1964) (see appendix 3) covered, and placed at 20° C in the dark.

After 24 hours, any females that had burst, were discarded and those remaining, were observed daily. The physiological solution was changed daily under sterile conditions. Eggs produced by each female daily, were collected using hair loops not to damage the eggs, and placed on sterile water agar on small petri-dishes with 2 drops of sterile physiological solution, and kept moist inside a larger dish (see Fig. 14). They were incubated at 20° C, and egg development was observed daily.

Eggs were collected and treated this way for 2 weeks.

D. Special techniques

(i) making sieves for hatching experiments

Two ml plastic syringes were sectioned to form rings 3 mm high, 7 mm diameter. Nylon mesh (45 µ) was cut into 1 cm^2 squares, and placed onto a hot plate (150° C), with one ring on top. The ring was pressed onto the nylon mesh for a few seconds until the mesh was firmly stuck onto the ring, all round.

Such sieves floated in water and needed no "legs".

Glue was thus not used, avoiding possible effects of the chemicals on the eggs.

(ii) hair loops

One pasteur pipette was used to hold a hair firmly in position, forming a loop of any size desired. Both ends of the hair were inserted into the narrow part of the pipette and pushed inside gently, until the loop was the desired size. Melted wax was immediately introduced at the opening of the pipette to secure the loop permanently (see Fig.15).

Such hair loops have a very resilient shape and were used for

picking nematodes and eggs for extra safety, and for freeing eggs from gelatinous matrices. A meniscus is formed which carries the egg or nematode without mechanical damage.

According to the use intended (ie. how flexible the hair is required to be), different thicknesses of hair could be used.

(iii) estimating the number of live and dead eggs left in an eggmass

After the completion of an experiment, each eggmass was stained with 0.05% NBR (following Shepherd's (1962) technique), for 24 hours, washed in tap water, placed onto a glass slide in a drop of water, and squashed using fine needles. A coverslip was placed on top and pressed until the eggs were dispersed. The slide was placed into a cover of a plastic petri-dish, which was marked with parallel lines, 1 to 2 mm apart (see Fig. 16).

As the slide was scanned with the lines as guidance, the eggs stained dark blue were counted as dead, and the clear ones as live. Many live eggs would burst under the pressure (internal and external), and J_2 would be released and later start sluggish movements. If in doubt about any eggs, a gentle tap on the coverslip with a needle would burst them and the J_2 could be observed.

Turgid, moving or unstained juveniles were classed as viable whereas flacid, immobile or stained juveniles were classed as dead.

This staining technique proved satisfactory, easy to use and appeared to give consistent results. In preliminary experiments, eggs containing dark blue (dead) J_2 and unstained (live) J_2, were burst open to see if the staining was successful.

E. Statistical analysis of results

The percentage hatched, live and dead eggs of each replicate in each experiment was transformed angularly before statistical analysis was done, using the Imperial College Computer Center and the University of London Computer Centre computers. Genstat and Minitab statistical analysis computer programs were used for F test analysis of variance (one-, two-, three-, four- and five way), see appendices 4 and 5.

All values shown in the results and graphs, are the means of the angularly transformed data after they were detransformed.

Six replicates were used for each hatching experiment using individual eggmasses.

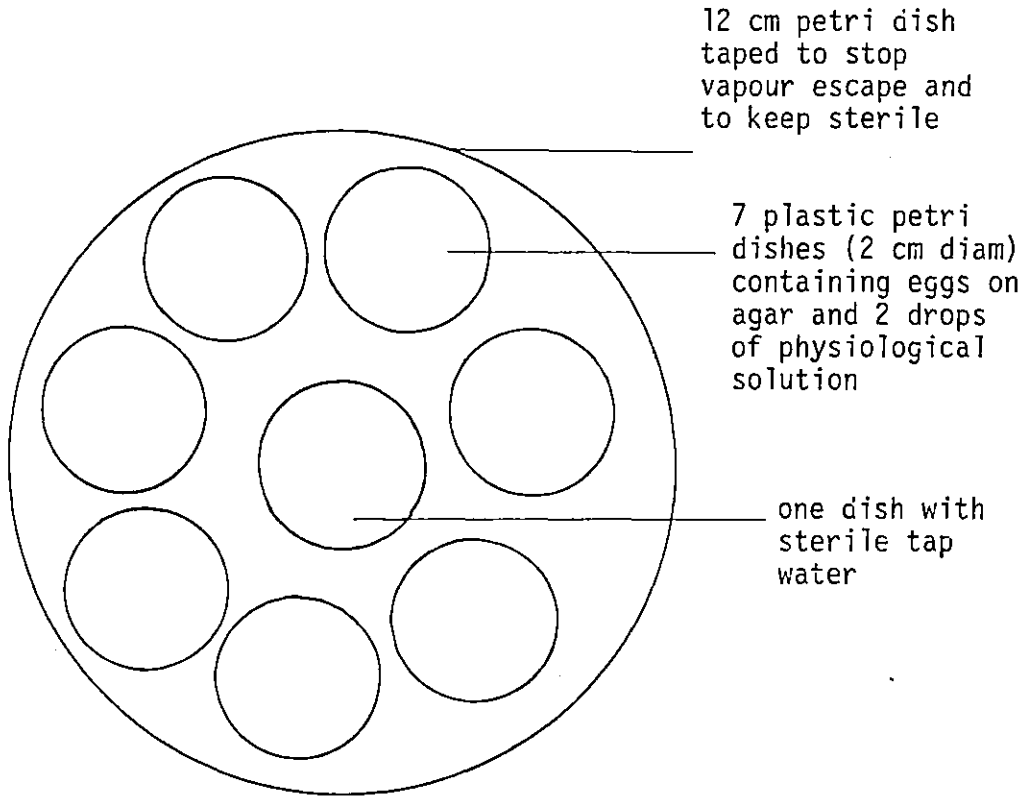


Fig. 14 Storing agar dishes containing eggs.

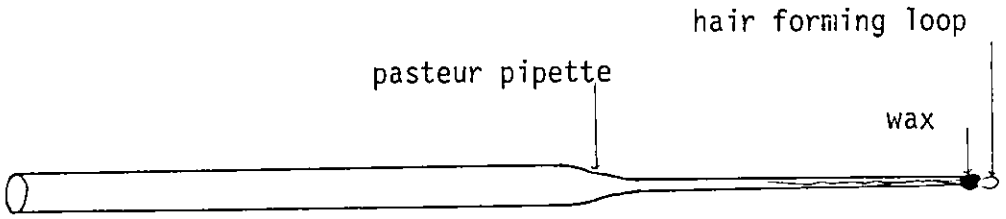


Fig. 15 Hair loop picker

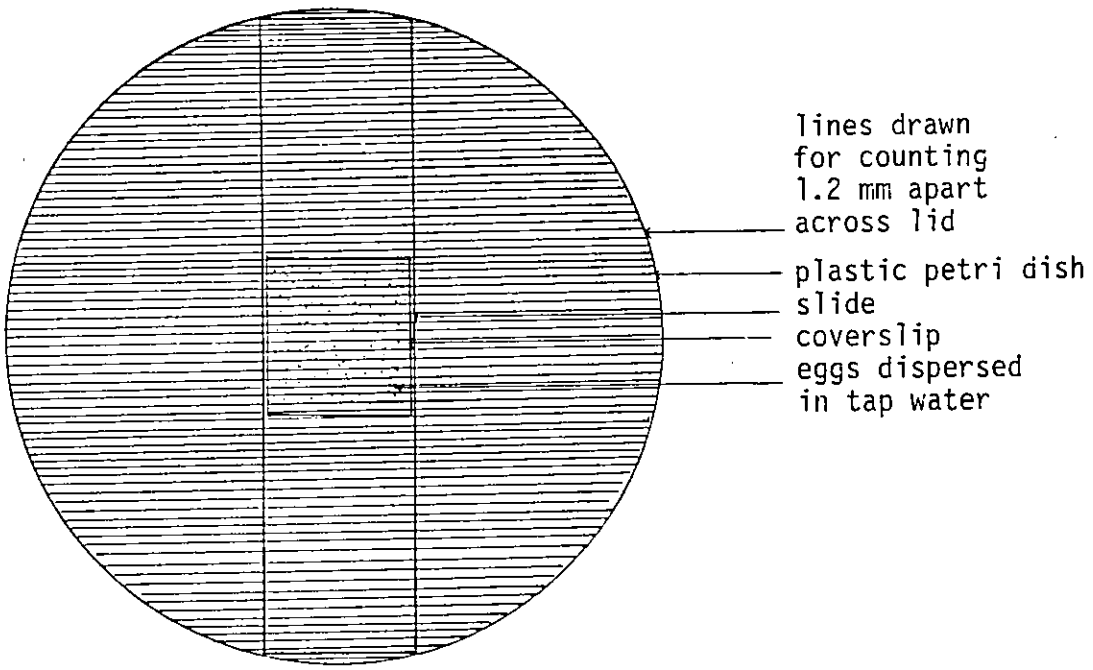


Fig. 16 Set up for counting nematode eggs or juveniles

2.II. The hatching system

introduction

Under natural conditions when rising soil temperatures in spring are associated with hatching of *M. naasi* J_2 secondary environmental factors may interfere to impose a quiescence on the J_2 in eggs, which might otherwise hatch (eg. lack of water, oxygen, or osmotic stress) (see section 1.C. page 16). In UK for example, high *M. naasi* populations have been reported in fields where waterlogging was prominent (Franklin, 1979).

To anticipate such interference with diapause, any experimental factors which seemed likely to influence the system were studied in advance in order to make allowances for them in the main experiments. Such factors under investigation were: drying of the eggmasses, frequency of changing the medium in which the eggmasses were incubated, the choice of that medium, and hatching eggs mechanically.

A. Effect of desiccation on the hatching behaviour of *M. naasi*.

populations used: Belgian, French, German, New Zealand, Californian, Welsh.
pretreatment chilling: none.
generation: F_1.
culturing medium: 50% loam, 50% river sand.
temperatures used for hatching experiments: 5° C, 20° C.

To study the hatchability of the eggs of *M. naasi* following storage under wet and dry conditions, 24 eggmasses from each population were set to hatch spontaneously at 20° C (see section 2.I.C(i)), after removal from the host. Two weeks later, the eggmasses were split randomly into 4 groups, with 6 eggmasses per group, in individual dishes:
A: 5° C dry; B: 5° C wet; C: 20° C dry; D: 20° C wet.

The "wet" dishes (controls) were placed in the appropriate incubators (relative humidity 100% inside closed dishes), and incubated for 7 weeks as normal (see section 2.I.C(i)).

The "dry" dishes were allowed to dry in air for 2 days at room temperature (relative humidity 60%, temperature 20° C), and incubated without water for 7 weeks.

After 7 weeks incubation, the eggmasses of both groups were covered in fresh tap water and hatched for 2 weeks at 20° C in the dark, before

the eggmasses were stained in NBR (see section 2.I.D(iii), to estimate numbers of live and dead eggs remaining.

Results

Hatching occurred only in treatment B (5° C wet) (see Table 11).

Some eggs hatched spontaneously at the begining of the experiment but they were not taken into account. All eggs in the dried eggmasses were dead. Incubation at 20° C resulted in more evaporation than at 5° C. For a well to dry completely at 20° C when covered, more than 3 weeks storage was necessary.

B. The effect of the frequency of changing the water in the hatching dishes, on the hatching behaviour of *M. naasi*.

populations used: Welsh (2)
pretreatment chilling: 15 weeks at 5° C
generation: F_1
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

To test whether the frequency of changing the water in the hatching dishes during an experiment had any effect on the rate of hatch of the eggmasses, the following experiment was done.

Using only the Welsh population (F_1), eggmasses that had been chilled for 15 weeks at 5° C were set to hatch at 20° C as described in section 2.I.C(i). Each sieve contained 4 randomly chosen eggmasses. Treatments, each with 6 replicates, were numbered 1 to 6 according to the frequency with which water in the dishes was changed, and after 2 weeks incubation at 20° C, the eggmasses were stained in NBR (see section 2.I.D.(iii)).

Results

The frequency of changing the water in the hatching dishes had a significant but inconsistent effect on the total percentage hatch obtained from the 6 treatments ($p < 0.005$) (see Fig. 17). Maximum hatch occurred after 4-day incubation of the eggmasses in the water containing their leachates.

Egg mortality was not affected by treatment.

Table 11 : Percentage hatch at 20°C for 2 weeks after treatment shown.

population	A 5°C dry	B 5°C wet	C 20°C dry	D 20°C wet
BEL	0%	2.2%	0%	0%
FRA	0%	2.5%	0%	0%
GER	0%	1.1%	0%	0%
NZL	0%	10.3%	0%	0%
CAL	0%	7.6%	0%	0%
WAL(1)	0%	4.8%	0%	0%
WAL(2)	0%	2.6%	0%	0%

Average of 6 replicas per treatment per population.

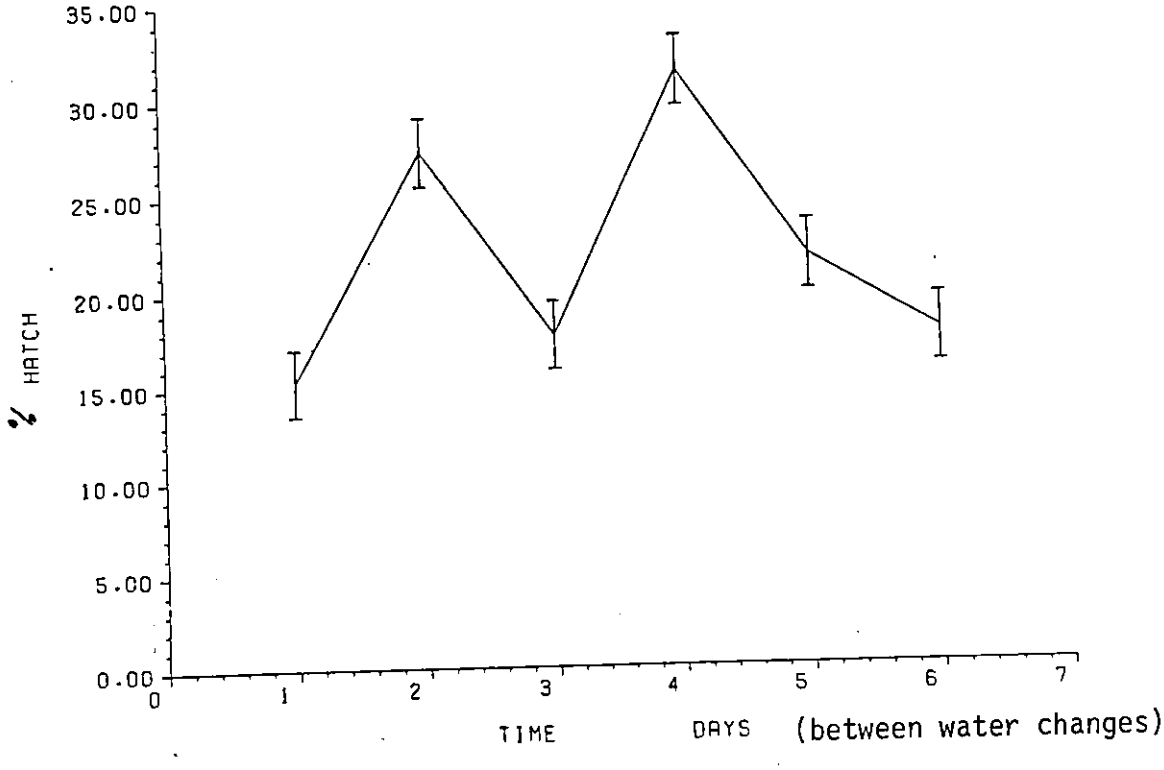


Fig. 17: Effect of "leaching" on the hatching behaviour of *M. naasi*.

C. The effect of medium on hatch of *M. naasi* eggs

populations used: French, German, Welsh(2)
chemicals used: Na-dehydroacetate (500 ppm), Streptomycin sulphate (1 mg/ml), Penicillin-G potassium (0.06 g /100 ml), Xylene, Benzene, Absolute alcohol. Controls: tap-water, distilled water
pretreatment chilling: 0 and 7 weeks chill at 5° C
generations: F_2
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

To determine which hatching medium interfered least with dormancy, the following 3 hatching tests were set up.
(i) Brown eggmasses of the French population (F_2), which had been stored at 5° C for 7 weeks (see section 2.I.C(i)), were set at 20° C in the dark individually in hatching dishes containing 2 ml of the following chemicals or mixtures of chemicals, known to protect the hatching medium from pathogen attacks, in equal volumes. Six replicates were used for each treatment. (a) distilled water (control). It was used by Watson and Lownsbery (1970) and Ogunfowora and Evans (1977); (b) tap water (control); (c) sterile tap water; (d) Na-dehydroacetate (500 ppm). It was used by Ishibashi *et al.*,(1973) for hatching *H. glycines*. (e) Streptomycin sulphate (1 mg/ml). It was used by Siddiqui and Taylor (1970) for *M. naasi* egg studies; (f) Penicillin-G potassium (0.06 g /100 ml); (g) Penicillin and Streptomycin; (h) Penicillin and Na-dehydroacetate; (i) Streptomycin and Na-dehydroacetate; (j) Penicillin, Streptomycycin and Na-dehydroacetate.

The chemical was renewed weekly and egghatch was assessed.

After 7 weeks incubation at 20° C, during which percentage spontaneous hatch was estimated (H_1), the eggmasses were chilled at 5° C for 7 weeks, transferred to 20° C for 7 weeks for further hatching (H_3), then rechilled for 12 weeks and finally hatched again at 20° C for 6 weeks (H_4).
(ii) The experiment was repeated, using brown eggmasses from the German population (F_2), which had been stored at 5° C for 7 weeks. Only tap water and Na-dehydroacetate were used, to test whether the inhibitory effect in hatching observed in the French population was repeated in the German population.
(iii) Using the Welsh (2) population (F_2), the effect of Xylene (Sulphur

free), Benzene and Absolute alcohol on egg hatch was studied. These chemicals are known lipid solvents. They were used in an attempt to dissolve the lipid layer of the egg-shell, which is believed to protect the J_2 from environmental hazards (Bird, 1971).

Twelve freshly picked brown eggmasses were placed individually in 45 μ mesh sieves, and vigorously shaken for 30 seconds in one of the chemicals, then washed thrice in tap water, and set to hatch in tap water at 20° C (see section 2.I.C(i)).

Spontaneous hatch (H_1) was studied at 20° C for 2 weeks before the eggs were chilled at 5° C for 7 weeks, after which hatch was assessed at 20° C for 6 weeks (H_3). Then the number of live and dead eggs remaining in each eggmass was estimated using NBR (see section 2.I.D (iii)).

Results

Na-dehydroacetate inhibited hatch significantly ($p < 0.001$) in both the French and German eggmasses, on its own, or in combination with Penicillin or Streptomycin, both of which had a stimulatory effect on hatch of French eggs ($p < 0.001$) (see figs. 18, 19 and 20).

Penicillin alone, stimulated hatch more than Streptomycin, but when the two chemicals were combined, the stimulation was less than Penicillin alone (see Fig. 19).

Stimulation occured during the first period of incubation (H_1) for Penicillin, and after the first chill (H_3) for Streptomycin and in its combination with Penicillin or Na-dehydroacetate (see Fig. 18). The lowest percentage hatch was observed in distilled water (4.3%),(see Table 12).

After the second chilling treatment, percentage hatch (H_4) dropped in all 7 cases compared to the control ($p < 0.001$).

Absolute alcohol, Xylene and Benzene produced statistically similar hatching results to that of the controls in the Welsh isolate.

Egg mortality was significantly affected by the various treatments (see section 5.F and Table 12).

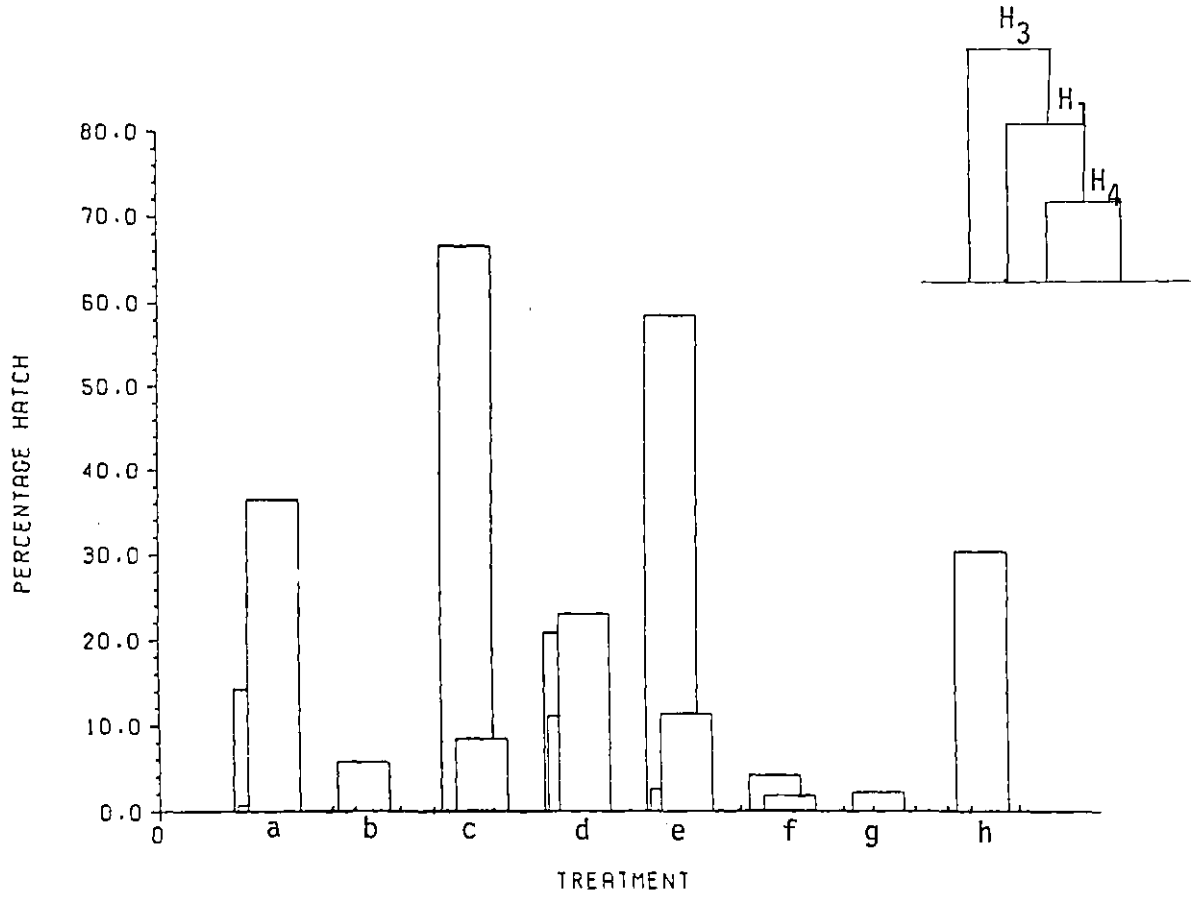
Na-dehydroacetate is a fungicide, Penicillin and Streptomycin are bacteriocides .

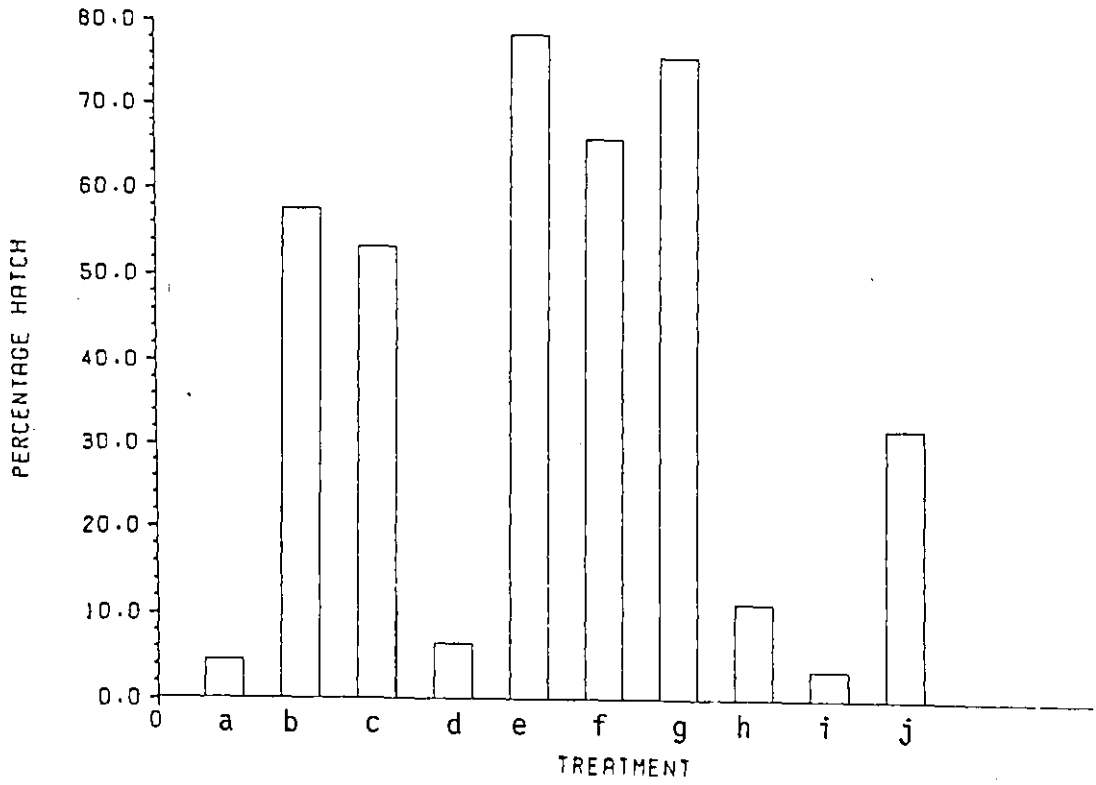
Table 12 : Mean percentage hatch and mortality of *M.naasi* eggs after 7 weeks chill in different hatching media. French population.

hatching medium	% total hatch	% dead eggs
distilled water	4.25%	4.39%
tap water	56.3%	4.68%
sterile tap water	51.9%	4.18%
Na-dehydroacetate 500 ppm	6.18%	40.3%
Penicillin-G potassium 0.6 g/lt	76.8%	3.84%
Streptomycin sulphate 50 ppm	64.6%	5.61%

Fig. 18: Hatchability of *M. naasi* eggs of the French population in: a: tap water; b: Na-dehydroacetate; c: Penicillin; d: Streptomycin; e: Penicillin with Streptomycin; f: Penicillin with Na-dehydroacetate; g: Streptomycin with Na-dehydroacetate; h: Penicillin with Streptomycin with Na-dehydroacetate.
After spontaneous hatch at 20° C (H_1), 1 chilling treatment (H_3) and 2 consecutive chilling treatments (H_4). (see page 74, section 2.II.C).

Fig. 19: Total percentage hatch of *M. naasi* eggs of the French population in: a: distilled water; b: tap water; c: sterile tap water; d: Na-dehydroacetate; e: Penicillin; f: Streptomycin; g: Penicillin with Streptomycin; h: Penicillin with Na-dehydroacetate; i: Streptomycin with Na-dehydroacetate; j: Penicillin with Streptomycin with Na-dehydroacetate.
After chilling treatment (see section 2.II.C, page 74).


H3
H1
H4
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
PERCENTAGE HATCH
0
a
b
c
d
e
f
g
h
TREATMENT


80.0
70.0
60.0
50.0
40.0
30.0
20.0
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PERCENTAGE HATCH
0
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TREATMENT

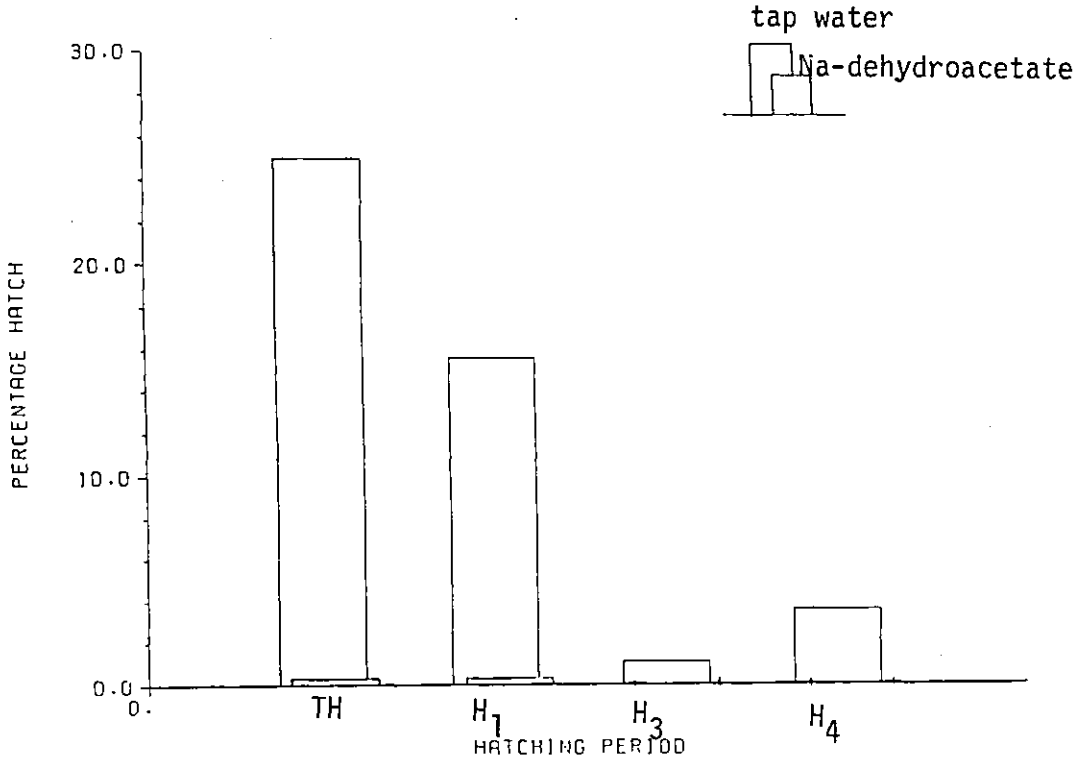


Fig. 20: Hatchability of *M. naasi* eggs of the German population in tap water and Na-dehydroacetate. TH: total percentage hatch; H_1: percentage spontaneous hatch; H_3: hatch after 7 weeks chill; H_4: hatch after 12 weeks chill following H_3. (see section 2.II.C, page 74).

Discussion

Evaluation of techniques

Before discussing criteria chosen for testing the hatching system, a few comments on the Materials and Methods are necessary.

Inoculations using either J_2, infested roots or infected soil, all gave very successful cultures. The most favourable soil mixture used, was 50% loam, 50% river sand. For this reason, in all experiments, unless otherwise stated, the wheat was planted in this mixture. With 100% river sand, suitable moisture and aeration was difficult to achieve, and fungal infections were sometimes encountered. Although the nematodes which invaded in such cultures produced big brown eggmasses, the overall percentage invasion was much lower than in the loam-sand mixture. The 50% river sand and 50% coarse sand mixture, gave an even lower J_2 invasion. The 100% loam medium became too hard and was difficult to clean the roots during uprooting.

For culturing, 11 cm diameter, 10 cm height plastic pots were used successfully, with about 10 wheat plants in each. For experiments, single plants using the petri-dish technique, proved most successful offering good observation facilities and good galling.

The 4-chamber pots, achieved good galling, but were difficult to uproot. They were used as indicators to observe nematode development on the roots, to help decide when to uproot the experimental material.

Hydroponic cultures were very successful and proved difficult to keep the wheat growth under control. Many new roots were produced and covered the invaded roots. This technique was used to collect the male nematodes as they escaped from the roots into the solution, in order to estimate male to female ratio for each population. This culturing technique could be very useful in studying reproduction in the genus *Meloidogyne*. The collected males could be used in cross fertilization studies between the different sexually reproducing species. In the present study, it was confirmed that *M. naasi* reproduces parthenogenetically (Triantaphyllou, 1969).

The hydroponic cultures produced similar eggmasses to cultures in soil (see section 4.B.), although the males were not able to fertilize the females. Few males were produced in hydroponic cultures, which might have favoured female production.

In the closed canister technique, the host plant was kept under stress (lack of light). Juveniles which invaded the roots failed to

develop beyond the swollen J_2 stage, and no moulting was seen despite otherwise favourable conditions.

Development of the hatching system

Of the features tested for the hatching system, it seemed that desiccation and the choice of the medium in which the eggs were incubated, were most critical.

Effect of desiccation on the hatching behaviour of *M. naasi*.

Rapid drying of eggmasses which proved lethal during this experiment, is unlikely under natural conditions, unless the eggmasses were at the soil surface.

Slow drying induces quiescence in many nematodes, including *Meloidogyne* species (see section 1.C. page 16). Franklin *et al*,(1971) and Ogunfowora (1979), found that moist soil was essential for extraction of *M. naasi* juveniles.

Finding thresholds for desiccation-induced quiescence, and for death of eggs, might give useful approaches for field control.

Choice of the hatching medium

The most favourable hatching medium, appeared to be the Penicillin-G potassium solution. However, whether it protected the eggs from bacterial infections or also stimulated them to hatch is unknown. Streptomycin sulphate had a similar effect, but Na-dehydroacetate proved lethal to over 40% of the eggs.

Some fungal growth was observed, in the hatching dishes containing Penicillin and/or Streptomycin, so it is possible that fungi and not the chemicals themselves were responsible for stimulating egg hatch.

If egg hatchability is to be studied, then these 3 chemicals should not be used in the hatching medium for egg protection. However, if protection is required during storage of the eggs at low temperatures, which could be used for other purposes than studies on hatching, then Penicillin or Streptomycin could be used.

The inner lipid layer of the nematode egg-shell, is believed to be responsible for the permeability propeties of the egg (Bird and McClure, 1976; Perry and Clarke, 1981). Wallace (1966), suggested that enzymes from juveniles dissolve the lipid layer in eggs of *M. javanica* making the egg-shell more flexible and permeable, letting water in, and eventually enabling the J_2 to hatch.

Xylene, Benzene and Absolute alcohol, are known to be able to dissolve lipids, but egg hatch after treatment with these chemicals was unchanged from the controls. The chemicals may not have penetrated the outer layers of the egg-shell to reach the lipid layer, or the time allowed was too short for them to be effective.

The effect of the frequency of changing the water in the hatching dishes, on the hatching behaviour of *M. naasi*.

The results did not show a clear trend due to treatment effects. The inconsistency of the results is not apparently attributable to the experimental technique. More studies, using more replicates are necessary before any serious conclusions can be reached.

Thus, a suitable hatching system was adopted as a result of the above observations.

— Hatching dishes were checked weekly, and the hatching medium was renewed.

— Only one eggmass was used per sieve and the hatching medium in the wells was changed twice during the first week of incubation at 20° C and once every week for the following weeks to avoid severe leaching effects.

— Tap water was chosen as the hatching medium most resembling natural conditions, without any protective chemicals, to allow the presence of at least some microorganisms.

CHAPTER 3

ENVIRONMENTAL FACTORS AFFECTING THE HATCHING BEHAVIOUR OF *M. NAASI*

Introduction

To study factors affecting hatch of *M. naasi* eggs, a detailed investigation of the effect of constant temperature (in the range 0° to 40° C); alternating temperatures (5° alternating with 20° C); and interrupted chilling treatment (5° C interrupted by short periods of 20° C), was carried out using the 6 isolates.

Temperature requirements may be different for embryonic and juvenile development (to the J_2 stage), and for hatching (eclosion). Banyer and Fisher (1971) divided hatching of the eggs of *H. avenae* into these two phases suggesting that the optimum temperature for juvenile development was 10° C and for eclosion 20° C. Juvenile development inside the eggs of *M. naasi* was examined during incubation at all temperatures used during this work, to find whether any of the temperatures blocked development.

In nature, the eggmasses are left in the soil after the host plant senesces in autumn, until conditions become favourable for the eggs to hatch. In the soil, apart from temperature changes, osmotic stress might be experienced, usually due to moisture fluctuations and the type of soil in the field. A range of NaCl concentrations (1 M to 2 mM) was chosen to study possible effects of osmotic stress on the hatching behaviour of *M. naasi* eggs.

Apart from the effects of macroenvironment, effects of changes in the relation of the nematode to its host were also studied: stress was exerted on the host by decapitating the plant at different times during nematode development; the J_2 were allowed to invade the host at different host ages and also at different times after hatching; the females were removed from the host at the time of egg production and allowed to produce eggs in a nutrient solution. The hatching behaviour of eggs produced under these conditions was studied and compared to the controls.

Parallel to egg hatchability, egg development and survival were also studied as an important adaptation to such conditions (see also chapter 5).

A. Effect of constant temperature on the hatching behaviour of *M. naasi*

populations used: Belgian, French, German, New Zealand, Californian, Welsh
pretreatment chilling: none
generation: F_2
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 0° to 40° C for 0 to 20 weeks

The 6 populations of *M. naasi* were reared in the laboratory under standardised conditions for 2 generations (see section 2.I.B)

Eggmasses (F_2) were collected from the roots, placed in small closed glass vials with enough sterile tap water to cover them, and stored in a water bath, in constant temperature incubators at 0°, 5°, 10°, 15°, 20°, 25°, 30°, 35°, 40°C in the dark. To keep eggmasses at constant 0° C, the glass vials were kept in melting ice, in a vacuum flask in a refrigerator. The temperatures were maintained constant in the incubators, with an accuracy of 1° C.

Every week (for 20 weeks), individual eggmasses were taken at random from all the vials and set to hatch at 20° C in tap water, as described in section 2.I.C(i). Keeping the vials in water baths, reduced temperature changes during the short period of time taken to transfer the eggmasses to the hatching dishes. Six replicates per population per temperature were set each week for 0 to 20 weeks. Sterile tap water kept infection of eggmasses during storage to a minimum and no losses due to any pathogens occured during the experiments.

Hatched J_2 (at 20° C), were first counted on day 3 and then every week for 8 weeks. After each count, fresh tap water at 20° was used to replace the old water containing the juveniles, in order to keep contamination to a minimum.

To assess spontaneous hatch at each temperature (as a control for the above experiments which followed hatch after transfer to 20° C), 6 eggmasses per population were set to hatch at each temperature for 8 weeks, using the same methods as above.

In all cases, care was taken to minimize temperature fluctuations during counting. For this, all eggmasses in hatching dishes were kept in water baths, and the hatching dishes were kept outside the incubators for only 5 to 10 minutes every time counting was done. The fresh water used to replace the water in the hatching wells was at the appropriate

temperature. Under these experimental conditions, no large temperature changes occured during the 5 to 10 minutes outside the incubator. The eggmasses were removed from the hatching dishes and placed in another dish in the water bath while the J_2 in the wells were counted and the water changed.

After 8 weeks of counting, the eggmasses were stained in NBR (see section 2.I.D(iii)), to assess the number of dead and live eggs left in each eggmass.

For a detailed study of the hatching behaviour of *M. naasi* at two critical temperatures: 20° C (hatching temperature), and 5° C (chilling temperature), counts of hatched J_2 were taken daily during a similar experiment, in order to construct hatching curves for each population. This way, the rate of egghatch (spontaneous and after chill) in the 6 isolates, was investigated and compared.

Results

spontaneous hatch: After incubation of the eggmasses at constant temperatures between 0° and 40° C, most populations showed some spontaneous hatch in nearly all temperatures tested (except 0° and 40° C). Percentage spontaneous hatch of all populations was significantly different at the 9 temperatures ($p < 0.001$). Greatest spontaneous hatch occured at 25° C for the French, New Zealand, Californian and Welsh populations and at 20° C for the Belgian and the German populations (see Table 13). It varied from 1.0% (German population), to 29.0% (Belgian population). After a detailed study of spontaneous hatch at 20° C for 25 weeks, the graph in Fig.21 was constructed. In all populations studied, most of the spontaneous hatch occured in the first 2 weeks of incubation, then declined towards zero with occasional hatch from individual eggmasses throughout this period. Spontaneous hatch at 5° C over 35 weeks, totalled only 0.3%, all occuring during the first 10 days of the incubation.

Hatch at 20° C following constant temperature exposures: Both temperature and length of incubation at the various constant temperatures significantly influenced the numbers of juveniles subsequently hatching at 20° C, for all populations tested ($p < 0.001$) (see Fig.22). Maximum hatch at 20° C occurred in all populations after incubation at 10° or 5° C for 13 to 19 weeks (see Table 13).

The data is presented in detail in Appendix 6, while the results

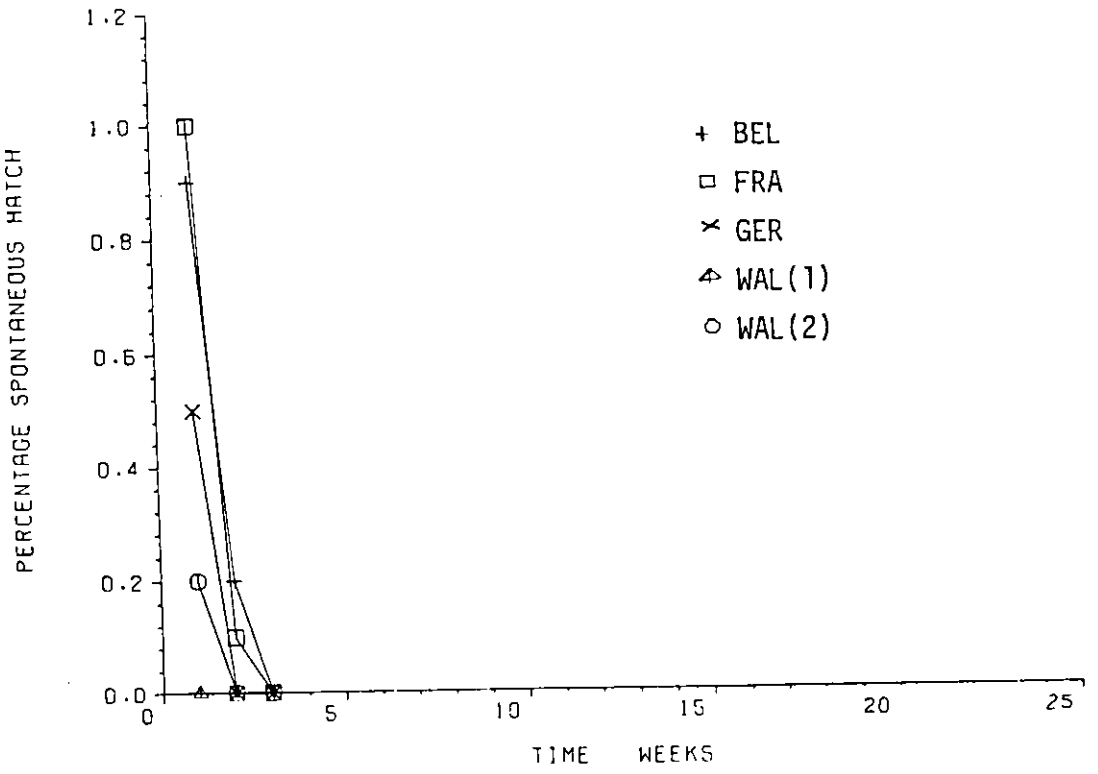


Fig. 21: Mean percentage spontaneous hatch of the various populations of *M. naasi* after incubation at 20° C for 25 weeks.

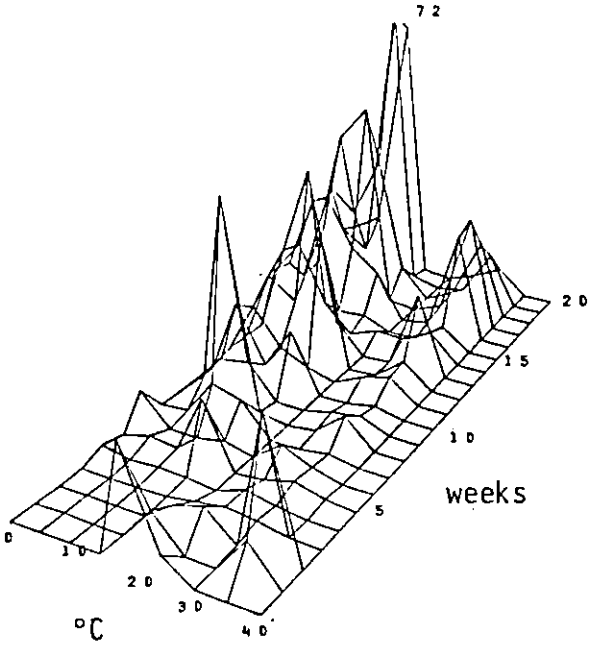
Table 13 : Spontaneous hatch and hatching following chilling treatment of 6 *M.naasi* populations.

population	A		B		
	% hatch	temp.°C	% hatch at 20°C	incubation temp. °C	incubation time weeks
BEL	29.0	20	72.3	10	19
FRA	2.3	25	73.1	10	17
GER	1.0	20	45.3 45.3	5 5	13 17
NZL	13.4	25	89.2	10	16
CAL	8.7	25	80.1	10	15
WAL	2.6 2.2	10 25	72.7	10	18

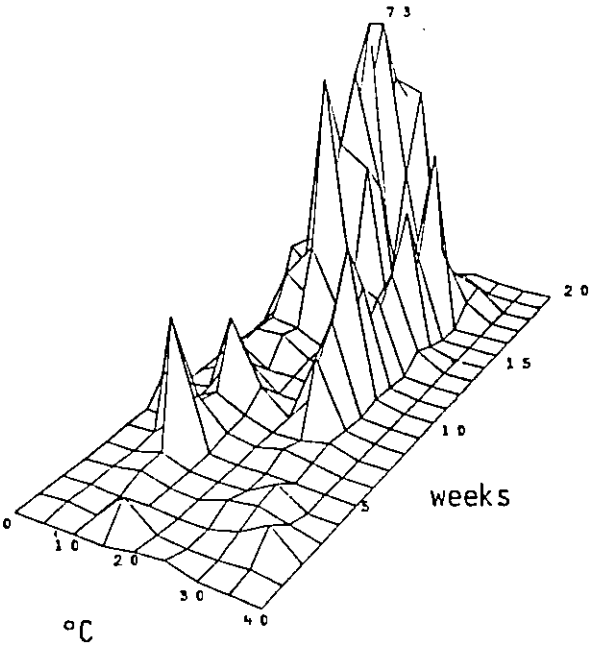
A: Average maximum percentage spontaneous hatch at the most favourable temperature, accumulated during incubation for 8 weeks.

B: Average maximum percentage hatch after incubation at the most favourable chilling temperature and incubation period.

Fig. 22: The hatching behaviour of the 6 populations of *M. naasi* after incubation at temperatures between 0° and 40° C for 0 to 20 weeks, and allowed to hatch at 20°C for 8 weeks. Actual values should be taken from Appendix Table 6 rather than scaled from the Figures, since the Figures are in perspective view.



PERCENTAGE HATCH BELGIAN POPULATION



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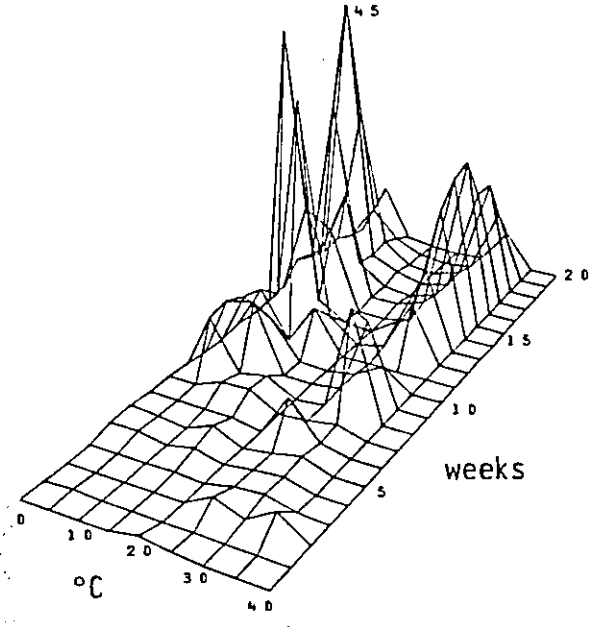
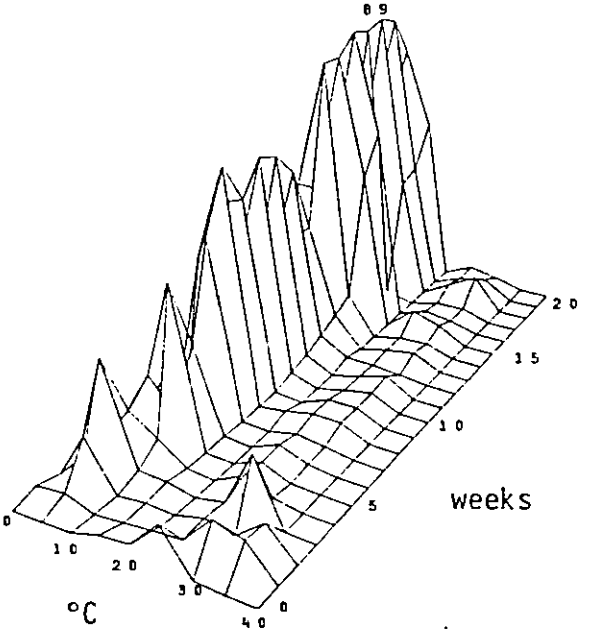
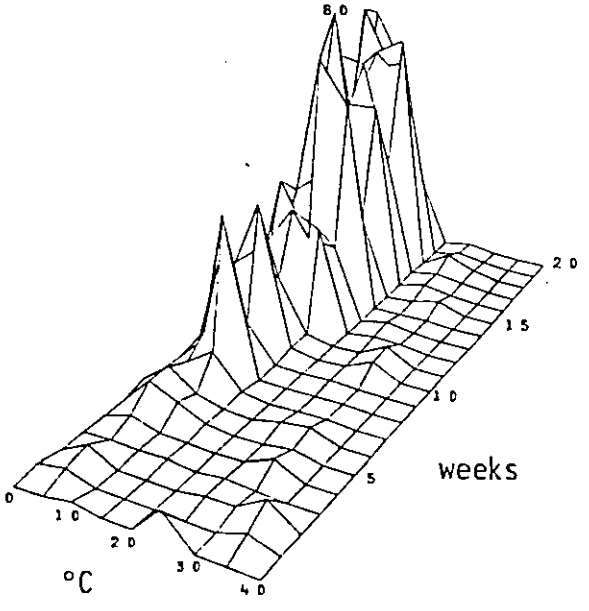


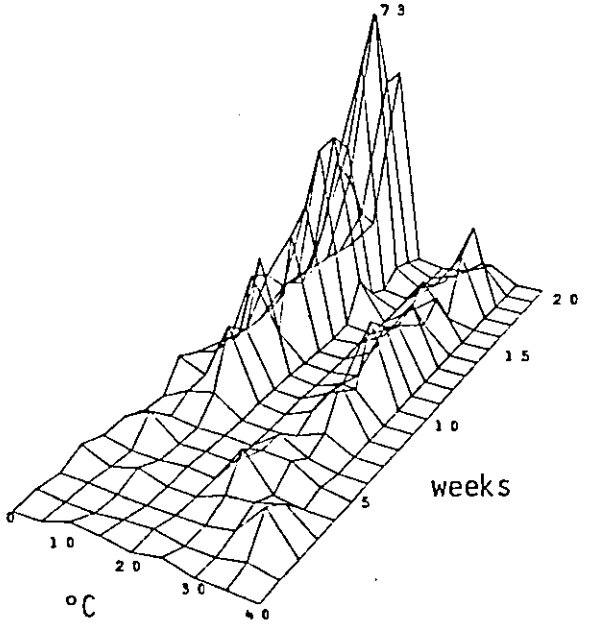
PERCENTAGE HATCH GERMAN POPULATION



PERCENTAGE HATCH NEW ZEALAND POPULATION



PERCENTAGE HATCH CALIFORNIAN POPULATION



PERCENTAGE HATCH WELSH POPULATION

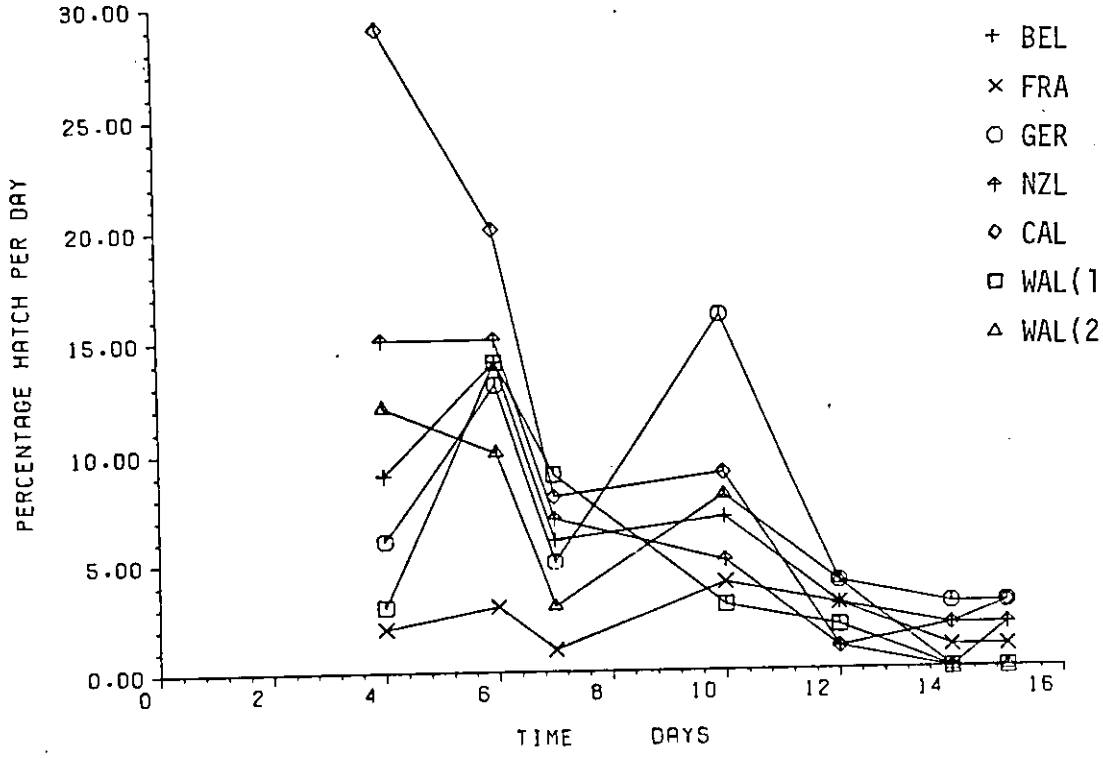


Fig. 23: An example of a typical hatching curve over 15 days for each population of *M. naasi* at 20° C, after chilling at 5° C for 17 weeks.

Table 14 : Average maximum percentage hatch at 20°C after incubation at temperatures between 0°C and 40°C for the most favourable incubation period for each population of *M. naasi*.

population	0°C		5°C		10°C		15°C		20°C		25°C		30°C		35°C		40°C	
	hatch %	weeks	hatch %	weeks	hatch %	weeks	hatch %	weeks	hatch %	weeks	hatch %	weeks	hatch %	weeks	hatch %	weeks	hatch %	weeks
BEL	26.5	18	43.4	17	72.3	19	34.1	12	29.0	0	7.7	2	28.3	17	33.8	2	0	-
FRA	15.9	15	32.6	18	73.1	17	53.1	19	1.4	0	46.0	16	5.6	18	6.7	2	0	-
GER	4.3	15	45.3 45.3	13 17	37.1	12	7.2	11	1.0	0	6.8	6	24.8	16	3.5	2	0	-
NZL	32.7	20	55.5	9	89.2	16	59.5	16	4.0	0	13.4	0	20.1	3	9.9	1	0	-
CAL	19.7	18	72.0	19	80.1	15	67.0	18	0.7	17	8.7	0	4.5	11	6.4	2	0	-
WAL	7.1	20	33.8	17	72.7	18	9.8	15	1.4	1	10.1	4	20.7 20.7	10 18	10.0	2	0.3	1

Table 15: Maximum percentage hatch of different *M. naasi* populations after incubation for 0 (spontaneous hatch) to 20 weeks.

weeks incubation	population	maximum % hatch	temp. °C
0 spont. hatch	BEL	29.0	20
1	NZL	12.5	30
2	BEL	33.8	35
3	NZL	33.5	5
4	NZL	12.9	5
5	NZL	49.0	10
6	NZL	26.2	10
7	NZL	54.5	10
8	NZL	70.8	10
9	NZL	58.4	10
10	NZL	67.4	10
11	NZL	64.7	10
12	NZL	57.0	10
13	BEL	45.4	10
14	NZL	84.3	10
15	NZL	83.9	10
16	NZL	89.2	10
17	NZL	87.4	10
18	NZL	89.0	10
19	NZL	86.3	10
20	CAL	69.2	5

are represented graphically in Fig. 22, and summarised in Tables 13, 14 and 15.

Peak hatch after incubation at the chilling temperatures (0° to 15° C), increased with incubation time. A second tendency to hatch after incubation at temperatures between 25° and 35° C was also seen in all isolates (see Fig.22).

The Belgian *M. naasi* showed 2 peaks clearly, but both were very scattered at the first 6 weeks incubation (see Fig.22 A). The peak hatch after chilling temperatures was 72.3% after 19 weeks at 10° C. The maximum hatch after the warm (above 20° C) temperatures, was 33.8% after 2 weeks incubation at 35°C.

The French population showed 2 uniform peaks, but the peak after warm temperatures merged more closely with the chilling peak than is seen in other populations (see Fig.22 B). There was nearly zero hatch at 30°, 35° and 40° C, unlike all other populations. Maximum hatch after chilling was 73.1% after 17 weeks at 10° C, similar to the other populations. Maximum hatch after warm temperatures was 46% at 25° C, after 16 weeks incubation.

The German population had 2 clear peaks, with a very uniform increase in hatch during incubation in warm temperatures, reaching 24.8% maximum at 30° C, only after 16 weeks (see Fig.22 C). The peaks after chilling were scattered, with 45.3% as the maximum at 5° C after 13 or 17 weeks incubation.

The New Zealand population had 2 clear peaks (see Fig.22 D), due to a large and steadily increasing hatch after chilling (with a maximum of 89.2% after 16 weeks at 10° C), and a modest peak after warm temperatures, moderately high at first (maximum of 20.1% at 30° C after 3 weeks), levelling off to lower numbers with increasing time of incubation.

The Californian population had a distinct hatching peak following chilling which reached the maximum of 80.1% after 16 weeks at 10° C (see Fig.22 E). It showed a relatively even increase in hatch as the incubation period progressed. However, the hatch after warm temperatures, was very low although the tendency was clear. After 18 weeks at 25° C, it reached a maximum of 4.2% hatch.

The Welsh population had 2 distinct peaks (see Fig.22 F). After chilling, one peak started at low levels with short incubations, and increased with time, to reach a maximum of 72.2% after 18 weeks at 10° C. The peak after the warm temperatures was reached more uniformly,

although hatch was uneven during the first 6 weeks incubation. It reached 2 maxima, of 20.7%, after incubations of 18 and 10 weeks at 30° C.

All populations gave 0% hatch at 40° C, except the Welsh population (0.3% hatch after 1 week).

After incubation at temperatures other than 20° C followed by a transfer to 20° C, maximum egg hatch from each replicate of eggmasses, occured within the first week of incubation at 20° C, with the exception of the French and German populations, which had their peak hatch on the second week (see Fig. 23).

The optimum incubation time at each constant temperature, before percentage hatch reached a maximum, varied for each population (see Table 14). At 0° C, all populations except the Welsh, required a long incubation before they reached their maximum percentage hatch at this temperature. The same was true for the 5° C temperature, with the New Zealand isolate as the exception. The French population required a long incubation at warm temperatures of 25° and 30° C before reaching its maximum hatch, while the other populations gave their highest hatch after short incubations at 25° C, with New Zealand at 30° C as the only exception.

The New Zealand population in most cases gave the highest hatch at each incubation period, with 10° C being the best prehatch treatment (see Table 15).

Juvenile development at each temperature tested: Embryonic development appeared to stop at 0°, 5°, 35° and 40° C, but it was completed in most cases when the eggs were transferred to 20° C (except in the cases when the eggs died). For egg mortality, see section 5.A.

After a chilling treatment, the majority of the eggs hatched during the first 2 weeks of incubation at 20° C, and then the percentage hatch dropped during the next 2 weeks to nearly zero. In contrast, after incubation in warm temperatures, egg hatch did not commence until after at least 2 weeks at 20° C and it lasted 2 to 3 weeks.

B. The effect of alternating temperatures on the hatching behaviour of *M. naasi*.

populations used: Belgian, French, German, Welsh (1) and (2)
pretreatment chilling: none
generation: F_1
culturing medium: original soil
temperatures used for hatching experiments: 5°, 20° C (see Fig. 24)

The 2 Welsh populations were used in this experiment, to investigate any differences in behaviour due to their culturing history. The Belgian population was chosen because of its high percentage spontaneous hatch compared to the other populations (see section 3.A).

Eggmasses from the Belgian, French, German, Welsh (1) and (2) populations (F_1), were collected from roots of cultures which were inoculated with only those J_2 which hatched after receiving from 0 to 7 weeks chilling at 5° C (0 to 14 weeks for the French population). Therefore, each population was considered as 8 groups (or 15 in the case of the French isolate), according to the weeks of chilling of the parent as juveniles.

Two sets of experiments were done: A and B (see Fig. 24). In set A, all 5 populations were used (47 groups comprising 4 populations of 8 groups each, and 1 population of 15 groups). Twelve eggmasses from each group were allowed to hatch spontaneously at 20° C (see section 2.I.C(i)), for 4 weeks, then 6 eggmasses from each group were retained at 20° C (A_1 set), while the other 6 eggmasses were transferred to 5° C for 2 weeks (set A_2). Eggmasses in set A_1 were incubated at 20° C for 20 weeks during which percentage spontaneous hatch (H_1) was assessed. Following this, they were chilled at 5° C for 7 weeks and transferred back to 20° C for 7 weeks to assess percentage hatch (H_3). They were rechilled for 7 weeks further, and percentage hatch (H_4) was assessed at 20° C.

Eggmasses in set A_2 (see Fig. 24), after incubation at 20° C for 4 weeks, were given alternating 2 week periods of 5° C and 2 week periods of 20° C, for 16 weeks all together. During this period, percentage hatch during the 20° C incubations was assessed (H_2). The eggmasses in set A_2 were then given a 7 week chill at 5° C, and a 7 week incubation at 20° C, during which hatch was estimated (H_3). They were rechilled at 5° C for another 7 weeks, and finally incubated

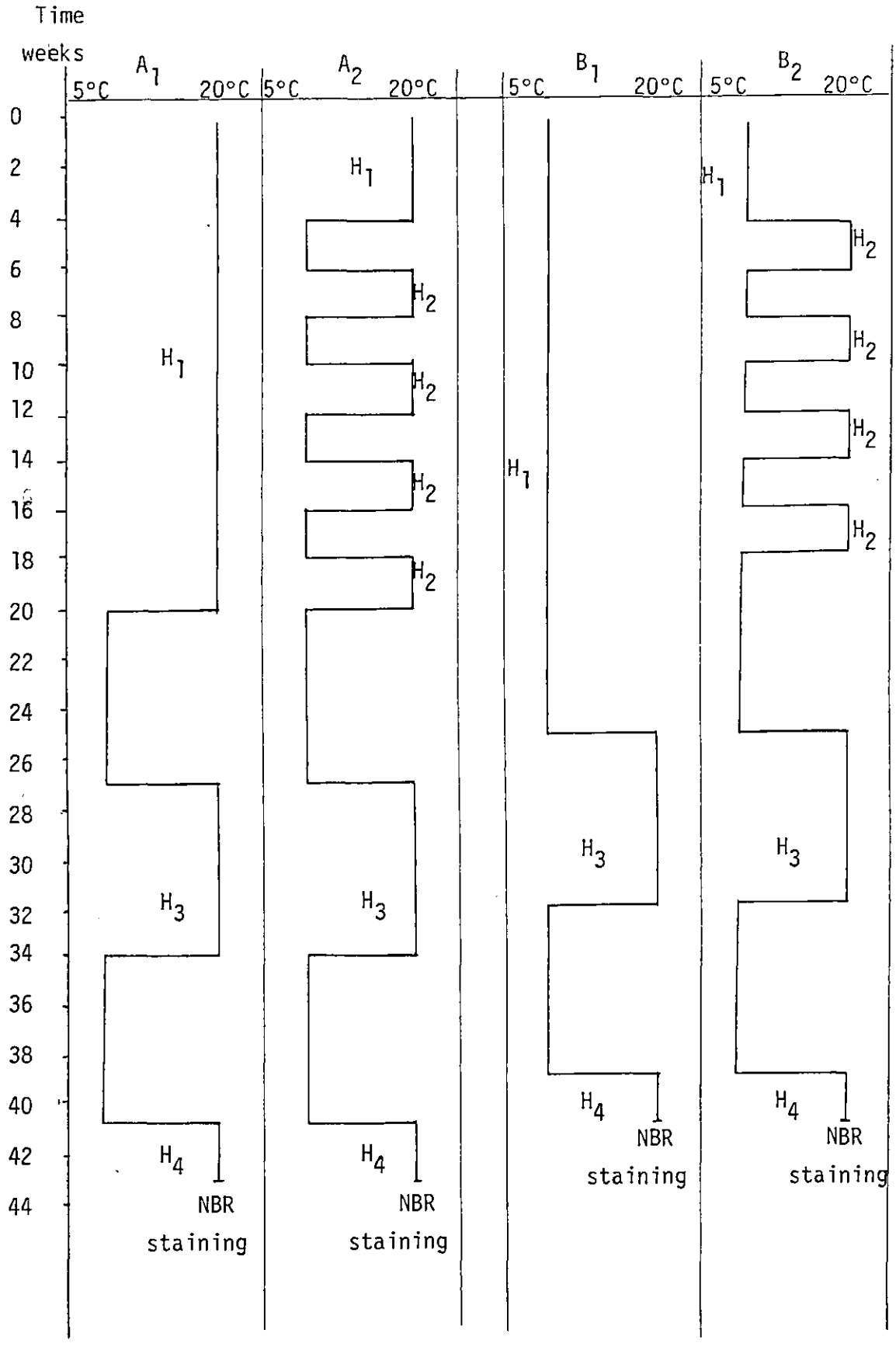


Fig. 24: The procedure followed in section 3.B.

at 20° C for 2 weeks to calculate hatch after chill (H_4).

The eggmasses were finally stained in NBR and the number of live and dead eggs remaining in each eggmass was estimated (see section 2.I.D(iii)).

The same procedure was followed for experimental set B, using only the Belgian population, but in this case the starting temperature was 5° C instead of 20° C (see Fig. 24).

Hatched J_2 were counted every week at first, but since hatching was found to occur only during incubation at 20° C, weekly counting was restricted to the 20° C incubations, with one count at the end of the 5° C incubation periods.

Results

Effect of treatment A_1 (constant temperature) and A_2 (fluctuating temperature), with 20° C as the first incubation temperature: The percentage hatch for the treatments A_1 and A_2 was compared for the different hatching periods: H_1: spontaneous hatch; H_2: hatch during incubation in alternating 5° and 20° C temperatures for 2 week periods each; H_3: hatch at 20° C after a 7 week incubation at 5° C following the alternating temperature period; H_4: final hatch at 20° C after 7 weeks chill at 5° C, following the H_3 period.

The percentage hatch obtained for each population was statistically similar for each corresponding hatching period (see Table 16), and therefore the results in Fig. 25 show the percentage hatch for the two treatments combined.

In treatment A_1, percentage hatch (H_2) was extremely low (average for all populations and groups 0.06%) and therefore was excluded from Table 16 and Fig.25 . It did not show any increase with the increased overall time the eggs were incubated at 5° C, so the interrupted chilling effect was not additive. H_3 was also omited from Fig. 25 (low values).

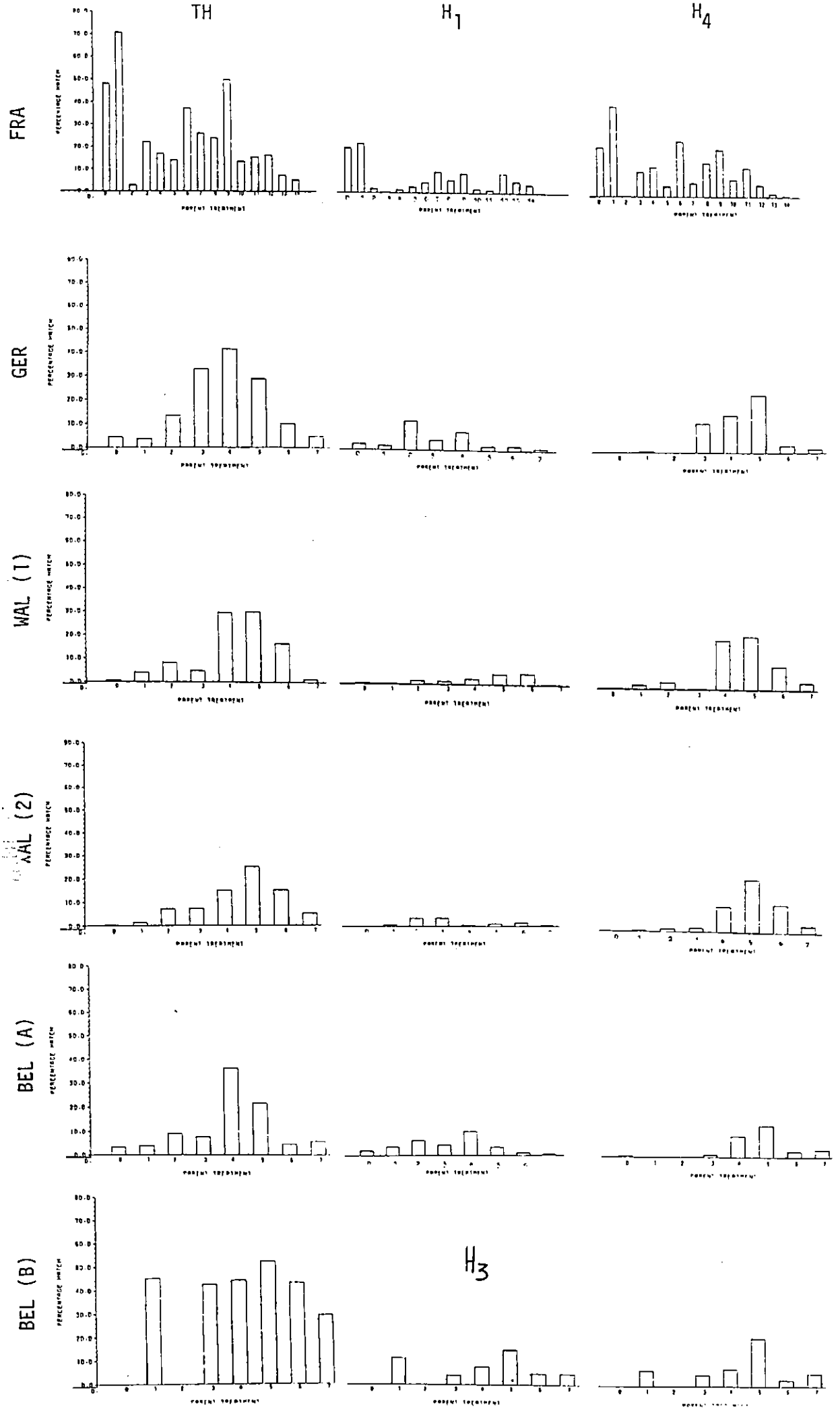
The 5 isolates however, gave statistically different percentage hatch in all hatching periods tested ($p < 0.001$). Parent treatment also influenced percentage total hatch and percentage final hatch (H_4) ($p < 0.001$). Parent treatment of 4 or 5 weeks chill at 5° C, gave the highest percentage hatch in all hatching periods, for all populations except the French (see Fig.25).

A continuous chill for 7 weeks at 5° C was included to induce egg hatch (see section 3.A). However, the percentage hatch obtained at 20° C (H_3) was lower than expected for both treatments A_1 and A_2

Table 16 : Average percentage hatch of *M. naasi* during temperature treatments A and B (see Fig.24).

	population	Total hatch		H 1		H 2	H 3		H 4	
		constant	altern.	constant	altern.	altern.	constant	altern.	constant	altern.
Treatment A		A_1	A_2	A_1	A_2	A_2	A_1	A_2	A_1	A_2
	BEL	9.7	9.8	4.0	3.1	0.06	0.1	0.1	1.7	2.9
	FRA	22.7	32.3	4.0	7.9	0.05	0.4	0.1	14.0	10.7
	GER	18.2	12.2	5.3	2.4	0.04	0.5	0.6	5.0	4.1
	WAL(1)	7.8	10.4	0.7	2.7	0.09	0.2	0.5	5.4	3.2
	WAL(2)	7.0	8.4	1.7	0.7	0.05	0.1	0.2	2.9	5.8
Treatment B		B_1	B_2	B_1	B_2	B_2	B_1	B_2	B_1	B_2
	BEL	25.1	60.7	0	0	31.4	4.0	12.1	7.6	6.6

Fig. 25: Average percentage hatch of the 5 *M. naasi* populations, from cultures obtained after inoculations using only those J_2 which hatched after receiving 0 to 7 weeks chilling at 5° C (0 to 14 weeks for the French population) (parent treatment). After chilling treatments (A_1 and A_2) and (B_1 and B_2) as shown in Fig. 24. TH: total percentage hatch; H_1: percentage spontaneous hatch; H_4: final percentage hatch after the chilling treatment.


TH
H1
H4
FRA
GER
WAL (1)
WAL (2)
BEL (A)
BEL (B)
H3

(maximum 0.6%) compared to 1.1% obtained for the same population after 7 weeks chill at 5° C (see section 3.A) (see Table 16).

The percentage hatch obtained after a further 7 weeks chill at 5° C, was relatively higher (maximum 10.7%) (see Table 16 ; Fig.25). This percentage hatch was higher than that obtained after a constant 7 week chill at 5° C, followed by incubation at 20° C, for all populations tested (see section 3.A; Appendix 6).

Effect of treatment B_1 (constant temperature) and B_2 (fluctuating temperature), with 5° C as the first incubation temperature: The effect of incubating the eggmasses at 5° C as soon as they were removed from the host's roots (treatments B_1 and B_2), gave a different pattern of hatch compared to the one obtained after incubating the eggmasses for 4 weeks or more at 20° C before applying chilling (treatments A_1 and A_2), in the Belgian population ($p < 0.001$).

The temperature treatments B_1 and B_2 gave significantly different results in all hatching periods ($p < 0.001$) (see Table 16 ; Fig.25).

Percentage spontaneous hatch (H_1) at 5° C during incubation for 25 or 4 weeks, was negligible (average 0.08%).

In treatment B_2, hatch during the alternating warm-cold period (H_2) was 31.4% (see Table 16). This was due to the expected high percentage hatch after the first incubation at 20° C following chilling at 5° C for 4 weeks. Hatch during the rest of the H_2 period, dropped to the same levels as that of the A_2 treatment (0.08%). After the alternating cold warm treatment and a 7 week constant chill at 5° C, eggmasses in B_2 treatment showed 12.1% hatch (H_3) (see Table 16). However, eggmasses in B_1 treatment, after 25 weeks constant chill at 5° C, gave only 4.0% hatch (H_3). Following a second chilling treatment (7 weeks), eggmasses in B_1 and B_2 treatments produced 7.6% and 6.6% hatch (H_4) respectively (see Table 16).

The highest total percentage hatch came after the B_2 treatment for the Belgian population, and was 60.7% (see Table 16). This was significantly different from that of the B_1 treatment (25.1%).

A summary of the hatching behaviour of the eggmasses of the 5 isolates after the 4 treatments, taking into account the treatment the parent population had received before hatching, is shown in Fig.25.

For egg mortality during these treatments, see section 5.B.

C. Effect of short daily breaks of the chilling treatment by periods of warmth

populations used: Welsh (2)
pretreatment chilling: none
generation: F_3
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

To determine whether short interruptions of the chilling treatment by periods of warmth each day, could abolish the chilling effect, the following temperature treatments were given to eggmasses.

Freshly picked brown eggmasses of the Welsh (2) population (F_3), were hatched spontaneously at 20° C for 4 weeks, before transfer to the 5° C incubator for interrupted chilling treatment (see section 2.I.C(i)). There were 5 groups of 6 eggmasses each, and each group was given daily periods of warmth (20° C) which interrupted the 5° C chilling treatment, as follows:A. 5 minutes; B. 10 minutes; C. 15 minutes; D. 30 minutes; E. 3 hours; F. 2 weeks. This consisted of placing hatching dishes at room temperature (around 20 to 24° C), with their lids removed. They were kept in the dark and allowed to reach room temperature (about 20 minutes were necessary for this). From then on, the eggmasses were left at 20° C for as long as their treatment required before their lid was replaced and they were returned to the 5° C incubator.

This procedure was repeated daily for 9 weeks and then the eggmasses were placed at constant 20° C for 6 weeks to hatch. Egg hatch was checked weekly during the incubation at both temperatures. At the end of the experiment, the eggmasses were stained in NBR and the number of live and dead eggs remaining in each eggmass was estimated (see section 2.I.D (iii)).

Results

Short periods of warmth every day (5 to 30 minutes), significantly stimulated hatching compared to the controls (constant chilling treatment) ($p < 0.001$). This stimulation occured during the interrupted chilling treatment (H_1) as well as after the eggmasses were transfered to constant 20° C (H_3) ($p < 0.001$) (see Fig. 26). They both showed an increase with the length of interruption.

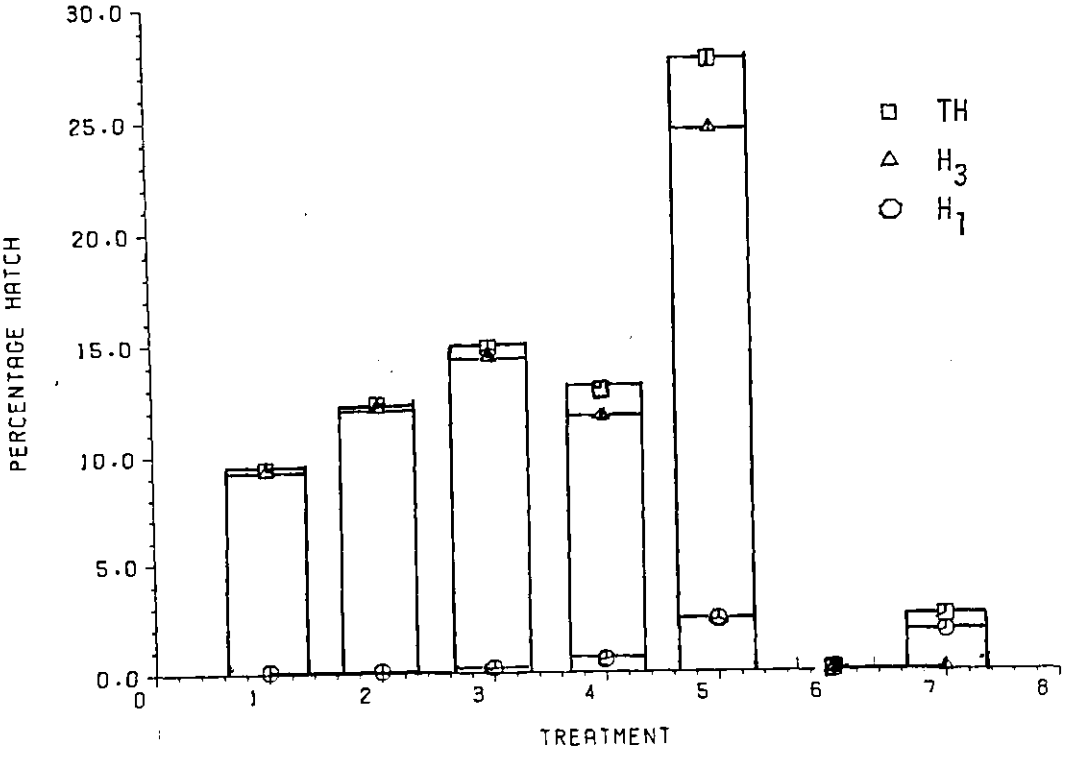


Fig. 26: The hatching behaviour of eggs of the Welsh population of *M. naasi* after receiving chilling interrupted by various periods of incubation at 20° C. 1: constant 5° C with no interruptions (control, data from section 3.A); 2: 5 minutes interruption; 3: 10 minutes; 4: 15 minutes; 5: 30 minutes; 6: 3 hours; 7: 2 weeks.
TH: total percentage hatch; H_1: percentage hatch during the interrupted chilling treatment; H_3: percentage hatch at 20° C following the interrupted chilling treatment.

The 3 hours interruption was inhibitory for egg hatch and gave similar results to the 2 week alternations of chill-warmth periods (see Fig.26).

During the short interruptions of the chilling treatments, the J_2 that hatched, did so evenly throughout the 9 weeks interrupted chilling period, except for the 30 minutes treatment where the number of eggs hatched increased as the treatment progressed.

For egg survival during the treatments, see section 5.B.

D. The effect of osmotic stress on the hatching behaviour of *M. naasi*

populations used: Welsh (2)
pretreatment chilling: none
generation: F_3
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

Osmotic stress is believed to induce quiescence in many nematodes (see section 1.C. page 24), including *M. naasi* in the egg stage.

To see whether osmotic stress interferes with the chilling treatment in terminating diapause and whether it induces more eggs to enter into diapause if it is applied before the chilling treatment, the following experiment was done.

Two treatments were set up, A and B, with 6 and 12 eggmasses freshly collected from the host roots, respectively.

In treatment A (see Fig.27), eggmasses were kept in the test solutions: 1 M, 250 mM, 50mM, 20mM, 10mM, 5mM, 2mM of NaCl, for 2 weeks at 20° C, and spontaneous hatch was assessed (H_1). At the end of this period, the eggmasses and dishes were washed 3 times using tap water, and the NaCl solutions changed to tap water. Hatch was estimated for 5 weeks after this change to see if the osmotic effect was reversible (H_2). The eggmasses were then chilled for 7 weeks to induce hatch (see section 3.A), before transfer to 20° C for 4 weeks to assess percentage hatch after chill (H_3).

In treatment B (see Fig.27), eggmasses were kept in the same test solutions for 7 weeks at 5° C and spontaneous hatch was estimated during osmotic stress at low temperature (H_1), to see whether chilling was effective under osmotic stress. The eggmasses were then transfered to 20° C, and after 1 and 4 weeks incubation during which hatch was

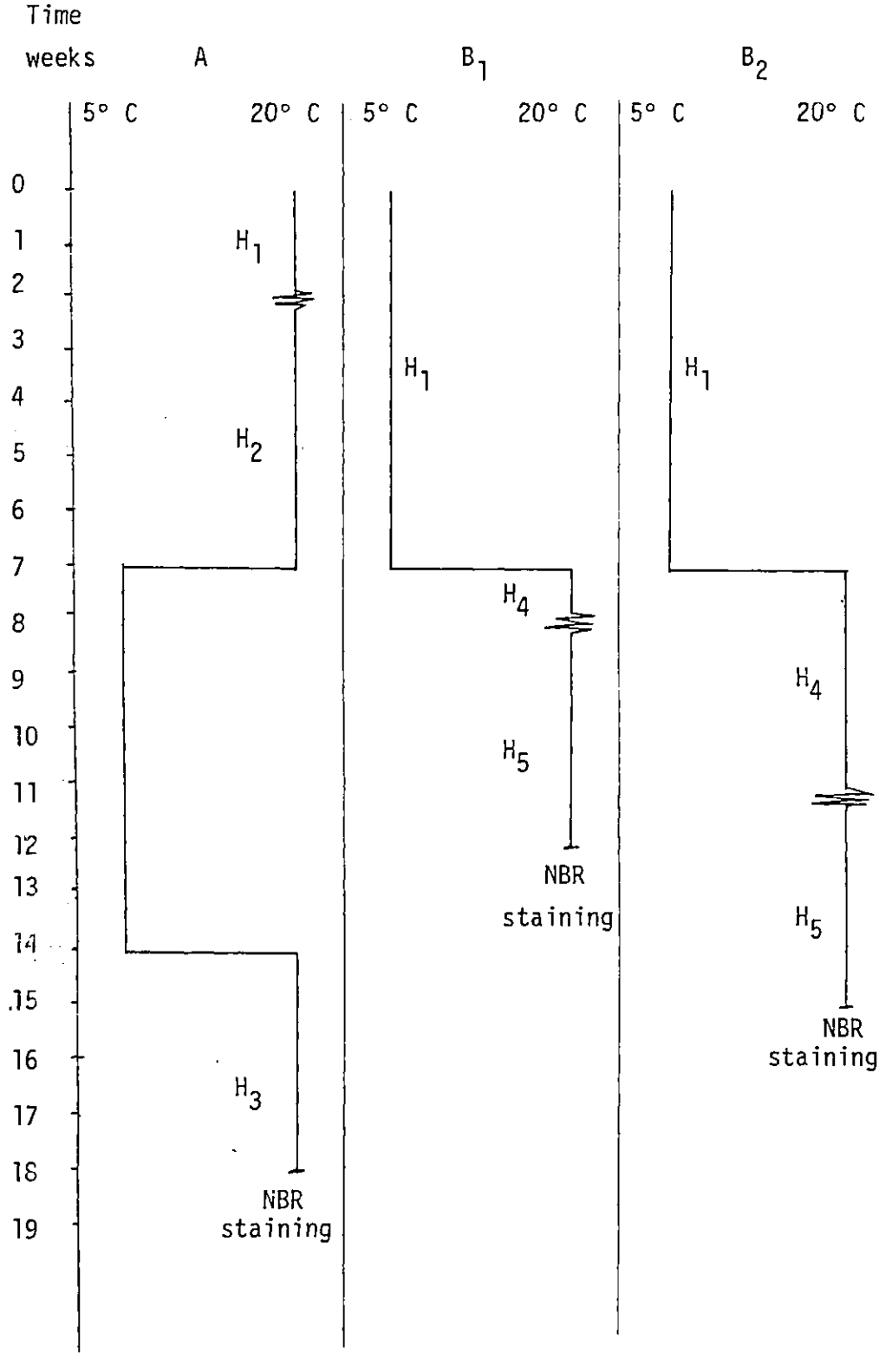


Fig. 27: The procedure followed in section 3.D.

Change of NaCl solution to tap water: ⇆

assessed (H_4), the 6 eggmasses from each NaCl treatment were washed and their NaCl solution changed to tap water (see Fig. 27). Hatch was then estimated for 4 weeks (H_5).

Distilled and tap water were used as controls for both A and B treatments.

Hatched J_2 were counted every 2 days after each temperature or hatching medium change, then weekly. After each counting, the NaCl solutions and water of the controls, were changed with fresh solutions at the appropriate temperature.
Finally, the eggmasses were stained in NBR and the number of live and dead eggs left in each eggmass calculated (see section 2.I.D(iii)).

Results

Concentration of NaCl did not significantly effect percentage hatch in any of the hatching treatment (see Table 17), which was very low in all cases compared to that in tap water ($p < 0.001$).

Some spontaneous hatch occured in all NaCl solutions tested at 20° C, even at the 1 M concentration. Eggs that hatched in this solution, did so over a 15 day period, but all J_2 hatched died. At 5° C, no J_2 hatched spontaneously in any of the solutions or the controls (see Table 17).

After the eggs were transfered from NaCl to tap water, still at 20° C (treatment A, H_2 hatching period), percentage hatch was zero, except for the 1 M and 10 mM NaCl concentrations (see Table 17). When the eggs were chilled in the NaCl solutions and transferred to 20° C still in the solutions, hatch occured in very low numbers, mainly in the second week of incubation at 20° C (H_4 in B_2 treatment, higher than H_4 in B_1 treatment). The hatch occured earlier in the lower than in the higher osmotic srength solutions (see Table 17).

Final hatch was much higher in the B treatments (H_5) than in A treatment (H_3), for nearly all cases ($p < 0.005$). The highest percentage hatch obtained was in tap water (control). Percentage hatch in distilled water was slightly higher than that of the NaCl solutions except for the final hatch (H_5) in the B treatments (see Table 17).

All unhatched live eggs contained J_2 in all treatments.

Egg mortality was greatly affected by NaCl concentration (see section 5.D).

Table 17 : Effect of osmotic stress on hatching behaviour of Welsh population of *M. naasi* (see Fig. 27).

hatching solution	H_1 A %	H_1 B_1&B_2 %	H_2 A %	H_4 B_1 %	H_4 B_2 %	H_3 A %	H_5 B_1 %	H_5 B_2 %
1 M NaCl	0.27	0	3.67	0	0	0.73	1.63	0
250 mM NaCl	0.00	0	0	0	0	0	0.14	1.59
50 mM NaCl	0.08	0	0	0	0	0.02	0.96	0.36
20 mM NaCl	0.00	0	0	0	0.02	0	1.71	0.06
10 mM NaCl	0.41	0	0.02	0	0	0.12	1.73	0.12
5 mM NaCl	0.04	0	0	0.03	0.05	0.39	1.33	2.04
2 mM NaCl	0.41	0	0	0	0.04	0.25	1.76	0.25
distilled water	0.54	0	0	0.29	0.49	0.70	0.82	0.82
tap water	1.03	0	0	0.44	0.73	0.76	4.03	4.03

E. Hatching behaviour of eggs produced under host stress

populations used: French, Welsh (2)
pretreatment chilling: 13 weeks at 5° C
generation: F_1
culturing medium: 100% river sand
temperatures used for hatching experiments: 5° C, 20° C

The females were forced to produce their eggs under stress by removing the part of the host plant above ground (host decapitation). The hatching behaviour of the eggs produced under such conditions was studied and compared to that of the controls, for untreated host plants.

Eggmasses from the French and Welsh (2) populations (F_1), which had been stored at 5° C for 13 weeks, were hatched at 20° C in small Whitehead trays (see section 2.I.C(i)). Hatched juveniles were collected daily and used to inoculate one-week old wheat seedlings which were growing singly in petri-dishes (see section 2.I.B(ii)). One thousand J_2 were used to inoculate each plant.

There were 4 treatments for the Welsh isolate, 4 replicates each. Plants in treatment A, had their stems cut off at the base, 4 weeks after inoculation. Plants in treatment B 6 weeks, and in treatment C 8 weeks after inoculation. Plants in D were controls and were grown normally.

For the French population, 5 treatments were set, 4 replicates per treatment. Plants in treatment A had their stems cut off at the base 7 weeks after inoculation, B after 8 weeks, C after 9 weeks, D after 10 weeks, E after 11 weeks. Plants in F were controls.

Twelve weeks after inoculation, the plants were uprooted. The eggmasses produced were counted and separated according to colour (brown or white), which has been used as an indication of their age (Ishibashi, 1969).

Six brown eggmasses were picked randomly from each treatment from the Welsh population and hatched at 20° C, and 6 eggmasses at 5° C. Spontaneous hatch was estimated at 20° C during 7 weeks incubation, and at 5° C during 32 weeks incubation. The eggmasses at 20° C were chilled for 7 weeks at 5° C and percentage hatch after chill was estimated at 20° C for 6 weeks.

The eggmasses at 5° c were transferred to 20° C for 6 weeks and hatch after chill was estimated.

Eggmasses from the French population were hatched at 20° C, 6 replicates for each treatment. Spontaneous hatch was calculated for 3 weeks before transfer to 5° C for 7 weeks, to estimate hatch after chill during 3 weeks at 20° C.

Finally, the eggmasses were stained in NBR and the number of live and dead eggs left in each eggmass estimated (see section 2.I.D(iii)). The total number of eggs produced per female under each treatment was also compared.

Results

The number of eggmasses produced after each treatment

The earlier the stem was cut off from the plant, the fewer the eggmasses produced in both populations ($p < 0.001$) (see Fig.28). The controls produced the maximum number of eggmasses in the Welsh population, but in the French population, decapitation of the host 11 weeks after inoculation gave the maximum average number of eggmasses per plant (see Tables 18 and 19). However, the difference between treatments E and F (11 weeks and control), was not statistically significant.

Eggmass age

The eggmasses produced were classified according to age, using eggmass colour as a guide (Ishibashi, 1969): (i) old (brown), (ii) young (white).

In the Welsh population, the controls produced around 50% brown and 50% white eggmasses during the 12 weeks growing period, while the 3 treatments had more brown than white eggmasses (see Fig.30). The French population' s controls, produced nearly 70% white and over 30% brown eggmasses, while the 5 treatments had more brown eggmasses, except for the first treatment which gave nearly identical results to that of the control (see Fig.31). Note however the average number of eggmasses produced in treatments A and F (see Table 19). It appears from these results, that stress from the host, induced the females to mature and produce their eggs sooner than the controls.

The number of eggs produced by each female in the various treatments

Earlier decapitation of the host plant, produced significantly smaller number of eggs per female ($p < 0.001$), except for the French population, where in the controls the number dropped slightly, resulting in the biggest number for treatment E (see Fig.29).

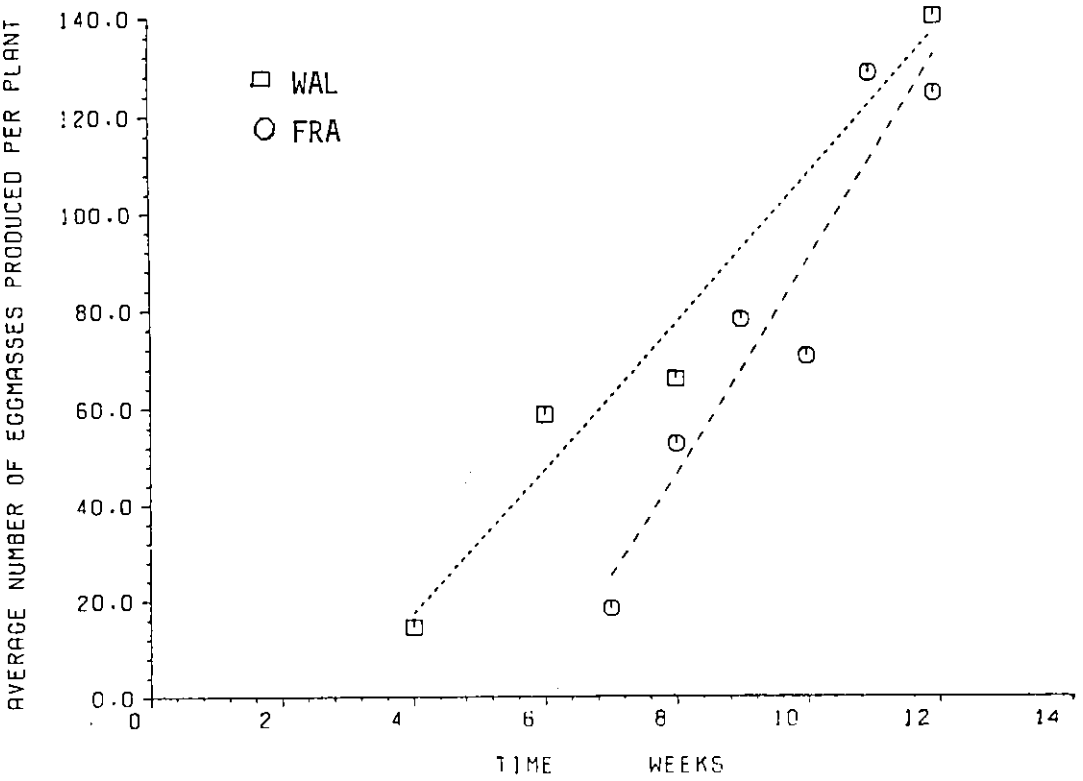


Fig. 28: The effect of decapitation of the host plant at various lengths of time after inoculation, on the number of *M. naasi* females reaching maturity.

Table 18 : Effect of host decapitation on the female population on its roots. Welsh population of *M. naasi*.

treatment	decapitation time weeks	total n° eggmasses	brown eggmasses	white eggmasses	eggs per eggmass	state of root system
A	4	14.5	9.75	4.75	26.3	small, unhealthy
B	6	58.5	45.2	13.2	87.3	small, unhealthy
C	8	65.8	45.5	20.2	71.2	small, unhealthy
D	control	139	69.5	70.0	247	extensive, healthy

Table 19 : Effect of host decapitation on the female population on its roots. French population of *M. naasi*.

treatment	decapitation time weeks	total n° eggmasses	brown eggmasses	white eggmasses	eggs per eggmass	state of root system
A	7	18.2	5.50	12.8	128	senescing
B	8	52.5	38.5	14.0	210	small, healthy
C	9	77.8	54.2	23.5	231	small, healthy
D	10	70.2	60.8	9.50	251	extensive, healthy
E	11	128	97.2	30.8	356	extensive, healthy
F	control	124	40.5	83.2	314	extensive, healthy

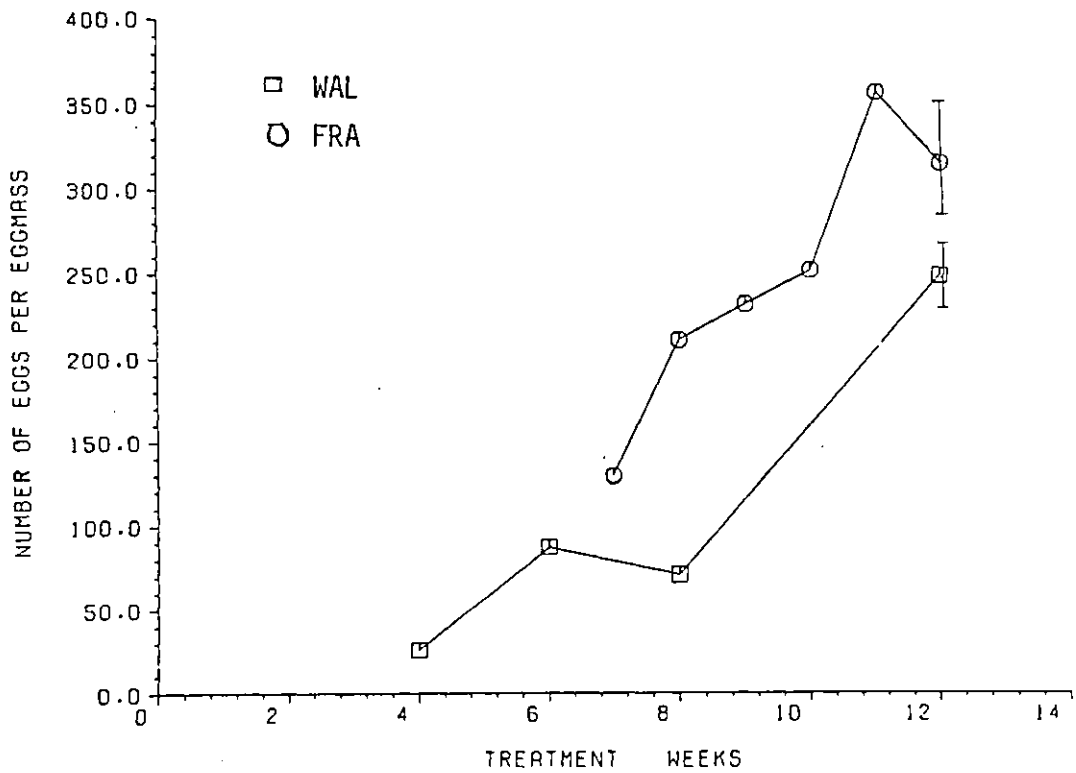


Fig. 29: Effect of decapitation of the host plant at various lengths of time after inoculation, on the number of eggs produced by females of *M. naasi*

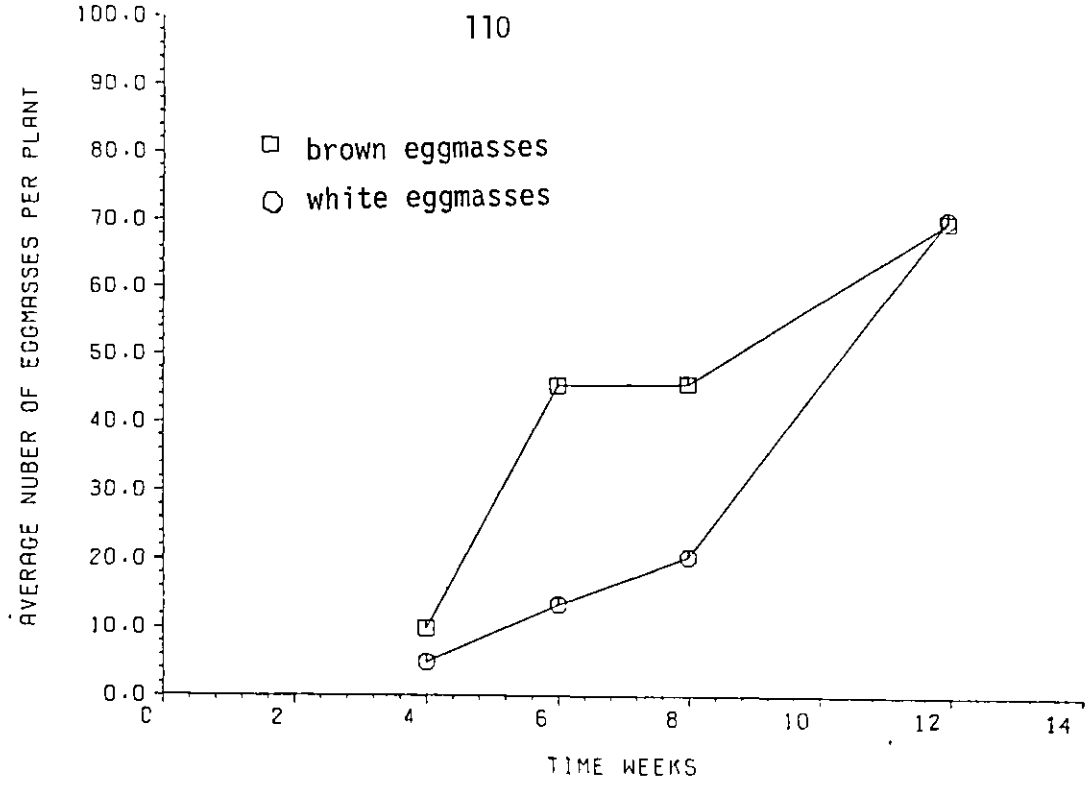


Fig. 30: Effect of decapitation of the host plant at various lengths of time after inoculation, on the colour (age) of the produced eggmasses after 12 weeks culturing, for the Welsh population of *M. naasi*.

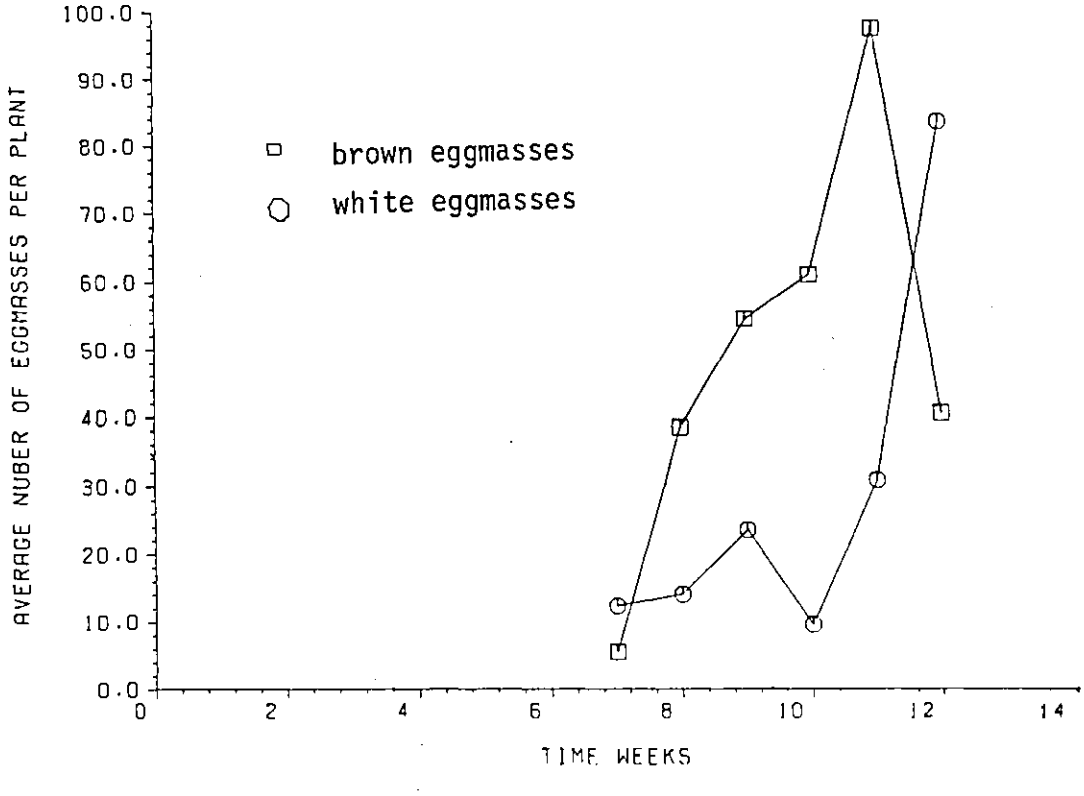


Fig. 31: Effect of decapitation of the host plant at various lengths of time after inoculation, on the colour (age) of the produced eggmasses after 12 weeks culturing, for the French population of *M. naasi*.

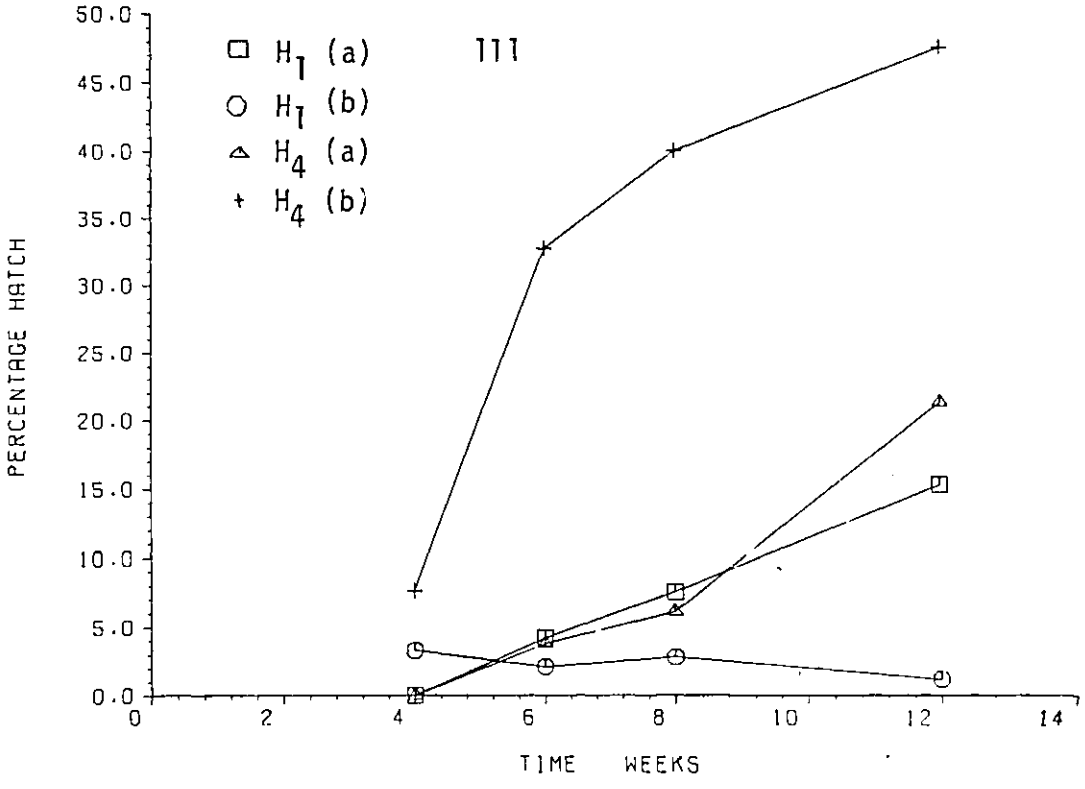


Fig. 32: Effect of decapitation of the host plant at various lengths of time after inoculation, on the hatching behaviour of the Welsh population of *M. naasi*. H_1: percentage spontaneous hatch; H_4: percentage hatch after chilling treatment. (a) 20° C original incubation temperature; (b) 5° C original incubation temperature.

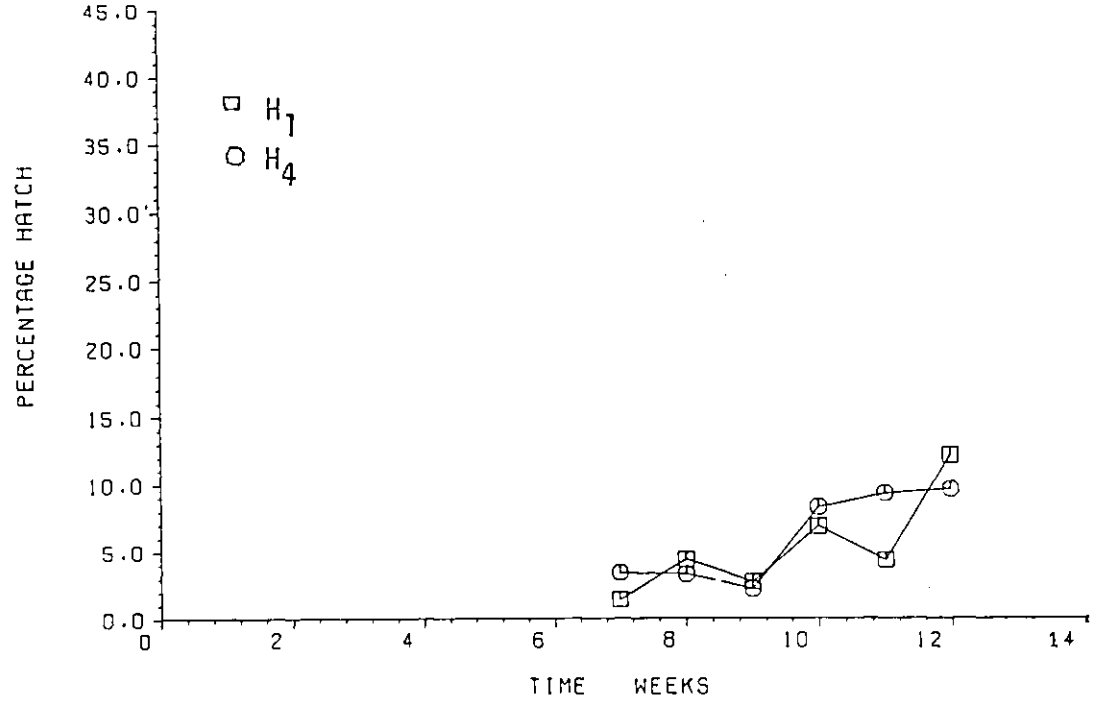


Fig. 33: Effect of decapitation of the host plant at various lengths of time after inoculation, on the hatching behaviour of the French population of *M. naasi*. H_1: percentage spontaneous hatch; H_4 percentage hatch after chilling treatment

The hatching behaviour of the eggs produced

When the eggmasses produced after the treatments described were hatched spontaneously at 20° C, the controls gave a significantly higher percentage hatch in both populations ($p < 0.001$) (see Figs 32 and 33).

The later the host plant was decapitated, the higher the percentage hatch obtained, either spontaneously or after the chilling treatments, in both populations ($p < 0.001$) (see Figs 32 and 33).

F. Hatching behaviour of eggs in relation to age of the host plant during invasion by the parent as J_2

population used: French
pretreatment chilling: 13 weeks at 5° C
generation: F_1
culturing medium: 100% river sand
temperatures used for hatching experiments: 5° C, 20° C

Host plants of different ages at the time of inoculation were used to produce eggs of *M. naasi* under different conditions. The effect of this stress on the J_2 at the time of invasion, was sought in the hatching behaviour of their eggs.

About 500 eggmasses from the French population (F_1) were chilled for 13 weeks at 5° C, then placed at 20° C to hatch (see section 2.I.C(i)). One thousand, one-day old J_2 were used to inoculate each of 12 sand containing petri-dishes supporting 1 wheat seedling each (see section 2.I.B(ii)).

Plants of 4 different ages were used, 3 replicates each: A: 7-day old (controls); B: 14-day old; C: 50-day old; D: 123-day old. The plants in treatment D were at the inflorescence emergence stage (Tottman, *et al.*, 1979).

The plants, before and after inoculation, were grown at 20° C (see section 2.I.B(iii)). Ten weeks after inoculation, the plants were uprooted due to the senescence occuring in the plants of treatment D. Galling was assessed and the eggmasses produced in each plant were classified as brown and white (see section 3.E).

One plant from each treatment was taken randomly, and 12 brown eggmasses were picked from each. Six were set to hatch individually at 20° C, and 6 at 5° C.

The eggmasses at 20° C, after 7 weeks incubation during which

spontaneous hatch (H_1) was assessed, were chilled at 5° C for 7 weeks. They were transferred to 20° C and percentage hatch was assessed for 7 weeks (H_2). The eggmasses were rechilled at 5° C for 12 weeks before transferring them to 20° C to estimate the final percentage hatch (H_3) during 6 weeks incubation.

The eggmasses originally set to hatch spontaneously at 5° C, during which spontaneous hatch was assessed (H_1), were transferred after 33 weeks incubation, to 20° C and percentage hatch was assessed for 6 weeks (H_3).

Finally, the eggmasses were stained in NBR and the number of live and dead eggs left in each eggmass was estimated (see section 2.I.D(iii)). The total number of eggs per eggmass was also calculated to compare the size of eggmasses produced after each treatment.

Results

The number of eggmasses produced after each treatment

The age of the host plant at the time of J_2 inoculation, significantly affected the number of eggmasses produced ($p < 0.001$). The younger the host, the greater were the number of J_2 which developed successfully to the female stage and produced eggs (see Fig. 34).

Eggmass age

After 10 weeks culturing at 20° C, few of the eggmasses produced on all plants were brown (see Fig.34). However, the older the plant at the time of inoculation, the greater the percentage of the eggmasses that were produced, were brown ($p < 0.001$) (see Fig.34).

The number of eggs produced by each female in the various treatments

The age of the plant at the time of invasion, affected significantly the size of the produced eggmasses ($p < 0.005$). The number of eggs per eggmass was generally low after only 10 weeks culturing at 20° C, compared to the usual 12 weeks. Females on the youngest plants, produced 167.4 eggs on average, and on the oldest plants 31.6 eggs (see Fig.35).

Hatching behaviour of the eggs produced

At 20° C original hatching temperature, the 4 treatments gave similar percentage spontaneous hatch (H_1). Percentage hatch after 7 weeks chill at 5° C (H_2), was significantly different for eggmasses

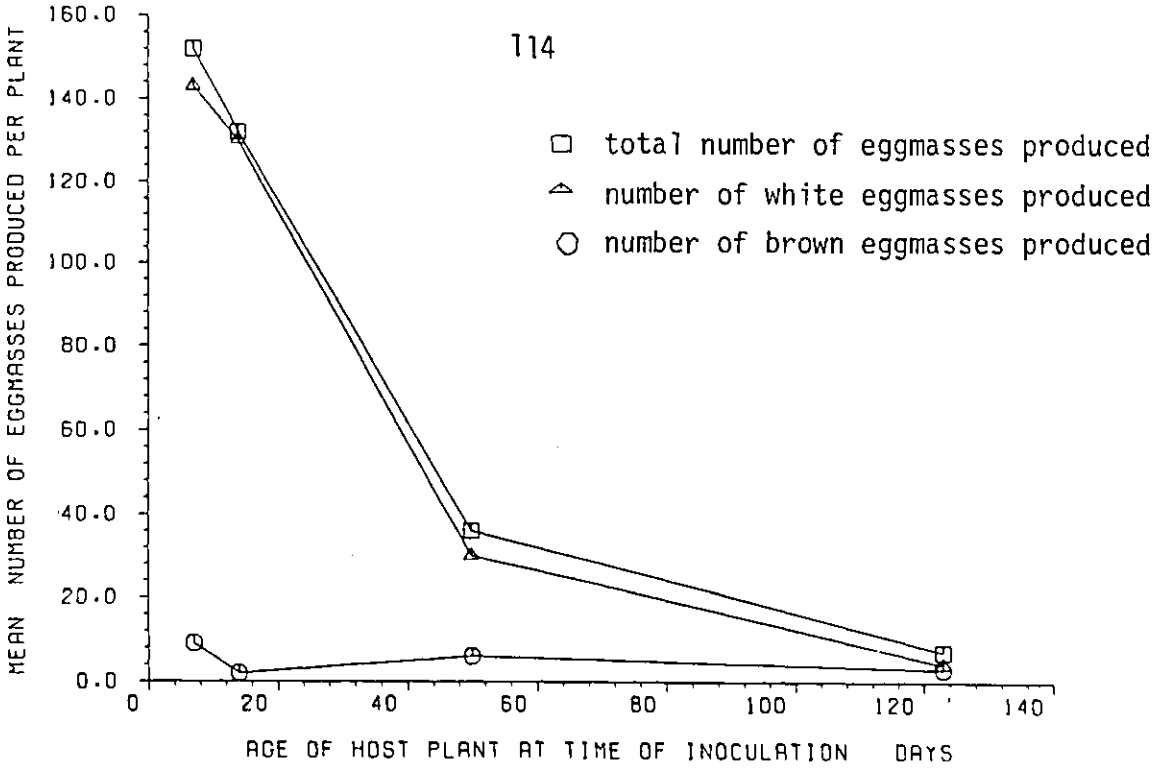


Fig. 34: Effect of host age at time of inoculation, on the establishment of *M. naasi* females on the roots. (French population).

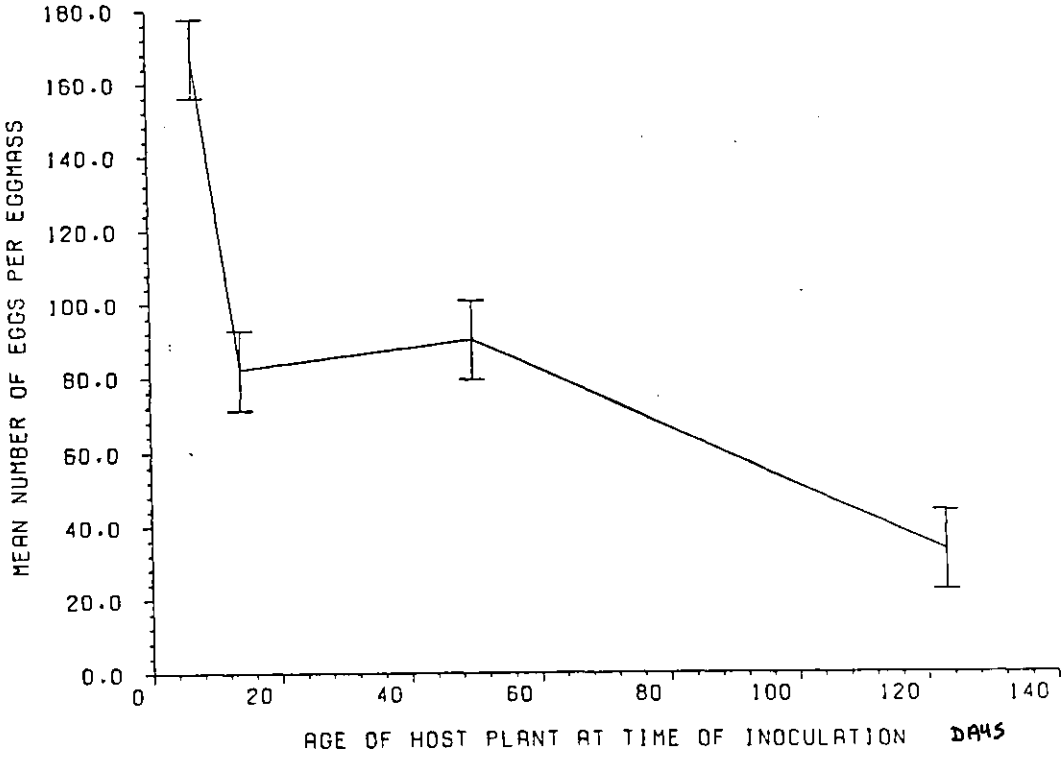


Fig. 35: Effect of host age at time of inoculation, on the number of eggs produced by the females of *M. naasi*. (French population).

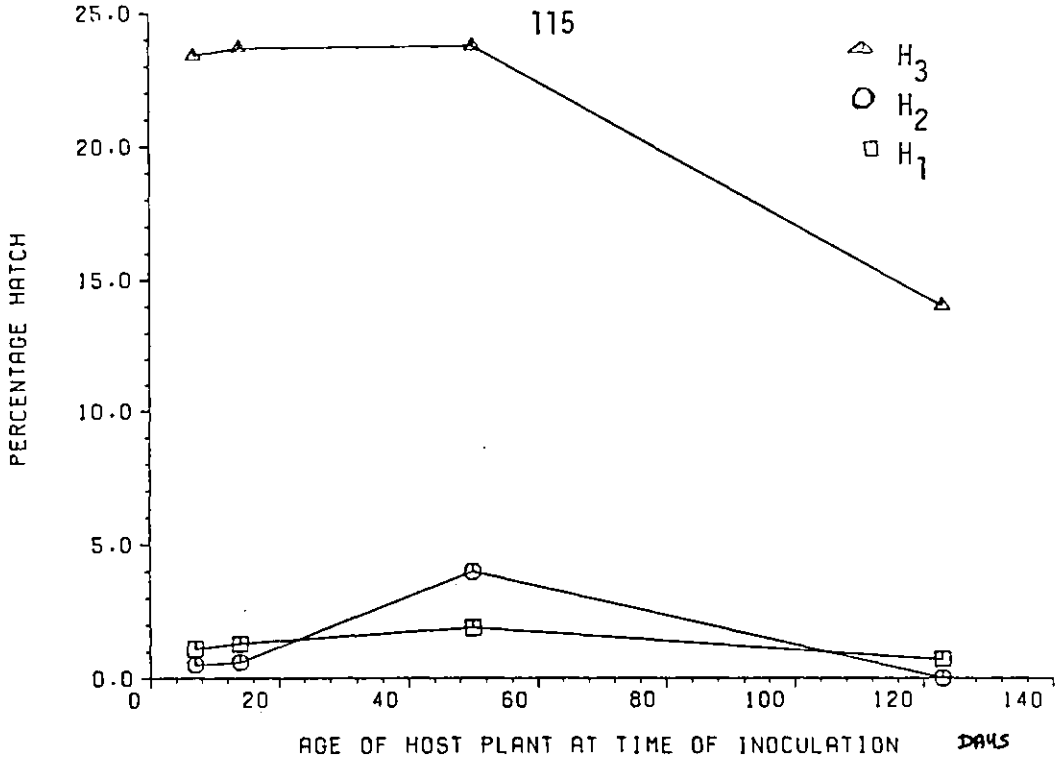


Fig. 36: Effect of host plant age at time of inoculation, on the hatching behaviour of *M. naasi* eggs. (French population). H_1: percentage spontaneous hatch; H_2: percentage hatch after 7 weeks chilling at 5° C; H_3: percentage hatch after 12 weeks chilling at 5° C following H_2.

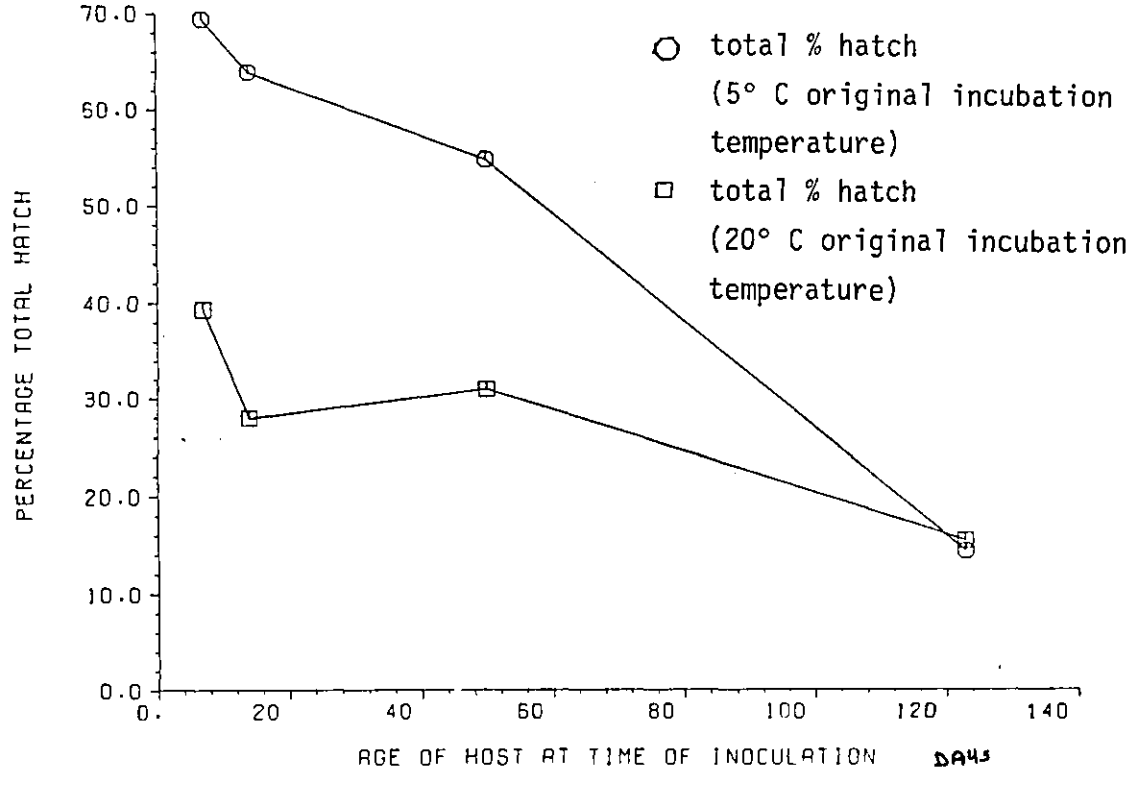


Fig. 37: Effect of host age at time of inoculation, on the hatching behaviour of *M. naasi* eggs. (French population).

produced on the 4 different age groups of hosts ($p < 0.001$). This hatch (H_2) was zero in eggmasses from the oldest plants.

After 12 weeks chill however, percentage hatch (H_3) was much higher, and again significantly different ($p < 0.001$).

In all 3 hatching periods (H_1, H_2, H_3), 50-day old plants at time of inoculation, gave rise to eggmasses with the highest hatch (see Fig. 36 ; Appendix 7).

Total percentage hatch was higher when the eggmasses were chilled at 5° C for 33 weeks before being allowed to hatch at 20° C, compared to the percentage total hatch obtained from eggmasses set to hatch at 20° C originally, and then chilled (see Fig.37). This was not true for eggmasses from treatment D (oldest plants), in which percentage total hatch was nearly the same for the 2 cases. Overall, the total percentage hatch dropped, the older the age of the host plant during inoculation.

Overall, percentage total hatch was significantly affected by treatment ($p < 0.001$), and by original incubation temperature treatment (20°C or 5° C) ($p < 0.001$). The same effects were observed in section 3.E).

Egg mortality was similar in the 4 treatments, in both hatching experiments.

G. Hatching behaviour of eggs in relation to parental "age" at invasion

populations used: French, New Zealand
pretreatment chilling: 9 weeks at 5° C
generation: F_3
culturing medium: 50% loam, 50% river sand
temperatures used in hatching experiments: 5° C, 20° C

The following experiments were done for two purposes: (i) to study the infectivity of J_2 of different ages; (ii) to compare the hatching behaviour of their progeny.

French and New Zealand eggmasses which were stored at 5° C for 9 weeks, were hatched at 20° C in small Whitehead trays (see section 2.I.C(i)). The hatched juveniles were stored in tap water (water column 2 cm hight), in small beakers at 20° C in the dark, and were used to inoculate one-week old wheat seedlings when the J_2 were: 1 to 8 days old. The seedlings were grown in plastic petri-dishes (see section

2.I.B(ii)), in 50% loam and 50% river sand mixture. Three hundred J_2 in suspension were used to inoculate each plant. There were 4 replicates for each J_2 age group, from each population. The plants were grown for 12 weeks (see section 2.I.B(iii)) before they were uprooted. The number of eggmasses produced on each plant was estimated, and 6 brown eggmasses from each set of plants (picked randomly) were set to hatch at 5° C for 7 weeks (see section 2.I.C(i)), then transferred to 20° C, and percentage hatch assessed over a 3 week period. After 3 weeks, the eggmasses were stained in NBR and the number of live and dead eggs left in each eggmass was estimated (see section 2.I.D(iii)).

Results

Infectivity of the J_2 of different ages at time of inoculation

The age of the J_2 at the inoculation time, did not affect their infectivity significantly for either population. This infectivity was estimated by considering the number of mature females given rise to by the J_2, and ignoring males or unsuccessful females.

The size of the eggmasses produced by the females of the 8 treatments did not vary significantly for either population, as shown by statistical tests.

The hatching behaviour of the eggs produced

The 8 treatments did not significantly affect the percentage hatch of the produced eggs, for either population. Overall, percentage hatch after 7 weeks chill at 5° C was much higher in the New Zealand eggs than in the French eggs (see Fig.38). A linear regression analysis however, failed to show a significant linear trend in either case.

Egg mortality did not vary significantly between the 8 treatments for either population.

H. Hatching behaviour of eggs produced by females under stress

population used: Welsh (2)
pretreatment chilling: 12 months at 5° C
generation: F_1
culturing medium: sterilized 100% river sand
temperatures used in hatching experiments: 5° C, 20° C

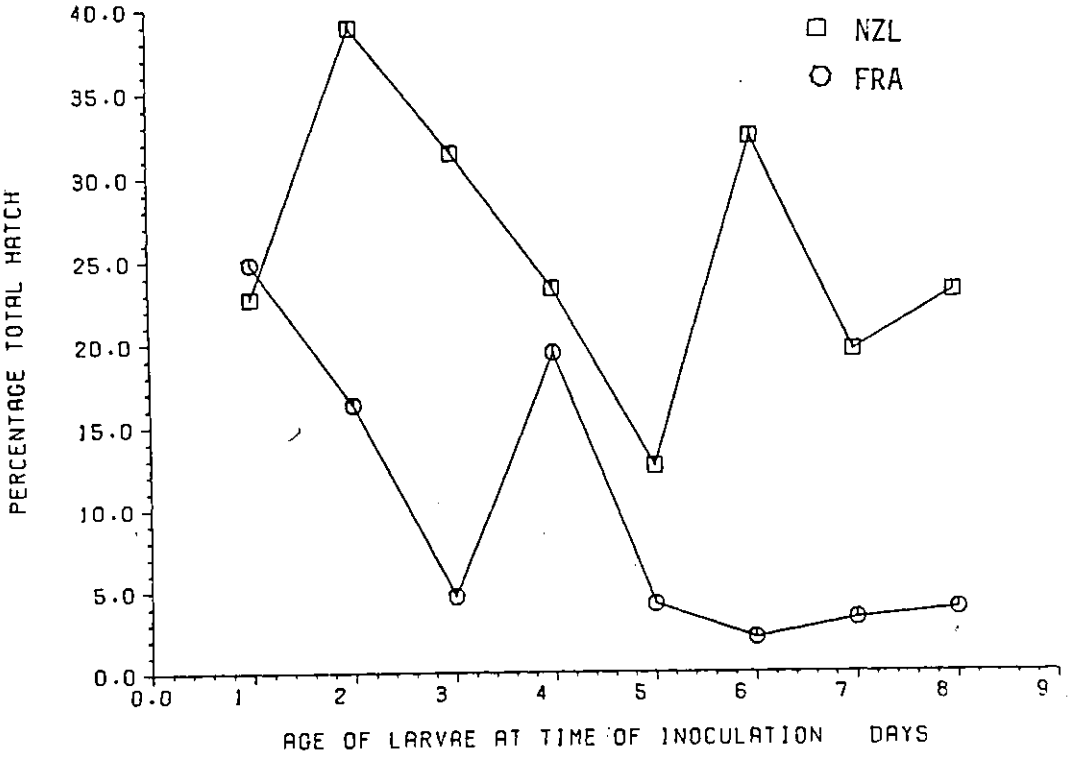


Fig. 38: Total percentage hatch obtained from eggmasses of *M. naasi*, which were produced by females of different age at time of inoculation as J_2.

Females were removed from the host just before egg laying started and were allowed to produce their eggs in a physiological salt solution (see Appendix 3), in order to investigate the effect of the absence of the host on: (i) the female egg production and (ii) on the hatching behaviour of her progeny.

Eggmasses from the Welsh population (F_1), stored at 5° C for 12 months, were set to hatch at 20° C on small Whitehead trays (see section 2.I.C(i)). Each day, the J_2 that hatched were used to inoculate 4 wheat seedlings grown individually under sterile conditions in petri-dishes (see section 2.I.B(ii)). About 1,000 J_2 were used for each plant. Nematode invasion was allowed for 3 days. The plants were lifted, the roots washed and replanted in sterilized sand.

Nematode development on host roots was observed very closely, and 9 weeks after inoculation, females about to start laying eggs were visible on the sides of the petri-dishes. Ten such females were very carefully dissected from the roots, and placed individually in physiological salt solution in syracuse watch glasses under sterile conditions at 20° C in the dark.

Eggs produced were collected daily using hair loops and placed at 20° C in the dark, onto sterile tap water agar with 2 drops of physiological salt solution. The agar was kept moist by adding sterile tap water, when necessary. For more technical details, see section 2.I.C(iii).

Females produced eggs this way for 2 weeks.

Eggs were allowed to develop and hatch spontaneously on the agar at 20° C for 3 weeks and then transferred to 5° C for 7 weeks before being allowed to hatch at 20° C for 8 weeks.

Finally, the eggs were burst open under water, still on the agar, using gentle pressure with a seeker. The escaped J_2 were observed for signs of movement to assess whether they were alive or dead.

Results

Overall, the 10 females produced 193 eggs, 7 of which hatched spontaneously within the first day (see Table 20). The maximum number of eggs produced by a single female during the 2 weeks incubation in the physiological salt solution, was 61. The same female produced the maximum number of eggs in one day (13) (see Table 20).

However, although nearly all of the eggs reached the J_2 stage and were alive at the completion of the experiment (90.2%), none of them hatched after the chilling treatment.

Table 20 : Egg productivity of *M. naasi* females in physiological salt solution, away from the host, and the fate of the progeny.

female	total n° of eggs produced	maximum eggs produced in one day	n° eggs hatched spontan/ly	n° eggs reached J_2 stage	n° eggs died
1	61	13	1	59	1
2	44	11	1	27	16
3	32	6	1	30	1
4	18	4	1	16	1
5	16	5	0	16	0
6	15	5	0	15	0
7	2	2	2	0	0
8	2	2	0	2	0
9	1	1	0	1	0
10	1	1	1	0	0
total	192	13	7	166	19

Discussion

Appraisal of the hatching system

The detailed experiments reported in this section, offered the first chance to appraise the hatching system, which was found to be easy to operate and to maintain at the appropriate temperatures. It was possible to run many experiments at the same time, due to the space saving hatching dishes, and the ease with which they could be handled.

Counting hatched J_2 in all experiments on day 3 and then weekly was done, to follow closely the rate of hatch of the different isolates. Frequent changing of the water in the hatching dishes, ensured control of infections by fungi, bacteria and viruses, especially at temperatures in the range of 20° to 30° C. Fungus was always found when gall tissue was attached to the eggmass, but egg mortality was not affected by the infection. It is however possible that the fungus may have an effect on the hatchability of the eggs. For this reason, sources of such infection were kept to a minimum by frequent water changes and by picking eggmasses free of gall tissue.

The eggmasses set to hatch were immersed in tap water but were close to the surface, for aeration. In nature, *M. naasi* eggmasses in the soil apparently require moisture and oxygen before hatch can take place, even at favourable temperatures, as dry or flooded soil induce quiescence after the breaking of diapause (Ogunfowora, 1979).

As a hatching temperature, 20° C was chosen, after suggestions from Ogunfowora and Evans's (1977a) work regarding the Welsh population of *M. naasi*. Watson and Lownsbery (1970) used 21° to 24° C as hatching temperature for the Californian population.

When no temperature changes were applied, any hatch occuring was considered spontaneous.

The reliability of the hatching system, gave confidence in the results obtained.

Experiments on the hatching behaviour of *M. naasi* eggs: Effect of constant temperature

The range of hatching temperature: "chilling" temperatures, below 20° C (0° to 20°); "warm" temperatures, above 20° C (20° to 40° C) were chosen with 5° C intervals.

Since similar eggmasses were placed at those 9 different

temperatures for a range of 0 to 20 weeks, a similar number of eggs should have hatched from all treatments, if temperature was not a limiting factor. However, the percentage of eggs hatched from each treatment were very different. This indicated that temperature treatments affected subsequent hatchability.

If some temperatures were providing stress, inducing eggs into quiescence, then when the eggs were removed from the "unfavourable" temperatures and placed at 20° C, a similar percentage hatch might have been expected from all temperatures (except for the case of 40° C, which proved lethal) (see following section 5.A). However, this again was not the case. Most of the eggs in each population, appeared to have an optimum incubation period at a specific temperature (10° or 5° C), before maximum hatch could be obtained at 20° C.

These phenomena can be explained by a diapause mechanism which predetermines the conditions required before dormancy is broken and hatch can take place.

In all populations studied, there was a strong tendency for the eggs to hatch after a long (more than 10 weeks) chilling period, with a more variable and generally weaker tendency to hatch following incubation at warm temperatures.

It is possible that the eggmass as a unit contains eggs capable of hatch in both cold and warm climates, and could build up populations under both conditions. Moreover, each population had its optimal conditions, which resulted in a maximum percentage spontaneous hatch and hatch after temperature treatment. This is probably due to the adaptation by natural selection of the particular isolate, to the specific environmental conditions it experienced over a long time.

Thermal stress on eggs of other *Meloidogyne* species has been reported by other workers. Brief heat treatment (46° C for 10 minutes) was found to suppress embryogenesis and hatch of *M. javanica*, but normal development followed those periods of suppression (Bird, 1974) (see section 1.C page 27).

Egg-shell permeability could be affected by high temperatures, due to changes in the lipoprotein membranes in the lipid layer. This property of the egg-shell might be responsible for the delay in hatch, but not for the delay in embryogenesis (Bird and McClure, 1976).

During the incubation of eggs in the warm temperatures, a delay in hatch was observed after their transfer to 20° C. This might be the reason why temperatures above 30° C were reported to inhibit hatch

in this nematode by other workers, since they only studied hatch for 2 weeks (Franklin, *et al*, 1971)

It would be interesting to find out whether a temperature other than 20° C is the best hatching temperature for each population. By repeating the above described experiments, but allowing the eggmasses to hatch at temperatures between 15° and 30° C, the temperature giving the maximum percentage hatch could be found. Such percentage hatch (and the required incubation time), may vary significantly in different hatching temperatures for each population. Lack of time did not allow me to extend the work to include these experiments.

Effect of alternating temperatures

Each eggmass appeared to contain eggs with a range of requirements regarding temperature treatment to break diapause (see section 3.A) This important evolutionary adaptation which ensures production of some progeny under almost any temperature, is however accompanied by the need for a continuous specific temperature treatment without extreme fluctuations before massive hatch can take place. Thus, alternating periods of warmth (20° C) and cold (5° C), interrupted the chilling treatment effects, and resulted in very low hatch. A long period of warmth followed by a long chilling treatment (7 weeks each), was necessary to remove the effects of the alternating temperatures. However, overall such a treatment delayed egg hatch, but otherwise was favourable since the final percentage hatch obtained was higher than that of a constant 7 weeks chill. Alternating temperatures also inhibited hatch in *H. avenae*(Banyer and Fisher, 1971).

The low percentage hatch (H_3) obtained for the control (A_1: 20 weeks at 20° C, followed by 7 weeks constant chill at 5° C), may be explained by the long incubation period at 20° C. This might have a delay effect in hatch, similar to the one obtained after incubation at warm temperatures (see section 3.A). Therefore, it appears that the alternating 5° and 20° C incubations had a similar effect to a long incubation at 20° C. However, when the starting temperature was 5° C, the constant temperature and the fluctuating temperature treatments gave different egg hatchability for the Belgian population. This is possibly due to the inhibition of hatch at the alternating 5° and 20° C periods, but the stimulation of hatch after 25 weeks at 5° C.

A period of embryonation at temperatures above 10° C seems necessary before the chilling treatment, if the latter is to be effective

in breaking diapause (Franklin, *et al.*, 1971) (see also following section 4.C). Eggs which were incubated at 20° C for at least 4 weeks before being given a chilling treatment (treatments A_1, A_2), gave a similar percentage total hatch. When the eggs were not allowed time at 20° C after removal from the host and were chilled at 5° C (treatments B_1, B_2), the percentage total hatch obtained in each treatment was different, and depended on the amount of time they were allowed at 20° C during the chilling treatment.

If diapause is controlled by the production of enzymes, hormones or other substances by the J_2 inside the egg, it is possible that the overall time spent in cold and warm temperatures, is important for the completion of their production. If hatching is indeed divided into 2 phases: (i) development; (ii) eclosion.(Banyer and Fisher, 1971), then the requirements for each phase might be different, making the whole process more complicated. It is possible that a specific number of heat units above 10° is essential for the completion of embryonation (Tyler, 1933; Franklin, *et al.*, 1971), and a number of heat units below 10° C is essential before the J_2 can respond to the hatching temperature. This hypothesis requires a very detailed study, which is complicated by the fact that not all eggs in any egg-mass or population have the same requirements before hatching.

A system which measures time might on the other hand control the breaking of diapause, as has been suggested in the case of insects (Lees, 1955).

It was evident from the data (see Fig. 25)that the chilling treatment experienced by the parent population before hatch, affected the hatching behaviour of the progeny; a possibility not studied before in *M. naasi*.This is an important consideration, which suggests that the ability of an egg to enter diapause might be determined genetically. If this is true, then by storing the eggmasses at 5° C before using them for the various experiments, I have been selecting for diapause. However, it proved impossible to set up cultures from spontaneously hatched J_2 due to the small number of such juveniles hatching, and to their low infectivity (see section 4.F). Such cultures would have been useful for comparative purposes rather than to overcome the problem itself.

It is possible too, that the female is responsible for the provision of substances to the eggs during egg production, which determines the specific amount of cold or warmth necessary before the putative internal

processes were activated or inactivated to break dormancy. Such chemicals could have been produced to a lesser or greater extent when the female was under stress. The amount of chemicals per egg could also have depended on the order in which the eggs were produced by the female (see section 4.D).

Effect of short daily breaks of the chilling treatment by periods of warmth

Interruptions of the chilling treatment by warm periods of up to 30 minutes length, appeared not to abolish the chilling effect, but on the contrary, made it more effective. However, when this warm period was longer (3 hours), the interruptions of the chilling treatment, caused an inhibition in egg hatch.

If the effect of chilling on the egg system was a biochemical one, then it is possible that the short period of warmth during the chilling treatment aided the process, while the longer periods interrupted it.

It would be interesting to repeat this experiment with more time intervals between 30 minutes and 3 hours, to find the exact point at which hatch stimulation stops and inhibition starts.

Effect of osmotic stress

Due to the overall low percentage hatch obtained during this experiment, a more detailed study is necessary before any conclusions can be reached. Ogunfowora and Evans (1977a), reported that osmotic stress induced quiescence in *M. naasi*, thus inhibiting hatch (see also section 1.C page 24).

The low hatch obtained in this experiment could be due to possible egg sensitivity to osmotic pressure, which was caused by the culturing practices used in the laboratory. The eggmasses were collected without being allowed to mature and dry up on the host roots as they would in nature. This might have resulted in eggmasses with softer gelatinous matrices, which controlled water potential in the eggs differently than dry matrices.

Hatching behaviour of eggs produced under host stress

The root systems of the decapitated wheat plants, survived in the soil, even in the case of early decapitation (4 weeks after inoculation, ie 5-week old plants). However, the earlier the stems were cut off, the smaller and less healthy was the root system recovered, yet a

small number of females was still able to reach maturity and produce viable eggs.

Females under such stress from the host, appeared to be able to mature and produce their eggs sooner than the controls. The size of the eggmasses produced however, was smaller than that of the controls, possibly due to lack of nutrients.

The results of the hatching experiments indicated a significant drop in percentage hatch (spontaneous or after chill), the earlier the host plant was decapitated. This suggested an effect of stress on the hatching behaviour of the eggs produced, either coming from the female or from the host through the female. This factor of stress may act on the eggs to influence its hatching behaviour, either independently, or in combination with other factors, like temperature for example.

Other experiments involving stress on the female via the host, could be done to study their effect on the hatching behaviour of their progeny. Such studies could be very useful in finding out more about the way diapause is brought about in *M. naasi* eggs, and possibly also related species of *Meloidogyne* Stress on the plant host could be induced by varying photoperiod, nutrients, temperature, water availability during the period of egg production.

The hatching behaviour of *G. rostochiensis* and *G. pallida* was reported to be affected by the daylength in which the host was growing (Franco and Evans, 1979).

Hatching behaviour of eggs produced by J_2 stressed during invasion

The older the host plant was at the time of inoculation, the smaller the number of juveniles which successfully invaded and produced eggmasses. This might be due to the state of the root system at the time of J_2 invasion. In the oldest plants, the leaves had started to die and inflorescence started to emerge; this might have affected the nutrient and the supply of other substances to the roots from the above ground parts of the plant, making the J_2 establishment and normal development difficult. The number of new rootlets produced by the senescing hosts might have dropped, thus increasing the competition between J_2 for sites of entry and feeding.

The youngest plants supported around 40 times more eggmasses than the oldest host plants, but only 1.5% of them were brown.

From the data in this section, and in section 3.E, it appeared that possible depression in the supply of nutrients due to decapitated or old plant hosts, did not delay development of the females, but rather

speeded it.

Females on the older host plants, produced a smaller number of eggs, either due to shortage of nutrients or because they were induced by the senescing host, to stop egg production.

Overall, percentage hatch whether the temperature of the thermal treatment was 20° or 5° C, dropped with the older aged host plants (higher stress), a phenomenon observed in section 3.E.

Hatching behaviour of eggs in relation to parental "age" at invasion

There was no significant difference in infectivity and hatching behaviour between the 8 different age groups of parent *M. naasi* used.

The recorded age of the J_2 at inoculation, does not correspond to their precise age at the time of invasion.

There was a lot of variation in the results from each group, which suggested the need for the repetition of the experiments, with a greater number of replicates, before any conclusions could be reached. However,to avoid any possible variation in the results, of either infectivity or hatching behaviour in all subsequent experiments, one-day old J_2 were used.

Hatching behaviour of eggs produced by females under stress

Females removed from roots, successfully laid some eggs in physiological salt solution. However while a few of these eggs hatched spontaneously, other chilling or 20° C incubations did not induce any hatch.

Thus the method worked technically, but failed to demonstrate additional factors leading to hatch of *M. naasi* eggs.

Egg laying in the physiological salt solution, stopped after 2 weeks. Franklin, *et al.*,(1971), reported that adult females continued to feed and lay eggs for 4 to 6 weeks in the field. Possible lack of host nutrients did not allow further egg production by the females, which had exausted their supplies.

Eggs were first kept moist in physiological solution for 3 days to provide a medium more resembling the female's young gelatinous matrix. They were then covered with sterile tap water in order to remove any possible osmotic effects of the solution. Eggs were successfully kept free of infections by working under sterile conditions.

This type of investigation could be pursued by obtaining a larger number of eggs in this way and increasing the number of treatments known

to stimulate hatch in normally produced *M. naasi* eggs. By providing the eggs on the agar with extracts from squashed females which were allowed to develop and feed normally on host roots, it is possible to find out whether the lack of chemicals from the female was responsible for the inhibition of hatch in this experiment.

Experiments in this section have shown that environmental temperature is the main stimulus used by *M. naasi* to terminate its diapause in the field, and that stress on the female via the host has some influence in the final proportion of eggs entering diapause.

Having considered the effects of temperature and stress on the hatching behaviour of the 6 populations of *M. naasi*, attention was given in the next chapter, to a range of "biological" factors, which may also influence egg hatch.

CHAPTER 4

BIOLOGICAL FACTORS AFFECTING THE HATCHING BEHAVIOUR OF *M. NAASI*.

Introduction

The possibility that egg hatch in *M. naasi* is influenced by biological factors, is suggested by a consideration of its life history (see section 1.B).

Temperature and other climatic changes could take place during the period of female egg production, especially at the end of the egg-laying season. The stage of the female, of the embryonic development of the egg and the composition of the gelatinous matrix when autumn chilling begins, might be important in determining whether the eggs, already produced, will become dormant or will be able to hatch spontaneously.

Inbuilt mechanisms in the female or in the J_2 may be present, which detect environmental changes and influence further egg laying or embryonic development, accordingly. Females may require from the host "nutrients" throughout egg-laying, so early removal from the host might interfere directly or indirectly with development and hatching behaviour of the eggs. Providing the host, and therefore the female with excess nutrients and water (hydroponic cultures), might induce an early hatch or longer diapause in the eggs produced.

The effects of such factors, not directly environmental, but which involve the biological system of *M. naasi* and its relationship with its host, were investigated, without attempting to study the biochemical mechanisms.

The infectivity of mechanically hatched J_2 was also studied and compared to that of spontaneously hatched J_2, and J_2 that hatched after a chilling treatment, to determine whether the egg-shell is the only barrier to further development (hatch) as suggested by Gooris and D'Herde (1977).

A. The effect of the "age" of the eggmass when removed from the host, on the hatching behaviour of *M. naasi* eggs

populations used: Welsh (2)
pretreatment chilling: none
generation: F_3
culturing medium: 50% loam, 50% river sand

temperatures used for hatching experiments: 5° C, 20° C

Eggmasses removed from the same host roots, differed in appearance according to the stage of development of the female. They appeared to be white and soft when young, containing a smaller number of eggs, and the females were still laying eggs. They appeared yellowish and harder when bigger, with females still laying eggs, and brown with the outermost part of the gelatinous matrix "shrunk", drier and harder when the female seemed to be empty of body contents.

The following experiment was done, to determine whether the "age" of the eggmass at the time it was removed from the host and chilled at 5° C, affected percentage hatch.

Eggmasses of the Welsh (2) population (F_3), stored at 5° C for 7 weeks were set to hatch at 20° C. One-day old J_2 were used to inoculate one-week old wheat seedlings in petri-dishes (see section 2.I.B(ii)). The plants were uprooted 12 weeks after inoculation, and brown and white eggmasses were picked from the roots and placed in tap water in 2 separate watch glasses.

Individual eggmasses were taken from each watch glass at random, and hatched at 5° C (see section 2.I.C(i)). Twentyfour eggmasses from each watch glass were used. After 9,10,11 and 12 weeks of chill at 5° C, 6 brown and 6 white eggmasses were transferred to clean hatching dishes at 20° C. The J_2 hatched were counted on day 3 at 20° C, and then weekly over a 6 week period.

Results

No detailed results are presented for this experiment, as the only significant points were:

From the sample of the 24 eggmasses from each age group, an average of 181 eggs per eggmass for the brown eggmasses and 135 for the white ones was estimated. The brown eggmasses were significantly bigger than the white ones ($p < 0.005$).

Overall, the white eggmasses resulted in a significantly higher percentage hatch after the chilling treatments than the brown eggmasses; 13.8% and 4.9% respectively ($p < 0.001$). However, the length of the chilling treatment (9, 10, 11, 12 weeks at 5° C) gave no significant difference in percentage hatch in either case.

The white eggmasses became darker in colour with time during the experiment.

For egg mortality during the treatments, see section 5.E.

B. The hatching behaviour of eggs produced in hydroponic cultures

populations used: Belgian, French, German, New Zealand, Californian, Welsh
pretreatment chilling: none
generation: F_3
culturing medium: water nutrient solution (see Appendix 2)
temperatures used for hatching experiments: 5° C, 20° C

To study whether the culturing method affected hatchability, eggmasses produced in water nutrient solution, and in soil medium were compared.

Eggmasses from the 6 populations (F_3), stored at 5° C for 10 weeks, were hatched at 20° C (see section 2.I.C(i)). About 1,000 one-day old J_2 were used to inoculate one-week old wheat seedlings growing in sand. After 3 days exposure to the J_2, the seedlings were transferred to the water nutrient solution (see section 2.I.B(ii)). There were 3 replicates for each population.

Ten weeks after inoculation, the plants were lifted, and 6 eggmasses picked randomly from each population, were hatched individually at 20° C for 6 weeks. At the same time, eggmasses from the Belgian population were also set to hatch at 5° C for 7 weeks before they were transferred to 20° C for 3 weeks.

The size of the eggmass, and the hatching behaviour of the produced eggs, were compared to that of eggmasses reared in 50% loam, 50% river sand mixture, from section 3.A.

Results

Eggmasses produced in hydroponic cultures were mainly enclosed in the soft white gall tissue. No brown eggmasses were obtained in any population. In the soil cultures, eggmasses were usually produced outside the root tissue and became darker with time (see section 4.A).

In the hydroponic cultures, all 6 populations produced similar sized eggmasses. The average number of eggs produced by each female, after only 10 weeks of culturing, was 182 (see Table 10).

Spontaneous hatch and hatch after chill did not vary between either population or culturing technique (hydroponics or soil). In the hydro-

ponic cultures, spontaneously hatched J_2 were recovered during the end of the tenth week of culturing. In the soil cultures however, it is not easy to detect small numbers of J_2 hatching spontaneously early in their life cycle. A proportion of such J_2 usually reinvades the roots and cannot be recovered.

Egg mortality obtained during the hatching experiments was similar in the eggmasses obtained from the 2 culturing techniques.

C. The hatching behaviour of eggs, in relation to the stage of embryonic development in the egg at the time the chilling stimulus was applied

population used: Welsh (2)
pretreatment chilling: none
generation: F_1
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

Eggmasses from the Welsh (2) population (F_1), were collected from freshly uprooted plants (see sections 2.I.B(iv) and (v)). They were placed in sterile tap water, and the eggs were separated from the eggmasses using hair loops to avoid damage.

As many eggs as possible were separated into watch glasses containing sterile tap water, according to their stage of embryonic development. As a guide to embryonic development of *M. naasi*, stages described by Siddiqui and Taylor (1970) were used. There were 7 categories: uteri containing eggs at 1 or 2 cell stage, 2, 3, 5 cell stage, gastrula, J_1 and J_2 egg stages. The eggs were transferred using small hair loops (about 1 mm diameter) (see section 2.I.D(ii)). The uteri containing eggs were obtained by squashing young females under sterile tap water, transferring the uteri onto sterile tap water agar and teasing them out to free the eggs. The remains of the tissues were left onto the agar.

Half of the eggs so collected (except the ones in the uteri), were surface sterilized using 0.26% NaOCl (see section 2.I.C(ii)). the other half using prolonged washing (see section 2.I.C(ii)).

A group of 20 to 30 eggs (according to availability), from each stage of development, and from each sterilization technique used, were placed onto a sterile tap water agar plate (2 cm diameter). The eggs were placed next to each other to make observation easy. Sterile

techniques and conditions were used throughout the experiment.

The plates were kept moist by adding sterile tap water every week and checked for egg development. They were stored in sealed petri-dishes (12 cm diameter) (see Fig.14).

The eggs in the plates were chilled at 5° C for 7 weeks, then transferred to 20° C for 6 weeks. They were rechilled for a further 7 weeks at 5° C and transferred at 20° C for 6 weeks. Egg development and hatch was assessed weekly throughout this time, by observing the eggs under the microscope in sterile conditions. Observation time was kept to a maximum of 10 minutes for each plate group each week, to avoid temperature fluctuations. Hatched J_2 and empty egg-shells were removed from the plates, to avoid substances from them reaching the other eggs.

At the end of the experiment, each unhatched egg was burst open by applying pressure using a seeker, to allow the J_2 to escape. The pressure inside a viable egg was high and releasing the J_2 was easy. In a dead egg, the pressure was low and the J_2 was unable to straighten up its body. The live J_2 contained lipid droplets and showed some movement. Thus, the number of live and dead eggs was estimated.

Results

The development of the embryo in the egg was very slow (if any at all) at 5° C (see Table 21). A few eggs developed slightly. However, the moment the eggs were transferred to 20° C, development was resumed, and nearly all eggs reached the J_2 stage within 2 weeks of incubation at this temperature. The rest of the eggs reached the J_2 stage by the end of the third week.

Although the eggs were at the J_2 stage at 20° C for at least 3 weeks, very few hatched, even after the second chilling treatment (see Table 21).

Under the conditions used, no fungal or other infections were observed in the dishes of either sterilization techniques. In both cases there was no difference in the trend of events observed, suggesting that contact with NaOCl for 30 seconds did not affect egg development or hatch.

The 2 plates containing eggs and the squashed uteri, did show some infection, probably resulting from the uteri contents , but it was limited and did not reach the eggs.

Table 21 : Embryonic development and hatchability of *M.naasi* eggs receiving chilling at different stages of development.

steril/on treatment of eggs	original stage when placed at 5°C	% of eggs at stage after 7 weeks at 5°C							% of eggs at stage after 1 week at 20°C							% of eggs at stage after 2 weeks at 20°C							final		
		1	2	3	4	5	gst	J_1	J_2	1	2	3	4	5	gst	J_1	J_2	1	2	3	4	5	gst	J_1	J_2	L	D	H
NaOCl	squashed uteri & 1,2 cell stg	5	25	70										75	20	5							60		40	30	15	55
NaOCl	2 cell stage		15	38	39	8								69	23	8									100	60	40	0
NaOCl	3 cell stage			36	19	19	26							64	18	18							9	91		64	36	0
NaOCl	5 cell stage					60	40							80		20							20		80	60	20	20
NaOCl	gastrula						78	22						18	45	37							13		87	47	45	8
NaOCl	J_1 stage							60	40						20	80									100	56	44	0
NaOCl	J_2 stage								100							100									100	50	47	3
prolonged washing	squashed uteri & 1,2 cell stg	13	40	47										68	12	20							30		70	31	19	50
	2 cell stage		25	35	50									75	15	10									100	75	25	0
	3 cell stage				35	24	41							59	29	12							6		94	76	24	0
	5 cell stage					80	20							60	20	20							20	10	70	80	20	0
	gastrula						85	15						30	50	20										65	20	15
	J_1 stage							86	14						14	86									100	29	71	0
	J_2 stage								100							100									100	67	25	8

L: % live eggs remaining D: % dead eggs remaining H: % hatched eggs

D. Hatching behaviour of eggs in relation to the order in which they were laid by the female

population used: Welsh (2)
pretreatment chilling: none
generation: F_1
culturing medium: sterilized 100% river sand
temperatures used for hatching experiments: 5° C, 20° C

The hatchability of the eggs produced by a single female was studied in relation to the order in which the eggs were produced. Eggs laid each day were collected and placed separately onto tap water agar at 20° C. Their embryonic development was observed weekly for 4 weeks, before they were chilled at 5° C for 7 weeks. Hatching was studied at 20° C for 6 weeks.

This experiment was described in detail in section 3.H. Here the study of the development of each egg produced under these conditions is described.

Results

Egg production by the females stopped 13 days after removal from the host roots. Egg laying spread evenly throughout the egg producing period and did not follow any significant pattern.

The majority of the eggs thus produced, reached the J_2 stage within 2 to 3 weeks at 20° C (see section 4.C). The eggs that failed to do so by the end of the 3 weeks, did not develop any further by the end of the experiment and they were considered dead (see Table 22).

No eggs hatched while on the agar plates. One to 2 hatched J_2 were found on several occasions in the dish containing the female, without any prior incubation (all eggs produced were removed daily from the dishes).

There was no significant difference in the pattern of embryonic development or hatchability, between the eggs produced each day.

In this experiment there was a relatively high egg mortality compared to other experiments (see chapter 5).

The females in the solution were considered dead and were discarded when they became empty of body contents and stopped producing eggs.

Table 22 : Female productivity in the physiological salt solution, the embryonic development and hatchability of the eggs produced.

female	n° eggs & hatched J_2 produced each day at 20°C in physiological salt solution														total n° eggs produced	% eggs reached J_2 stage	total % hatch	final % of live eggs	final % of dead eggs
	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
1	1	13	8 $1J_2$	7	9	5	1	7	4	3	1		1	*	61	91.8	1.6	47.5	50.8
2		11	5 $1J_2$	7	8	4	3	5						*	44	65.9	2.3	31.8	65.9
3			4 $1J_2$	4	6	2	4	4	2	4	1			*	32	87.5	3.1	43.8	53.1
4					3	3	3 $1J_2$	3	2	2	1			*	18	94.4	5.5	50.0	44.4
5			2	2	5	3	2	2						*	16	100	0	31.2	68.8
6			2	5	3	3	2							*	15	100	0	20.0	80.0
7			$2J_2$		*										2	100	100	0	0
8						2								*	2	100	0	0	100
9	1											*			1	100	0	100	0
10			$1J_2$									*			1	100	100	0	0

* indicates that the female died and was discarded.

E. Effect of the presence of the female in the hatching dish, on the hatching behaviour of her eggs

population used: Welsh (2)
pretreatment chilling: none
generation: F_3
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

To test whether chemicals from the female body might leak out and affect egg hatch, the following experiment was done.

Freshly picked eggmasses from the Welsh (2) population (F_3), were hatched spontaneously at 20° C for 2 weeks (see section 2.I.C(i)). The eggmasses were then transferred to clean hatching dishes in fresh tap water. Six eggmasses had the female body still attached to the gelatinous matrix (treatment: A. control, the situation most common in nature). Using fine hair loops the female body was removed carefully from the eggmasses without damage to the eggs (treatment: B). From 6 eggmasses, the female bodies were detached from the gelatinous matrix as described above, but the females were kept in the sieves with the eggs. Their bodies were burst open using fine forceps (treatment: C). For the last 6 eggmasses, the female body was also burst as above, but the eggs were dispersed using fine hair loops (treatment: D).

Any J_2 hatched on day 1 were discarded because of the possibility of mechanical hatch during the setting up of the experiment.

The eggmasses were kept in the same hatching medium throughout the experiment to avoid loss of any chemicals leaked by the female's body. Hatching was assessed for 4 weeks at 20° C (H_1), then the eggmasses were chilled at 5° C for 10 weeks and transferred to 20° C for 6 more weeks (H_4).

Results

Percentage hatch in the 4 treatments was significantly different ($p < 0.001$), with highest hatch from the eggmasses which had their eggs dispersed and a squashed female body in the hatching dish. When no female body was present, a very low hatch occurred (see Fig. 38). However, percentage hatch after chilling was zero for all treatments except for treatment D, which was very low compared to other experiments (see section 3.A)

Egg mortality was similar in the 4 treatments.

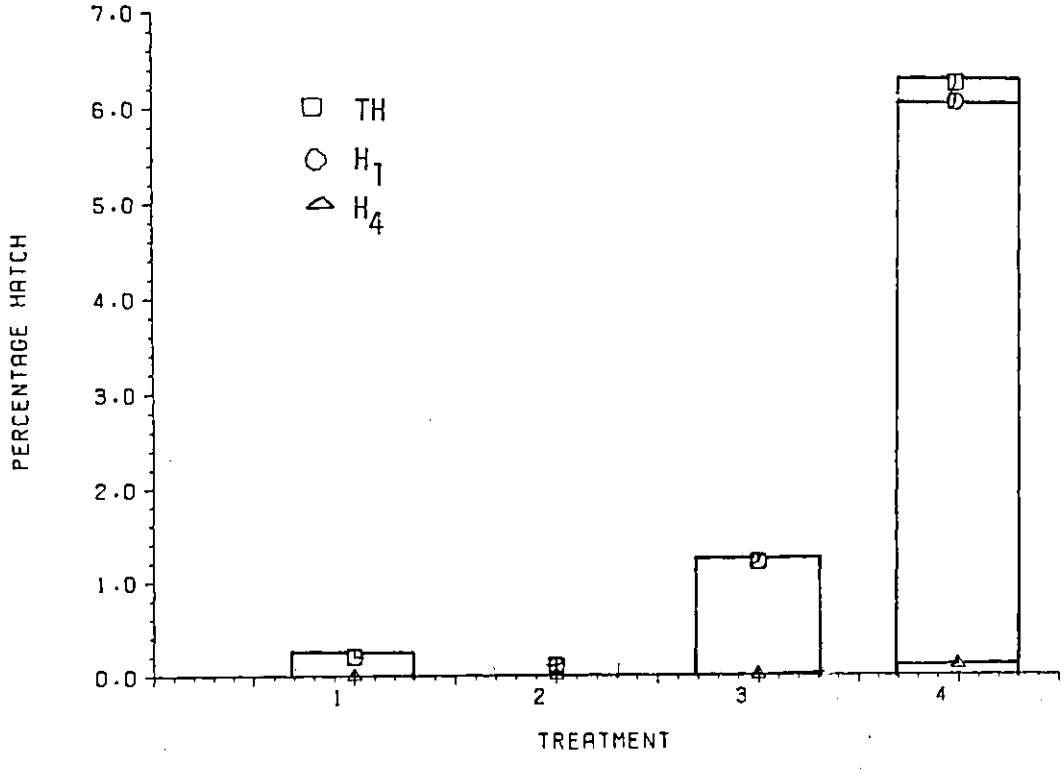


Fig. 38: The hatching behaviour of the Welsh population of *M. naasi* when the eggmasses were incubated: 1: with the female body; 2: without the female body; 3: with the female body squashed; 4: with the female body sqashed and the eggs freed from the gelatinous matrix.
TH: total percentage hatch; H_1: percentage spontaneous hatch; H_4: percentage hatch after chill.

F. The disposition of eggs to enter diapause through generations

populations used: German, Welsh (1)
pretreatment chilling: none
generation: F_2
culturing medium: 100% river sand
temperatures used for hatching experiments: 5° C, 20° C

The following experiment studied: (i) the infectivity of J_2 that hatched spontaneously, and (ii) whether these J_2 give rise to eggs with a disposition to enter diapause or to hatch spontaneously.

Eight different groups of parent treatment for each population (German, Welsh:F_2), were used. Juveniles that had experienced 0 to 7 weeks of chilling in the egg, at 5° C before hatching, were used to inoculate wheat seedlings (see section 2.I.C(i)). The infected roots were collected 12 weeks after inoculation, cut into 1 cm long pieces, and hatched in a Whitehead tray at 20° C for 8 days.

The J_2 obtained during the first 2 days of incubation at 20° C, were discarded, to avoid possible collection of J_2 that had hatched mechanically during the cutting of the roots. All J_2 hatched each day were used to inoculate one-week old wheat seedlings growing in petri-dishes (see section 2.I.B(ii)). There were 3 replicates for each parent treatment, for each population.

Twelve weeks after inoculation, the plants were uprooted. Brown eggmasses were picked randomly from each set of plants and were hatched individually at 20° C, and at 5° C. There were 6 replicates from each parent treatment, from each population for every hatching temperature.

The eggmasses originally set to hatch at 20° C were incubated at this temperature for 13 weeks and percentage spontaneous hatch (H_1) was assessed. They were then chilled for 7 weeks, and transferred to 20° C for 7 weeks to estimate percentage hatch (H_3). The eggmasses were then rechilled at 5° C for a further 7 weeks before they were transferred to 20° C for a further 8 weeks to estimate percentage hatch (H_4).

The eggmasses originally set to hatch at 5° C were incubated at this temperature for 27 weeks. Percentage spontaneous hatch at 5° C was evaluated before they were transferred to 20° C for 8 weeks and percentage hatch (H_3) was assessed.

To finish, the eggmasses were stained in NBR and the number of

live and dead eggs that remained in each eggmass was counted (see section 2.I.D(iii)).

Plans to study the disposition of eggs to enter diapause through 8 generations, by rearing 2 types of cultures: (i) reared from J_2 that hatched spontaneously; (ii) reared from J_2 that hatched after chill, failed. Very few J_2 hatched from eggmasses of the second generation of spontaneously hatched parents, and most of them died within one day after hatch.

Results

Juveniles that hatched spontaneously, from both populations and all parent treatments, were infective; varying from 0.7% to 27.7%, but generally being statistically similar in the 2 populations and all parent treatments. It was, however, generally lower than that of J_2 hatched after chilling ($p < 0.001$) (see Table 23).

A similar number of eggs per eggmass were produced by females which had hatched spontaneously as J_2 and from those that had experienced chilling.

Eggs produced by spontaneously hatched parents, gave rise to eggs that could both hatch spontaneously and hatch only after chilling. The same was true for eggs produced after chilling (see sections 3.B).

Percentage hatch (total, after 1 or 2 chilling treatments) was significantly lower for both populations tested (see Fig. 40); when the culture had been obtained from spontaneously hatched J_2, compared to that obtained from chilled J_2 ($p < 0.001$). This was not true for percentage spontaneous hatch, which was similar in the 2 populations under the conditions described, for all parent treatments. It was significantly lower in eggs produced by chilled parents ($p < 0.001$) (see Fig. 40).

Percentage hatch in all hatching periods tested, was similar in the 2 populations, but it was significantly affected by parent treatments ($p < 0.001$).

(see Appendix 8). At the same time, temperature treatment of the eggs during the hatching experiments (5° or 20° C original hatching temperature), significantly affected percentage hatch in all hatching periods ($p < 0.001$) (see Fig.39).

Egg mortality was not affected by population or parent treatment.

Overall, trends in infectivity, hatching behaviour and egg mortality

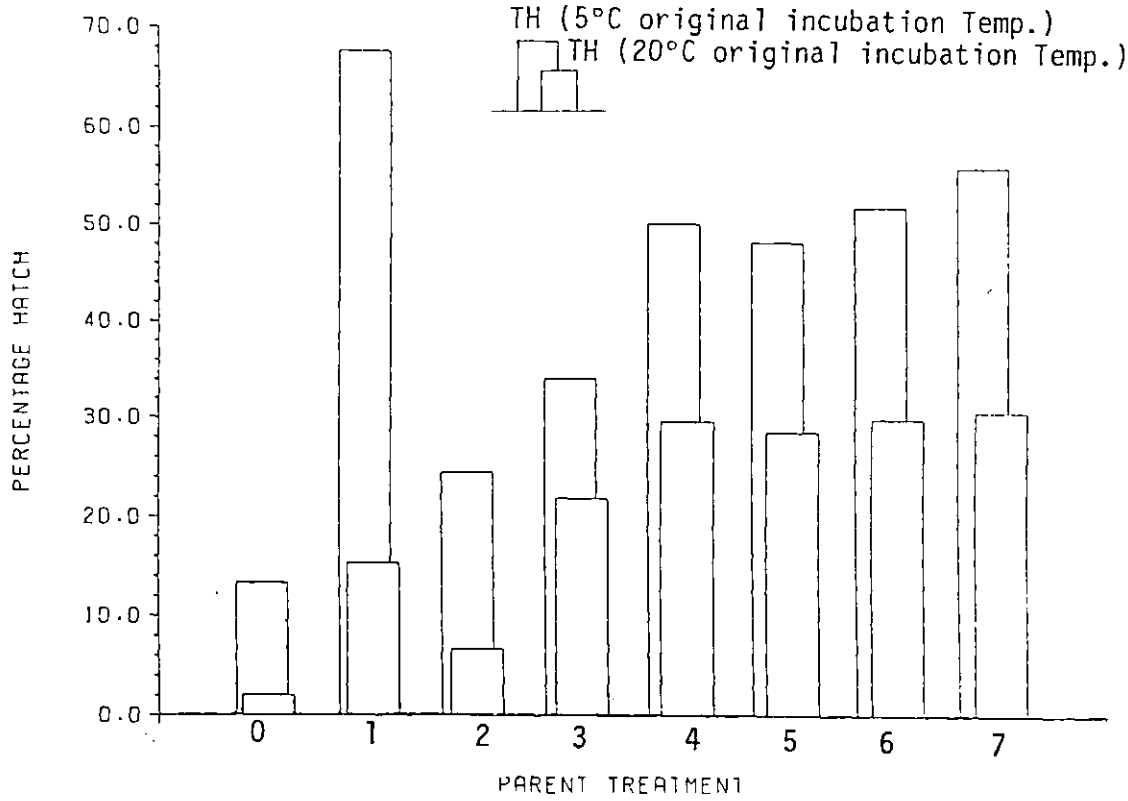


Fig. 39: Total percentage hatch of the progeny of the German and the Welsh populations of *M. naasi* considered together, after 0 to 7 weeks parent chilling treatment (see page 139).

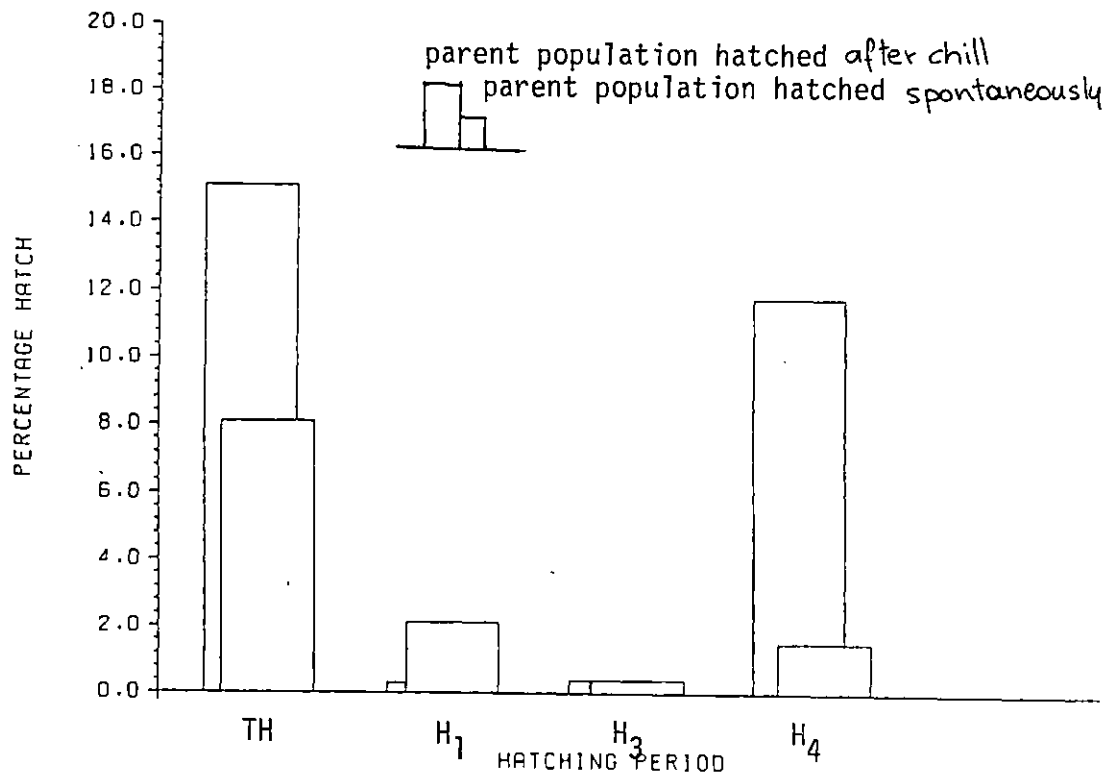


Fig. 40: The hatching behaviour of the German and the Welsh populations of *M. naasi* considered together, whose parent population hatched spontaneously or after chill (up to 7 weeks at 5°).

TH: total % hatch; H1: spontaneous % hatch; H3: % hatch at 20°C after 7 weeks chill at 5°C; H4: % hatch at 20°C after a further 7 weeks chill at 5°C.

of the 2 populations were not statistically different when the cultures originated from using spontaneously hatched J_2, and therefore the 2 populations were considered together in Figs 39 and 40 .

G. "Rest" period required before a second chill is applied

population used: Belgian
pretreatment chilling: 14 weeks at 5° C
generation: F_2
culturing medium: 50% loam, 50% river sand
temperatures used for hatching experiments: 5° C, 20° C

To study how long a "rest" is required (by the eggs that did not hatch after the first long chilling treatment), before a second chilling can be applied effectively, the following procedure was used.

Eggmasses from the Belgian isolate which were stored at 5° C for 14 weeks in glass vials in tap water, were set to hatch individually at 20° C (see section 2.I.C(i)). There were 8 treatments, 6 eggmasses in each. Eggmasses were left at 20° C for 2 weeks (treatment 1) to 16 weeks (treatment 8), each treatment was 2 weeks longer than the previous one. The J_2 hatched were not taken into account. After this period of time at 20° C ("rest"), a second chilling was applied (5° C for 9 weeks), and percentage hatch was studied after the eggmasses were returned to 20° C for 6 weeks (TH). At the end of this period, eggmasses were stained in NBR to estimate the number of live and dead eggs remaining in each eggmass (see section 2.I.D(iii)).

Results

The longer the period of "rest" allowed, up to 16 weeks, before a second chill was applied, the significantly smaller was the percentage of eggs hatched at 20° C and the significantly bigger the egg mortality obtained ($p < 0.001$) (see Fig. 41 ; section 5.C)

The percentage hatch obtained after the 2 weeks "rest" period followed by 9 weeks chill (9.2%), was higher than the percentage hatch obtained by the same population after a single 9 weeks at 5° C, without any previous chill and "rest" (7.5%) (see section 3.A).

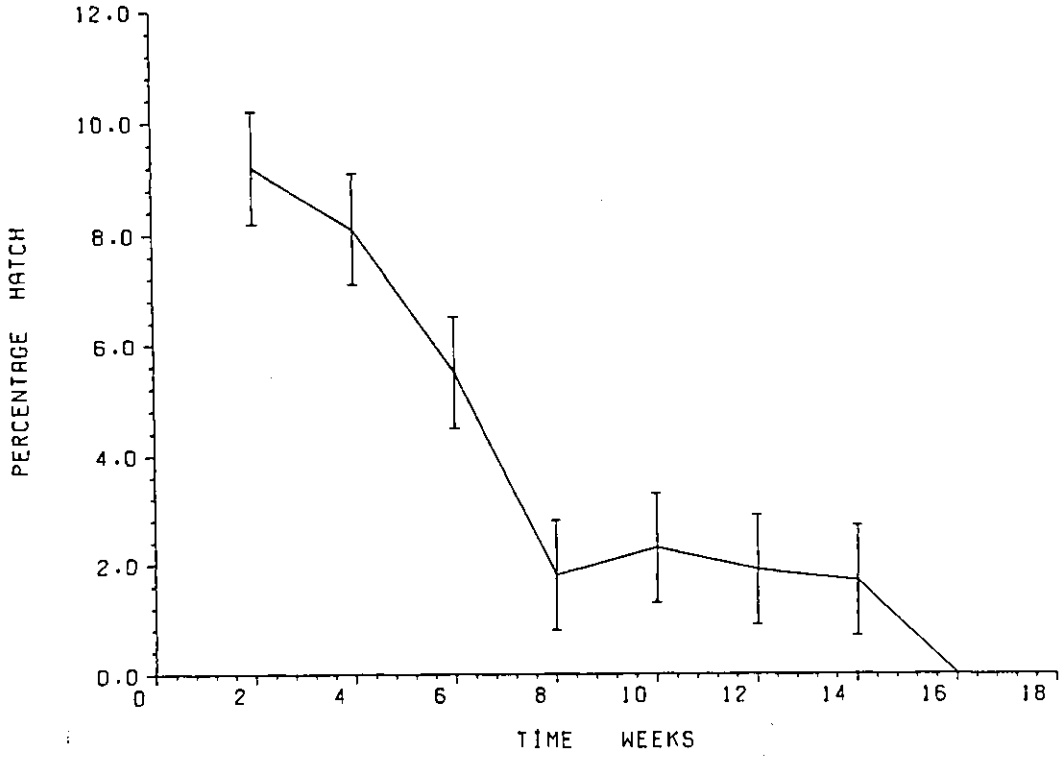


Fig. 41: The effect of the length of the "rest" period allowed (incubation of eggmasses at 20°C), before the application of a second chilling period (9 weeks at 5°C), on the hatchability of the eggs of the Belgian population of *M. naasi*.

H. Infectivity of mechanically hatched J_2

populations used: Belgian, Welsh (2)
pretreatment chilling: none
generation: F_3
culture medium: 100% river sand
temperatures used: 20° C

To study the infectivity of J_2 that hatched mechanically when they were believed to be in diapause, the following experiment was done.

Fourty seven eggmasses from the Welsh population and 30 from the Belgian population (F_3), were set to hatch spontaneously at 20° C as soon as they were collected from host roots, for 2 weeks. The J_2 hatched were discarded.

The eggmasses from each population separately, were then mechanically hatched using a glass homogenizer (see section 2.I.C(i) or Gooris and D'Herde, 1977). The contents of the homogenizer were emptied onto a single layer of kleenex tissue, onto a 90 μ sieve, placed into a 12 cm diameter petri-dish and just covered with tap water.

The 2 dishes (one for each population), were left overnight at 20° C to allow active J_2 to penetrate to the bottom of the petri-dish. Active J_2 were picked using a pipette and inoculated onto one-week old wheat seedlings growing in 100% river sand in plastic petri-dishes (see section 2.I.B(ii)). Two hundred J_2 per plant, 4 plants for each population. The plants were grown as described in section 2.I.B(iii).

After a 12 week growing period, the plants were uprooted (see sections 2.I.B(iv) and (v)) and the eggmasses formed on the roots, counted under the microscope.

The percentage infectivity obtained from mechanically hatched J_2, was compared to that of spontaneously hatched J_2 and that of J_2 hatched after receiving a 7 week at 5° C chilling stimulus (results from experiments described in section 3.A).

Results

Many J_2 hatched and penetrated the kleenex tissue paper, but died in the water 1 to 2 days later. From the active J_2 used for the inoculations, an average of 10.5% produced eggmasses from the Welsh and 12% from the Belgian population. This infectivity was lower than in J_2

hatched normally after receiving chilling treatment or after hatching spontaneously ($p < 0.001$) (see Table 23).

Table 23 : Infectivity of *M. naasi* J_2 after they were hatched mechanically, spontaneously or following chilling.

treatment	population	infectivity %
mechanically	BEL	12.0
	WAL (1)	10.5
spontaneously	BEL	12.2
	WAL (1)	22.7
after chilling 7 weeks at 5°C	BEL	37.3
	WAL (1)	54.9

Discussion

The possibility that egg hatch in *M. naasi* is influenced by biological factors, is suggested by a consideration of its life history, but several factors appear to be most critical.

The effect of the "age" of the eggmass at the time it was removed from the host on the hatching behaviour of *M. naasi* eggs

Eggs in white eggmasses gave higher percentage hatch than eggs in brown eggmasses after chilling. It is possible that the chilling treatment received by the female while still in the egg laying period (white eggmasses) induced her to provide a chemical signal to the eggs which broke diapause as soon as favourable conditions returned.

The experiment did not allow the study of spontaneous hatch in the 2 "types" of eggmasses, since the chilling stimulus had to be applied soon after the removal from the host's roots. It would be interesting to investigate this problem making sure that all early-hatching J_2 are included.

The fact that white eggmasses stored in water turned brown with age, supports Ishibashi's (1969) theory about *M. incognita* eggmass age-colour correlation. If moisture availability alone was the factor determining the colour of the eggmass (de Guiran, 1980), then it would be difficult to explain the fact that brown eggmasses contained a greater number of eggs than white eggmasses, all produced under the same conditions on the same host that the gelatinous matrix colour change with time, and that egg mortality was greater in the white than in the brown eggmasses. Females in the brown eggmasses probably had the time to complete their life span and prepare the matrix for the overwintering period in the soil. It might be possible that a mechanism exists for tanning the gelatinous matrix, similar to that found in the cysts of *G. rostochiensis* (Awan and Hominick, 1982), with the female or the matrix containing the necessary enzymes and substrates concerned.

The effect of the presence of the female in the hatching dish, on the hatching behaviour of her eggs

When the female body was squashed and left in the hatching dish with her eggs which were freed from the gelatinous matrix, the percentage hatch obtained was much higher than in all other treatments. Chemicals

stimulating hatch from the female body or simply hydrolytic enzymes from the disintegrating female body, might have acted on the egg-shell to soften it, or on the J_2 to stimulate it to hatch. Such effects might have also been brought about to a lesser extent by hatched J_2 or their egg-shells.

Ogunfowora and Evans (1977a) found that free eggs and eggs in the gelatinous matrix of *M. naasi*, produced the same percentage hatch, and they suggested that the matrix does not contain a hatching inhibitor as proposed by Gooris and D'Herde (1972).

After chilling, hatch dropped to zero. This might have been caused by the build up in the hatching medium, of inhibitory substances either from the female body, the hatched J_2 or the activity of micro-organisms. Although the hatching system allowed the exchange of oxygen and other gases with the atmosphere (see section 2.I.C(i)), the build up of populations of microorganisms in the water which was not renewed during this experiment, might have starved the eggs of oxygen and induced quiescence due to anoxybiosis (see section 1.C page 26). All those possibilities need further careful investigation.

The infectivity of mechanically hatched J_2

When eggs were hatched mechanically, a lower percentage of the obtained J_2 were infective compared to J_2 that hatched after chill or spontaneously. However, more studies are required to investigate whether the eggmasses each produces are of a similar size and nature, and whether the hatching behaviour of their eggs is similar.

In a similar experiment using the Belgian population of *M. naasi*, Gooris and D'Herde (1977) concluded that, since some mechanically hatched J_2 were infective, then the juveniles were in a quiescent state inside the egg and not in diapause. From their experimental results, approximately 10% infectivity could be inferred from the following calculations. Eggs were hatched mechanically (without allowing J_2 that would have hatched spontaneously to escape), and 40,000 J_2 were used to inoculate plants. From these, 9 weeks later, Gooris and D'Herde obtained 201,875 eggs. If each female produced 50 eggs (9 weeks growing period at 17.5° C), then 4,037.5 females had developed from 40,000 J_2 (10% infectivity). They compared this result to the number of eggs produced when the plants were inoculated using freshly picked embryonated eggs, without a previous chilling treatment. Therefore they compared infectivity of mechanically hatched J_2 to that

of spontaneously hatched J_2, and concluded that percentage infectivity of mechanically hatched J_2 was higher than that of embryonated eggs.

In nature, egg-shells have to be penetrated by the J_2 before hatch can take place, and very seldom are they broken mechanically. The fact that the penetration normally takes place at one particular time of the year only under natural conditions, or after receiving a chilling stimulus in the laboratory, indicates that a special mechanism from within the egg controls the phenomenon, which is therefore a diapause (see section I.C, page 31). Similar records of larval development proceeding after mechanical hatch occurs, is reported in insects. The *Bombyx mori* larva for example is believed to be in diapause inside the egg-shell. When the egg is broken artificially, then the larva resumes its development normally (Lees, 1955).

Banyer and Fisher (1972) found behavioural differences between mechanically hatched and normally hatched J_2 of *H. avenae* . The same authors (1980) reported that low temperature treatment of mechanically hatched J_2 transformed their behaviour into that of normally hatched J_2, indicating that the temperature treatment affected the J_2 and not the egg-shell, to induce hatch. Similar experiments would be useful in *M. naasi* to help us understand the effect of temperature on the hatching behaviour of the eggs.

To summarise, the chilling treatment was found to be necessary before hatch took place in *M. naasi*. Hatching eggs mechanically did not produce a great proportion of J_2 which were infective compared to normal hatching. Also, interrupting the chilling stimulus eliminated its effects.

The other biological factors studied did not appear to influence the hatching behaviour of *M. naasi*:

The hatching behaviour of eggs produced in hydroponics was similar to that of eggs produced in soil cultures, for all the populations tested. Thus, providing favourable conditions for the host and the female, did not eliminate diapause in the eggs produced.

The hatching behaviour of eggs in relation to the stage of embryonic development in the egg receiving the chilling stimulus. At 5° C, egg development was very slow (if any). However, the eggs completed their development within 14 to 21 days when placed at 20° C (see also Siddiqui and Taylor, 1970; Franklin, *et al.*, 1971). It is probable that

egg development was "blocked" (very slow rate) at 5° C and it recovered fully as soon as the eggs were placed at 20° C.

The younger embryos appeared to have completed development faster than the older ones, with the exception of the eggs taken from the uteri which took the longest time. It is possible that in nature, if egg development is blocked due to unfavourable temperatures, the younger the embryo is, the better it is suited for the retardation of development, and the faster it recovers when conditions become favourable. However, if embryonic development had progressed before conditions changed, then perhaps chemicals controlling development, which were produced, had to be inactivated, and produced again when conditions became favourable before development could proceed. The histochemistry of embryogenesis has to be known before any conclusion could be reached.

Chemicals from the female body or/and from growing microorganisms in the plate, might have stimulated hatch in the eggs obtained from the uteri, while lack of them might have inhibited hatch in the other plates.

The hatching behaviour of eggs in relation to the order in which they were laid by the female . Egg hatchability on the agar plates was zero and egg mortality high during this experiment. This might have been due to the presence of 400 ppm Na-dehydroacetate as a fungicide in the physiological salt solution, or the absence of special factors provided only by the host or the *M. naasi* female. Banyer and Fisher (1976) found that egg development in *H. avenae* was achieved only while the female was feeding. Similar reports on *M. incognita* are given by Ishibashi (1969) and de Guiran (1979a, 1980).
This could be possibly tested by adding squashed female bodies to the agar plates, using females which were allowed to develop fully on the host's roots.

To study egg laying by the female under natural conditions, it is necessary to keep the females on the host roots during the process. To do this, it might be necessary to observe the roots still attached to the host's root system, under sterile tap water. Extra care should be taken not to damage the roots, since this would introduce infections and could kill the root and female.

The disposition of eggs to enter diapause through successive generations was demonstrated in that there was a strong tendency for the majority of the eggs to require chilling before hatch. Juveniles which hatched spontaneously gave rise to a mixed progeny (J_2 hatching spontaneously or requiring chilling before hatch).

If the phenomenon of diapause in this nematode was controlled solely genetically, and assuming that asexual genetic mechanisms are operating in this parthenogenetic species, then the fact that spontaneously hatched J_2 did not give rise to 100% spontaneously hatched J_2, implies that the gene/s for spontaneous hatch is/are not recessive. However, it appears to be a complicated process, and whether it is controlled by genes or environment or both, is still difficult to speculate. Rivoal (1978) and El-Shatoury (1978) working with *H. avenae* and *G. rostochiensis* respectively, suggested that the phenomenon of diapause in these nematodes has a hereditary character (see section 1.C page 39). Mass matings of spontaneously hatched J_2 were found to result in sterile matings in *G. rostochiensis* by El-Shatoury (1977).

A new study is required, using fresh material from the field, to see whether cultures reared from only J_2 hatching spontaneously, are possible to set up and for how many generations.

The "rest" period before response to a second chill was shown to further inhibit hatch the longer it continued after 2 weeks.

In other experiments, short (2 weeks) chilling treatments interrupted by short (2 weeks) periods of "rest", resulted in inhibiting egg hatch (see section 3.B) but in this case, a long chilling period (14 weeks) interrupted by only 2 weeks "rest" was the most favourable treatment. It must be considered however, that in this case, the egg-masses had already experienced a long chilling treatment, at the end of which the eggs were allowed to hatch at 20° C. The 2 weeks "rest" period, during which eggs hatched as a result of the chilling stimulus, was not long enough to allow all the eggs ready to hatch to do so. Such eggs hatched after the second chilling treatment. However, eggs which were allowed a longer "rest" period, had more time to hatch and so few were left to hatch after the second chill. This might explain the lower percentage hatch and higher mortality obtained during this experiment, and not the effect of the length of the "rest" period alone. For a more realistic result, the eggs that hatched after the first chilling (14 weeks) during the "rest" period, should have been taken into account, in order to estimate the final percentage hatch.

Another important biological factor which might determine the fate of the J_2 inside the egg and which would be interesting to study in another project, is the rate at which the lipids are metabolised by the J_2, or the amount of lipid present in the egg before hatching.

It is possible to seek correlations between various metabolites in a J_2 inside the egg, and the percentage of J_2 with a disposition to enter diapause, by mechanically hatching J_2 as soon as they complete their embryonic development and determining the levels of metabolites. It is possible that hatch takes place when reserves in the J_2 reach a specific level and spontaneously hatched eggs are those which alredy have these required levels.

It is evident that at least some biological factors play a role in the induction and termination of diapause, in combination with the temperature stimulus. However, introducing artificial factors in the biological system, might have interfered with the normal development and behaviour of the eggs. If such factors are to be considered seriously, a more detailed study is required with the minimum possible amount of interference in the system. Although "it is impossible to maintain a culture of worms without subjecting it to selection pressures." *

In the next chapter, the mortality of the eggs during the various treatments is investigated, to determine whether eggs that failed to hatch after a treatment did so because the treatment was lethal or because it failed to stimulate them to hatch.

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* Michel (1982).

CHAPTER 5

EGG SURVIVAL DURING THE DESCRIBED TREATMENTS

Introduction

At the end of each hatching experiment, the eggmasses were stained in NBR (see section 2.I.D(iii)). The number of dead and live eggs left in each eggmass was counted. Thus the total number of eggs per eggmass was estimated, which consisted of: (i) the total number of eggs hatched, (ii) the total number of remaining as unhatched live eggs, and (iii) the total number of remaining as unhatched dead eggs.

Percentage hatch and percentage dead eggs was thus estimated, based on the total number of eggs per eggmass. Thus, the effect of each treatment on egg mortality (percentage dead eggs at the end of the experiment) was assessed.

Only the treatments which affected egg mortality significantly are presented in this chapter.

A. The effect of incubation at constant temperatures (0° to 40° C) on egg mortality

For experimental details, see section 3.A.

Results

Incubation temperature (0° to 40° C), length of incubation (0 to 20 weeks) and origin of population, affected egg mortality significantly ($p < 0.001$).

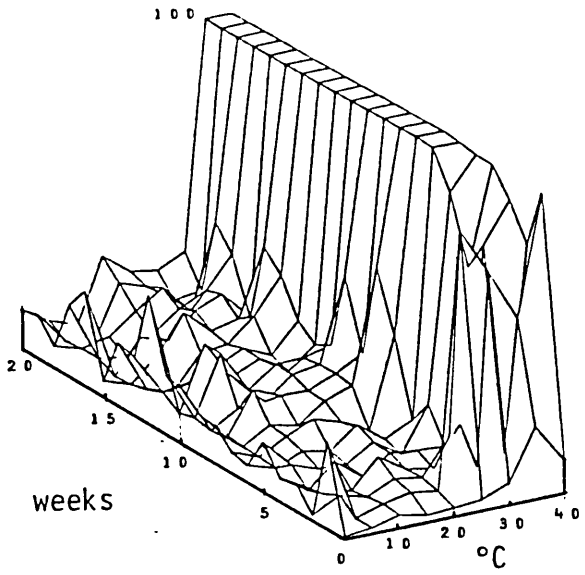
Overall, eggs of *M. naasi* survived best at the lower temperatures tested (0° to 15° C). The shorter the length of incubation, the better the survival obtained (see Fig. 42).

The minimum average percentage of dead eggs over all populations and lengths of incubation was at 5° C (5.2%) and the maximum, at 35° C (95.6%) (see Table 24 ; Appendix 9).

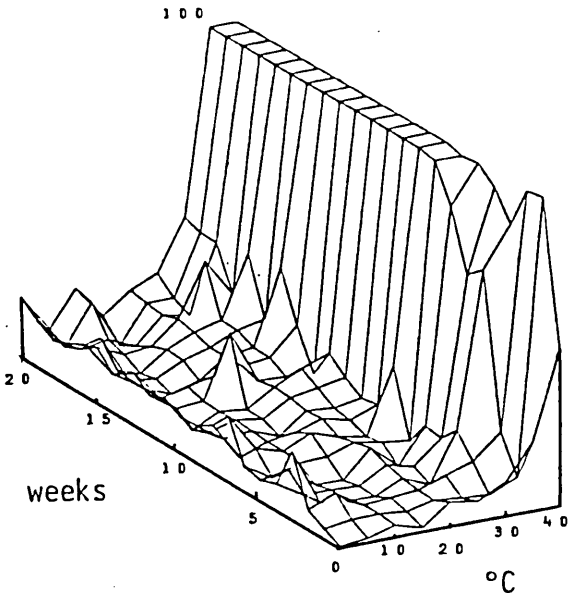
The lowest average egg mortality, when all temperatures and populations*considered together, was at 0 weeks (6.7%). The 0 weeks treatment represents spontaneous hatch at a specific temperature. That

* were

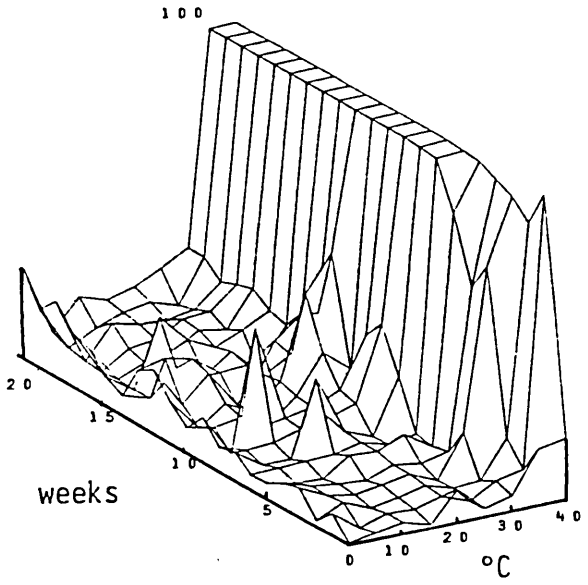
Fig. 42: Egg mortality of the 6 populations of *M. naasi*, after incubation at temperatures between 0° and 40° C for 0 to 20 weeks, shown in perspective view. For this reason, actual values should be taken from the Appendix Table. 9 rather than scaled from the Figures.



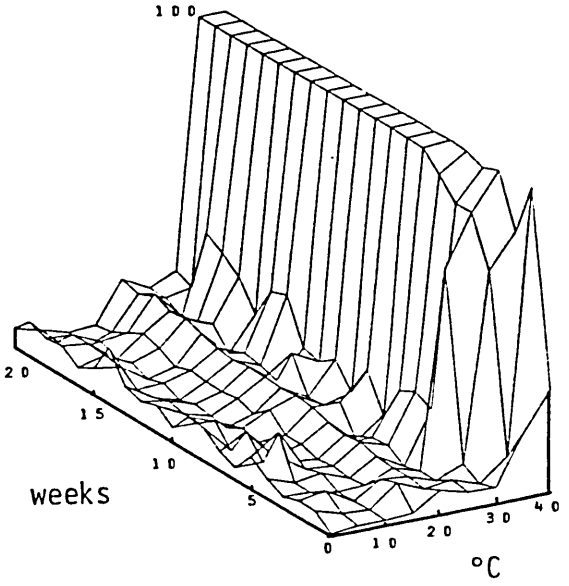
PERCENTAGE DEAD EGGS BELGIAN POPULATION



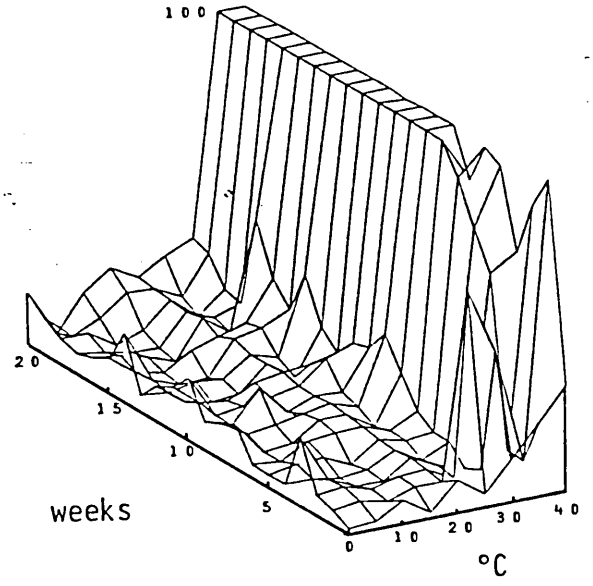
PERCENTAGE DEAD EGGS FRENCH POPULATION



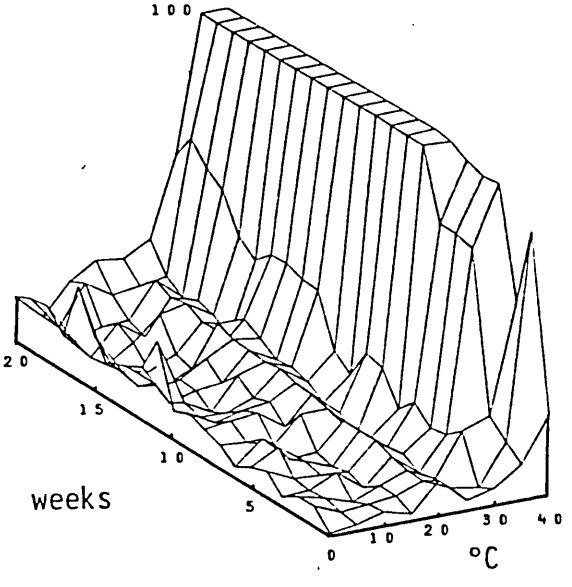
PERCENTAGE DEAD EGGS GERMAN POPULATION



PERCENTAGE DEAD EGGS NEW ZEALAND POPULATION



PERCENTAGE DEAD EGGS CALIFORNIAN POPULATION



PERCENTAGE DEAD EGGS WELSH POPULATION

Table 24 : Average egg mortality at each temperature, over all populations and lengths of incubation.

temp.	0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
% egg mortality	11.3	5.15	7.19	8.92	11.4	13.4	36.5	95.6	91.3

Table 26 : Average egg mortality during each incubation period, over all populations and temperatures.

weeks incubation	0	1	2	3	4	5	6
% egg mortality	6.65	18.6	13.2	19.0	19.5	23.9	26.2

weeks incubation	7	8	9	10	11	12	13
% egg mortality	31.1	33.5	33.4	34.5	36.8	34.6	36.6

weeks incubation	14	15	16	17	18	19	20
% egg mortality	35.0	36.2	37.7	37.0	37.1	36.8	40.0

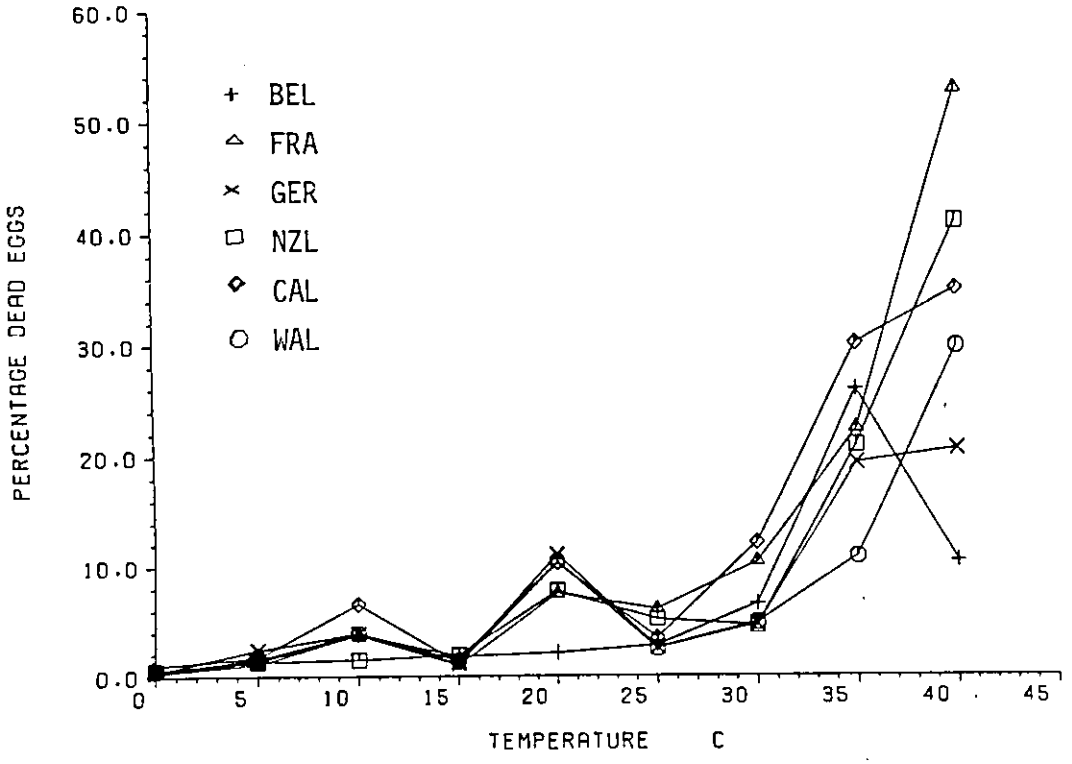


Fig. 43: Mean egg mortality for the 6 populations of *M. naasi*, after incubation at temperatures 0° to 40° C for 4 weeks.

is, the eggmasseswere set to hatch in that temperature for 4 weeks, and then stained, without being transferred to 20° C for hatch after temperature treatment. The highest average egg mortality was obtained after 20 weeks incubation of the eggmasses (40.0%) (see Table 26).

At 0 weeks incubation, egg mortality followed a similar pattern in all populations tested. It was very low at the lowest temperatures and rose steadily with the increase in incubation temperature (see Figs 42 and 43).

The minimum egg mortality was 0.3% for the French population at 0° C, and maximum 52.7% for the same population at 40° C (see Fig. 43).

In all populations, egg mortality reached 100% at 35° and 40° C after incubations longer than 7 weeks (see Appendix 9 ; Fig. 42).

At 30°, 35° and 40° C, many eggs that had failed to hatch but were still alive (did not stain with NBR), were found to be in the gastrula stage. Many dead eggs at those temperatures were also in the gastrula stage. Very few eggs which failed to hatch after incubation at 0° or 10° C were in the gastrula stage (1 to 3%).

B. Effect of incubation at alternating and interrupted temperatures on egg mortality

For experimental details, see sections 3.B and 3.C.

Results

Egg mortality varied significantly between populations and parent treatment ($p < 0.001$). Whether the eggs had experienced constant or alternating temperatures however, did not affect their mortality significantly (see Fig. 44).

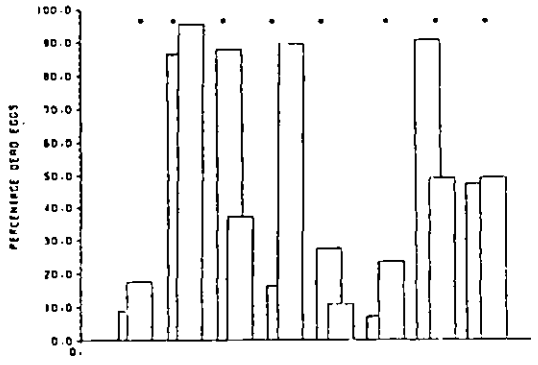
Overall, egg mortality after the alternating temperature treatments was 41.2% and after constant temperatures was 35.1%.

It appeared from the results, that eggmasses which were produced by parents which had hatched spontaneously, gave the maximum egg mortality in both Welsh populations tested (99.4% for Welsh (1) and 98.2% for Welsh (2)). (see Table 27). Eggs whose parents hatched after a short chill at 5° C (1 or 2 weeks), gave a maximum mortality in the 3 other populations (see Fig.44 ; Table 27).

The overall maximum egg mortality was obtained from 2 weeks chilled parents (69.6%). The lowest egg mortality was obtained in eggmasses

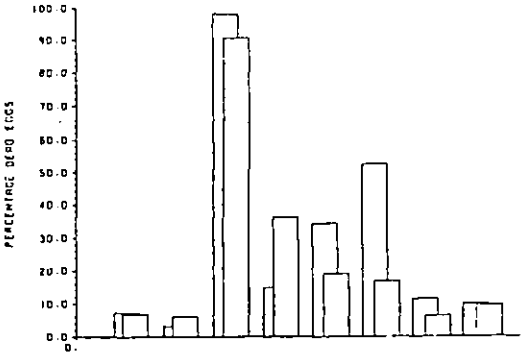
Fig. 44: Egg mortality after constant and alternating temperature treatments for the 5 isolates of *M. naasi*, whose parent population had experienced 0 to 7 weeks chilling before hatch.

PARENT TREATMENT 0 1 2 3 4 5 6 7

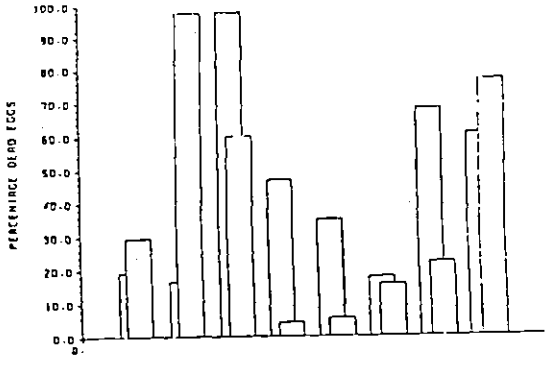


alternating Temp.
constant Temp.

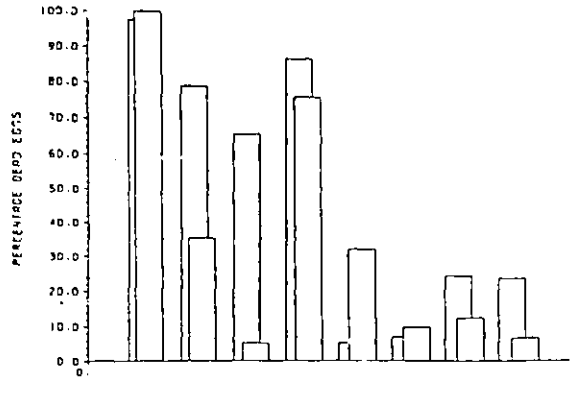
BEL



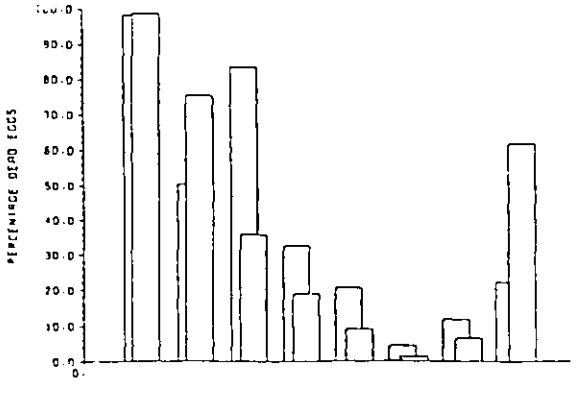
FRA



GER



WAL(1)



WAL(2)

Table 27 : Average egg mortality of each population of *M. naasi* for all parent treatments (see p. 93).

parent treatment weeks	average egg mortality %				
	BEL	FRA	GER	WAL(1)	WAL(2)
0	12.6	7.08	23.8	99.4	98.2
1	91.5	4.49	62.4	58.3	63.4
2	64.9	95.1	84.0	31.2	61.4
3	54.4	24.1	21.3	81.6	25.6
4	18.2	26.1	17.6	1.70	14.5
5	14.1	33.3	16.2	8.47	2.76
6	72.8	8.63	44.1	18.2	9.14
7	48.7	9.70	68.9	14.2	41.8

Table 28 : Average egg mortality after incubation in different concentrations of NaCl, at 20°C and 5°C.
Welsh population of *M. naasi*

temp.	NaCl concentration							
	1 M	250 mM	50 mM	20 mM	10 mM	5 mM	2 mM	tap water
20°C	30.7%	2.2%	2.9%	1.4%	3.4%	1.6%	2.9%	6.1%
5°C	65.6%	4.9%	10.4%	6.9%	14.1%	1.9%	16.4%	18.3%

whose parents experienced 4 weeks chilling for the Welsh (1) population (1.7%), 5 weeks chilling for the Welsh (2) population (2.8%), and 5 weeks chilling for the German population (16.2%). The Belgian and French isolates gave minimum egg mortality when the parent had hatched spontaneously (12.6% and 7.1% respectively) (see Table 27). The overall minimum mortality was obtained from 5-weeks chilled parents (13.4%). For an overall summary of the results, see Appendix 9.

During interrupted temperature treatment, egg mortality was statistically similar for the 5 minutes to 3 hour interruptions. However, the 2 week interruptions gave significantly higher egg mortality ($p < 0.001$).

C. The effect of the length of the "rest" period on egg mortality

For experimental details, see section 4.G.

Results

There was little difference in egg mortality up to the 14 week "rest" period, but for 16 weeks the percentage egg mortality obtained was significantly higher ($p < 0.001$) (see Fig. 45).

D. The effect of osmotic stress on egg mortality

For experimental details, see section 3.D.

Results

Osmotic stress caused by incubation of eggmasses in various NaCl concentrations, significantly affected the egg mortality of the Welsh population ($p < 0.001$). At 5°C, the highest NaCl concentration tested (1 M), resulted in 65.6% mortality, and the lowest concentration tested (0.002 M), resulted in 16.4% egg mortality (see Fig. 46). Tap water gave lower mortality than distilled water (5.9% and 18.3% respectively).

At 20° C, egg mortality was significantly lower than the mortality obtained at 5° C, for all NaCl concentrations ($p < 0.001$). At 20° C, it varied between 1.4% (0.02 M) and 30.7% (1 M). At 5° C, minimum mortality was obtained at 0.005 M NaCl concentration (1.9%), and maximum at 1 M (65.6%) (see Table 28).

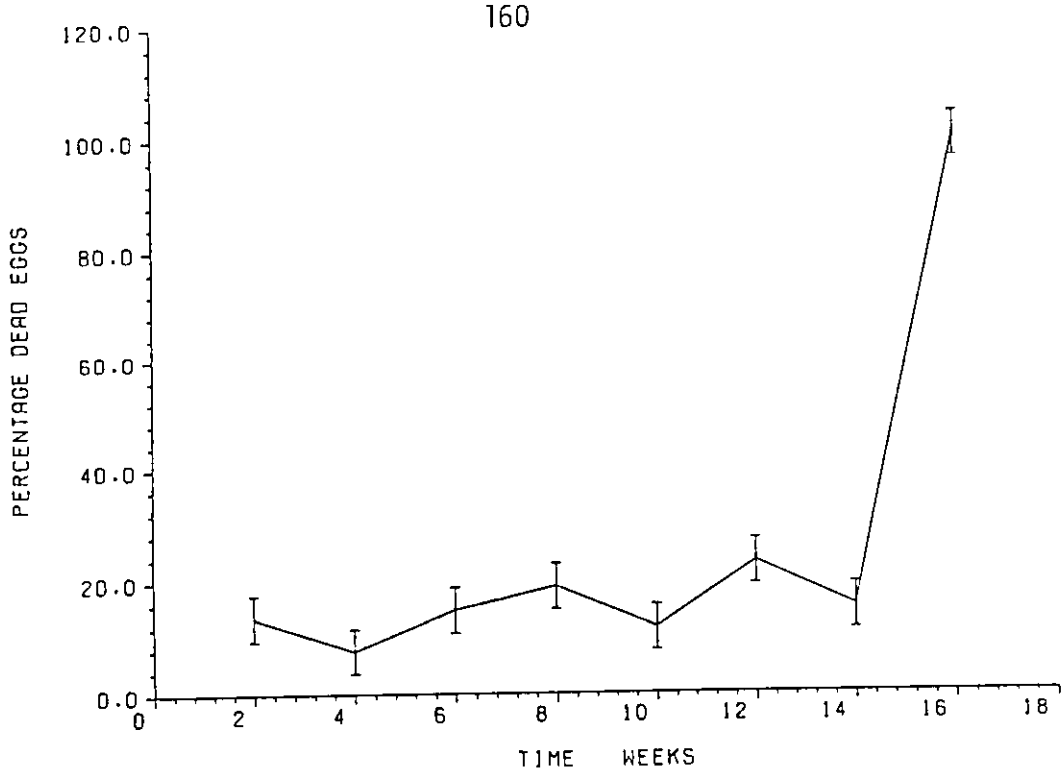


Fig. 45: Egg mortality obtained during 2 consecutive chillings, interrupted by various lengths of "rest" period. (Belgian population of *M. naasi*).

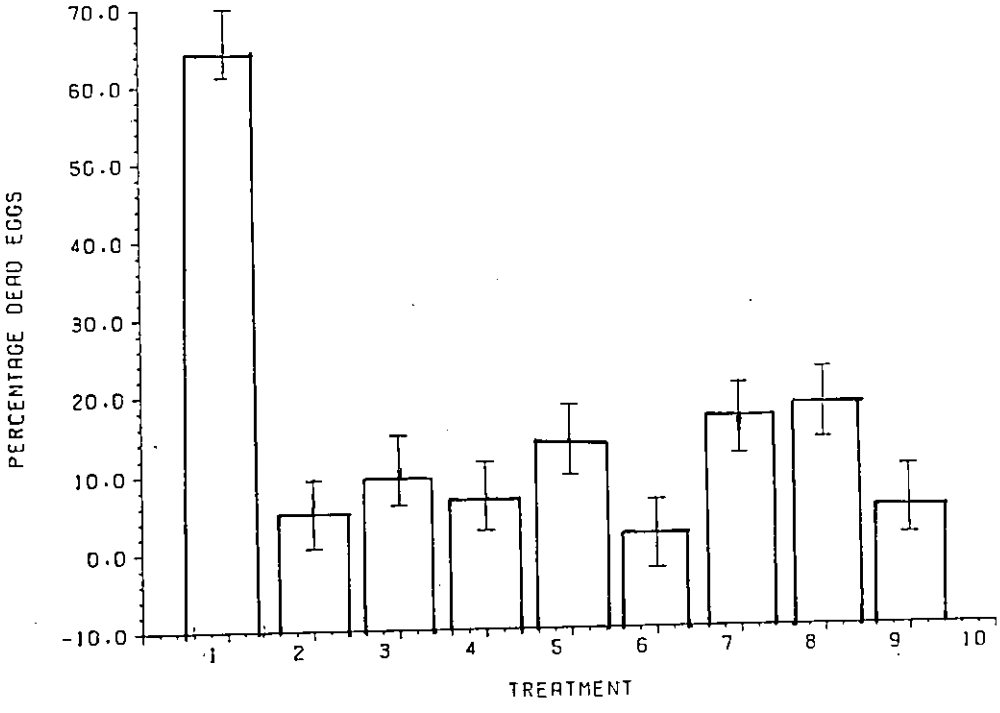


Fig. 46: Egg mortality obtained after incubation in various NaCl concentrations at 5° C. (Welsh population of *M. naasi*). 1: 1 M; 2: 0.25 M; 3: 0.05 M; 4: 0.02 M; 5:0.01 M; 6: 0.005 M; 7:0.002 M; 8: distilled water; 9: tap water.

E. The effect of the "age" of the eggmass during temperature treatments on egg mortality

For experimental details, see section 4.A.

Results

Egg survival during the 4 chilling treatments, was significantly affected by the age of the eggmass at the time it was picked from the host and chilled ($p < 0.001$).

During chilling treatments, eggs from the brown eggmasses appeared to survive better than eggs from the white eggmasses. In brown eggmasses, an average of 4.4% of eggs died during the 4 chilling treatments and 16.2% in white eggmasses. The length of chilling itself, did not affect egg mortality significantly.

F. The effect of various chemicals on egg mortality

For experimental details, see section 2.II.C.

Results

Egg mortality increased considerably when the eggs were incubated in Na-dehydroacetate, either on its own, or in combination with Penicillin or Streptomycin, compared to incubation in tap water, for the French population ($p < 0.001$).

The lowest egg mortality was obtained when eggs were incubated in Penicillin (3.8%). This was lower than the control (tap water, 4.7%) (see Fig. 47).

Incubation in Na-dehydroacetate also caused death in eggs of the German population; 49% of the eggs died in Na-dehydroacetate, and only 14% in tap water.

Xylene, Benzene and Absolute alcohol treatments did not affect egg mortality significantly (see Appendix 11).

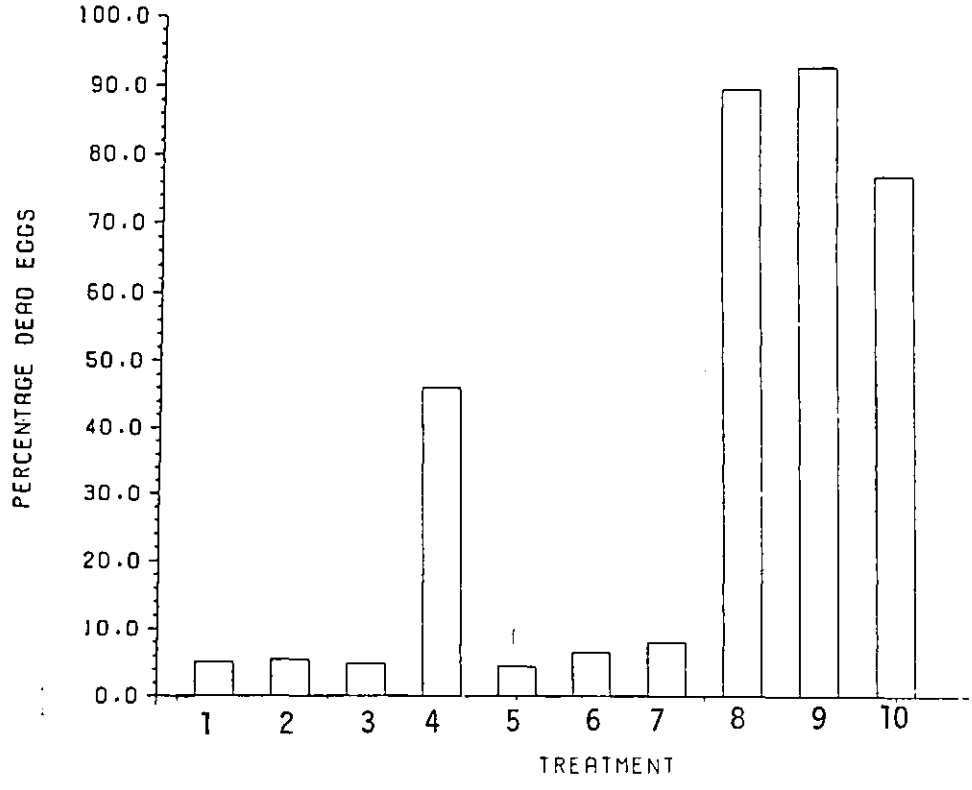


Fig. 47: The effect of various chemicals on egg mortality of the French population of *M. naasi.* 1: distilled water; 2: tap water; 3: sterile tap water; 4: Na-dehydroacetate; 5: Penicillin; 6: Streptomycin; 7:Penicillin with Streptomycin; 8: Penicillin with Na-dehydroacetate; 9: Streptomycin with Na-dehydroacetate; 10: Penicillin with Streptomycin with Na-dehydroacetate.

Discussion

Several treatments caused significant egg mortality in hatching experiments.

Incubation at constant temperatures (0° to 40° C) showed a different survival ability in the different temperatures tested, for each of the 6 populations used in constant temperature incubation experiments. This might be due to the adaptations each population has undergone in its country of origin to suit the particular climatological conditions of its environment.

Overall, 5° C appeared to cause less mortality among eggs from all populations of *M. naasi* tested. Eggs could be stored in this temperature for long periods of time in the laboratory, to be used at a later date. During this work, eggs were stored for longer than one year at 5° C (see section 3.H), and the majority of them were still alive and infective.

M. naasi eggs appeared to be adapted to survive in temperatures below 20° C. High temperatures were not favourable, and above 35° C were lethal.

Embryonation appeared to have been suppressed by the thermal stress. At 30° to 40° C, egg development in many eggs stopped at the gastrula stage. Bird (1974) also found suppression of embryogenesis and hatching in *M. javanica* as a result of thermal stress (46° C for 10 minutes).

Incubation at alternating compared to interrupted and constant temperatures. Whereas incubation of eggs in alternating temperatures produced similar egg mortality (average 41.2%) to that in constant temperatures (average 35.1%), this egg mortality was very high compared to incubation in interrupted chilling temperatures (by periods as long as 3 hours), where 3% to 6.2% died during the treatment (see Appendix 10).

Parent treatment before hatch, was found to be an important factor affecting egg mortality. Eggs that had experienced a long chilling treatment before hatching, produced progeny which survived hardship better than the progeny of parents that hatched spontaneously, or after a short chilling treatment. This suggested that eggs which had a "normal" diapause (ie a length of chilling treatment comparable in length to natural conditions) produced a more hardy progeny than the ones which hatched without experiencing such chilling treatment.

Length of the "rest" period. Overall, the longer the "rest" period allowed before a second chill was given, the higher the egg mortality obtained. However, 16 weeks at 20° C (the longest "rest" period tested), is an abnormally long summer for a temperate region (the Belgian population was used in this experiment). The high mortality obtained during this treatment, might be due to the fact that the population is not adapted for such long summers, but it might also be due to chance. More work is required, using longer "rest" periods to see whether this high mortality is consistent or whether it was due to chance.

Osmotic stress also affected egg mortality. Nematodes in general are known to tolerate fluctuations in the osmotic pressure of their environment (Wright and Newall, 1976), although there is little evidence on how they do so. Incubation in 1 M NaCl concentration, resulted in high egg mortality in the Welsh population, while lower osmotic stress did not significantly affect mortality, compared to that obtained in tap water.

Soil nematodes experience a range of osmotic pressure fluctuations depending on the type of soil and on rainfall. Eggs of plant parasitic nematodes might be expected to be even more tolerant than free stages, due to the long time they have to spend in the soil (especially eggs of *M. naasi* which have to stay in the soil over 2 different seasons), and so the egg-shell might be a limited protective barrier against such hazards.

The "age" of the eggmass during temperature treatments affected egg mortality. Brown eggmasses yielded a lower egg mortality than white eggmasses during chilling treatments. It is possible that many eggs in the white eggmasses, which had not completed their embryonation by the time the eggmasses were chilled, were killed by the treatment, so resulting in higher egg mortality. On the other hand, it is possible that brown eggmasses (older in age; see section 4.A) with their harder covering of gelatinous matrix, offered more protection than white eggmasses. Nevertheless, in the soil, the overwintering eggmasses are brown.

Chemicals like Na-dehydroacetate, should not be used in the storage or hatching medium, for *M. naasi* eggs, since they may be lethal for them. Penicillin could be used in the storage medium, but not if the its
hatching behaviour of the eggs is to be studied, because of interference with egg hatchability.

Thus certain factors can induce a very high egg mortality and may be responsible for variations in numbers of infective juveniles found in the field. If warm conditions in early spring for example were followed by late spring temperature decreases, the potential infective population could be reduced considerably. Information on egg sensitivity to different conditions in the field would be very useful in building population models for this nematode, to know the best time for sowing cereals, to achieve the minimum possible damage.

CHAPTER 6

GENERAL DISCUSSION

Several of the questions posed earlier in this thesis on the nature of dormancy in *M. naasi*, can now be re-evaluated. It is evident from the knowledge regarding *M. naasi* up to date, that this nematode exhibits diapause, as J_2 in the egg stage, when unfavourable low temperatures are experienced. The fact that the great majority of the eggs of this species in all 6 populations studied without exception, do not hatch after full embryonic development is completed (although favourable conditions prevail), unless a specific chilling treatment has been experienced for a limited period of time, agrees with the definition of diapause given in the literature (see section 1.C page 31).

If the eggs were not in diapause but were in a quiescent state, then they would be expected to hatch at any time favourable conditions were experienced, without having to obtain a specific stimulus first. Also, the chilling temperature incubations which this nematode requires before hatching can take place, were found to cause less mortality than any other temperature tested, and did not allow a considerable hatching to take place.

Thus, the nature of the diapause in *M. naasi* can be specified in that environmental temperature is the dominant exogenous factor which brings about the termination of dormancy. Indeed, in the soil medium, temperature could be the most reliable environmental factor indicating seasonal progression, particularly if the organism is not stimulated by host root diffusates to break its dormancy.

All populations tested in the laboratory, showed very low hatch when incubated at constant 0° to 15° C. When they were transferred from those chilling temperatures to 20° C however, high percentage hatches followed, especially after long chilling incubations. The 6 populations showed variability in their temperature and length of incubation requirements, before maximum hatch was obtained, but the general hatching trends were very similar (see Table 29).

All populations studied showed best survival at 5° and 10° C and highest mortality at 40° C.

The difference in their requirements could be a result of their adaptation to the climatic conditions and culturing practices encountered in their country of origin.

The above results agree with the findings of Ogunfowora and Evans (1977a) for the Welsh population, and those of Watson and Lownsbery (1970)

Table 29 : The major features of hatching behaviour of individual isolates of *M. naasi*.

origin of population	optimum temp. for spontan. hatch	maximum spontan. hatch	optimum chilling temp.	optimum chilling period weeks	maximum hatch after chilling	optimum warm temp.	optimum incubation period weeks	maximum hatch after warm temp	time before 100% mortality at 35°C weeks	time before 100% mortality at 40°C weeks
Belgium	20°C	29%	10°C	19	72%	35°C	2	34%	6	4
France	25°C	2%	10°C	17	73%	25°C	16	46%	6	1
Germany	20°C	1%	5°C	13 17	45%	30°C	16	25%	6	5
New Zealand	25°C	13%	10°C	16	89%	30°C	3	20%	6	1
USA California	25°C	9%	10°C	15	80%	25°C	spontan.	9%	6	7
Wales	10°C	3%	10°C	17	73%	30°C	10 18	21%	6	6

optimum: indicates the conditions which resulted in maximum percentage hatch during the experimental conditions tested.

for the Californian population. The different optimum temperature and incubation time for maximum hatch obtained by these workers (5° or 10° C for at least 7 weeks, and 6° to 9° C for 7 weeks respectively), might be due to the different experimental conditions or host used in both cases. Ogunfowora and Evans (1977a) used material collected directly from the field, grown under naturally fluctuating temperature, moisture and light intensity conditions, on barley. Watson and Lownsbery (1970) used material grown for 1 generation in the laboratory, on barley.

In all populations there appeared to be a second temperature regime, not previously suggested by experimental or field observations with a weaker tendency to promote hatch. This tendency appeared after incubation of the eggs in temperatures above 20° C (25° to 35°), and was much more variable in the 6 populations than that obtained after the chilling treatment (see Table 29). This response could be either a new phenomenon appearing with the relatively new cultivations of winter cereals, or a capacity which the nematode had before becoming specialized on cereal hosts, which is perhaps now being selected against.

The 6 populations used in this work, experience one of 2 types of climates: Mediterranean type (California, France, New Zealand), or cool temperate type (Northen Europe; Belgium, Germany, Wales). Temperature conditions are always favourable for plant growth in the Mediterranean climate, but rainfall is the determining factor. In Northern Europe, rainfall is favourable, but temperatures may fall too low to allow plant growth and so temperature is the determining factor for plant growth. In the field, both these factors may be important in determining the termination of dormancy in *M. naasi* eggs.

It would be of interest to explore these isolates' hatching behaviour under the particular climatological conditions they are found in, to reveal the potential that the eggs have for development and synchronization with their hosts.

In nature, temperature fluctuates throughout the winter period. Such fluctuations seem to play an important role in delaying hatch until a substantial amount of chilling is experienced before warm temperatures could effectively induce hatch.

It appears from the results, that *M. naasi* eggs, require a period

of warmth soon after they are laid by the female in order to complete their embryonation. This period should be at least 2 weeks at temperatures above 15° C (see section 4.C; and Siddiqui and Taylor, 1970). Only after embryonation is completed, can a chilling treatment be effective in terminating diapause. The chilling treatment should be exerted for at least 5 to 10 weeks (depending on the population), at temperatures below 15° C, without interruptions by warmth. Hatch can then take place at temperatures above 15° C.

These findings support a hypothesis similar to that proposed by Banyer and Fisher (1971), regarding *H. avenae* egg hatch. They divided the process into 2 phases: (i) juvenile development, and (ii) eclosion. The 2 phases have different temperature requirements, which are independent of each other. In the case of *M. naasi*, 3 phases may be suggested each with different temperature optima, independent of each other: (i) embryonic development (20° C); (ii) chilling requirement (5° to 10° C); (iii) eclosion (20° C). The particular temperature and length of incubation required to complete each phase, may be governed by biochemical pathways which have to be completed at those temperatures.

Evans (1974), while discussing the possible effects of chilling on the termination of diapause in *M. naasi*, suggested that a single enzyme, or systems of enzymes having their temperature optima around 10° C, might be activated in the nematode, due to the chilling treatment. Watson and Lownsbery (1970), also suggested a chemical rather than a structural effect of temperature on terminating diapause in this nematode, and they suggested the inactivation of a hatching inhibitor by the temperature effect. Biochemical work is required before any definite answers could be given to this question.

It was found that chilling or heat stress slowed down or stopped embryonation in this nematode which was nevertheless resumed normally as soon as eggs were placed at 20° C.

Evidence has also been found to suggest that the particular chilling treatment and stress experienced by the parent population, affected the infectivity, hatching behaviour and survival abilities of the produced eggs. This however, could be due to selection during the experiment, of individuals with particular tendencies. De Guiran and Villemin (1978; 1980), in similar work using *M. incognita*, found that the percentage of eggs in diapause increased when stress was experienced by the female. Using the same nematode, Ishibashi (1980) and

de Guiran (1979a; 1980), found that stress due to lack of nutriton in the plant, increased the occurence of eggs in diapause.

It seems possible that the disposition of eggs of *M. naasi* to enter diapause is determined genetically, and stress on the female during different stages in its life cycle may influence the final proportion of the eggs entering diapause. This would be a flexible hereditary system, which is affected by environment. Indeed, in many *Meloidogyne* species, environmental factors, such as temperature and stress, have been shown to play a very important role in the life cycle of the population, by altering its sex ratio (Ellenby, 1954).

Within and between the populations studied, there was variability among eggs in their temperature requirements before hatching. Neither the eggmasses nor the populations were homogenous. Some eggs hatched spontaneously, others required chilling of various lengths, some two consecutive long chillings before hatching. Hatch in some eggs occurred soon after removal from the host, or after the termination of the chilling treatment, while in others it occurred sometime after the conditions became stable. However, the majority of the eggs in each eggmass, of each population tested, required at least 5 to 10 weeks chilling at 5° to 10° C before hatch could occur. Thus, each eggmass appeared to contain eggs requiring a wide range of temperatures and incubation time conditions before terminating their diapause.

On the basis of the present results, one could put forward the hypothesis that the hatching behaviour of eggs in an eggmass, is roughly normally distributed with respect to the length of time they require at the appropriate chilling temperature (5° to 10° C) for each population (see Figs 22 and 48).

More data are required to establish this hypothesis however, especially for chilling periods longer than 20 weeks. Such a study is made more complicated by the fact that the effect of chilling is cumulative, so that after, for example, 20 weeks chill at 5° C, all the eggs that required less than 20 weeks chill would hatch once the temperature was raised to 20° C (delayed hatch) and not only eggs which required exactly 20 weeks chill. The same might be true for egg mortality. One would have to plot the rate of change of hatch against chilling time and not the hatch itself, to test the normal distribution hypothesis. To achieve significant values for the rate of change of hatch, a bigger sample may be required throughout the study.

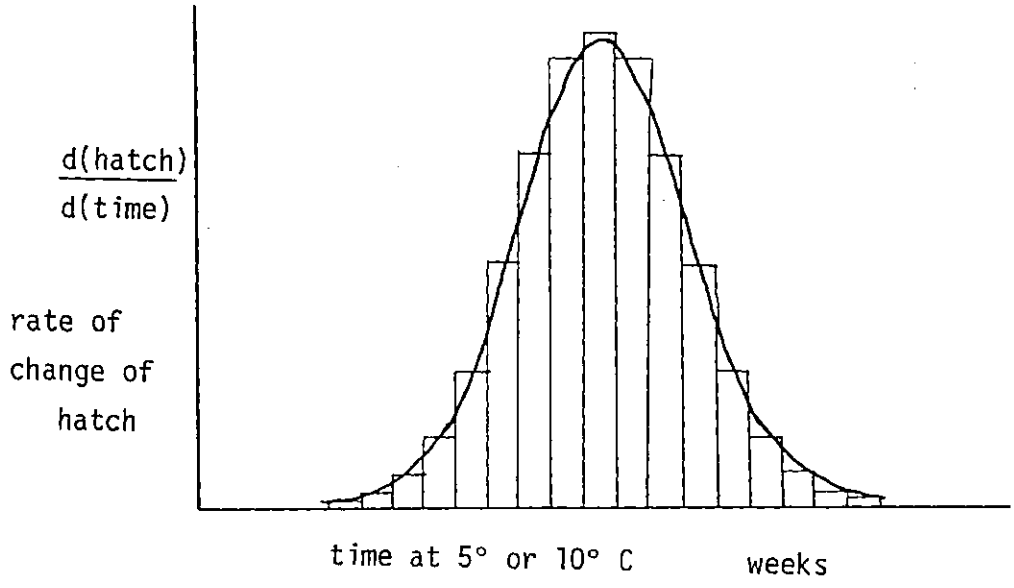


Fig. 48: A possible distribution of eggs of a single eggmass of *M.naasi* regarding their chilling requirements before hatch.

Such variability in the hatching behaviour of the eggs, ensures the presence of some individuals throughout the year in case favourable conditions are encountered, and reserves in case of misfortune; but the bigger proportion of the population occurs at the most favourable time of the year. Thus, some progeny capable of adapting to changing environmental conditions are provided in every generation.

Penicillin-G and Streptomycin were found to stimulate hatch considerably, while Na-dehydroacetate inhibited hatch and was found to be lethal to many eggs. Sodium hypoclorite solution (0.4% by weight) was reported by many workers to stimulate hatch in this and other species of *Meloidogyne* (Siddiqui and Taylor, 1970; Watson and Lownsbery, 1970). However, the effect of these chemicals on the egg system is still not understood. NaOCl has been used to induce hatch in *M. naasi* to obtain inoculum for studies of various kinds.

Other chemicals known to stimulate hatch in other plant parasitic nematodes (see section 1.C, Table 7), as well as insect hormones (Davey and Sommerville ,1974) could be tried on *M. naasi* eggs to study their effect on this nematode's hatching, by considering J_2 movement inside the egg, water content, lipid reserves, secretions and oxygen uptake, to help us understand whether the egg-shell or the J_2 are responsible for controlling hatch in this and maybe other nematodes as well.

Exogenous factors which might influence diapause of *M. naasi* eggs in the field, are summarised in Fig. 49. It is likely that in the soil environment, a combination of factors results in the termination of diapause, and not just the effect of the chilling treatment. However it is not yet possible to evaluate the effect of all the factors acting together under natural conditions, in experimental set ups. In nature, less clearcut changes take place in the soil, and the J_2 might be responding differently to the conditions applied in the laboratory than it would in the field. However, knowing the dominant factor/s, might be sufficient to enable us to interfere with the life cycle of this pest to control it in the field; and it appears that the temperature effect is indeed a necessary and sufficient factor to induce hatch and extraction of J_2 from field soil (Ogunfowora and Evans, 1977; Franklin, *et al* ., 1971) (see Table 30).

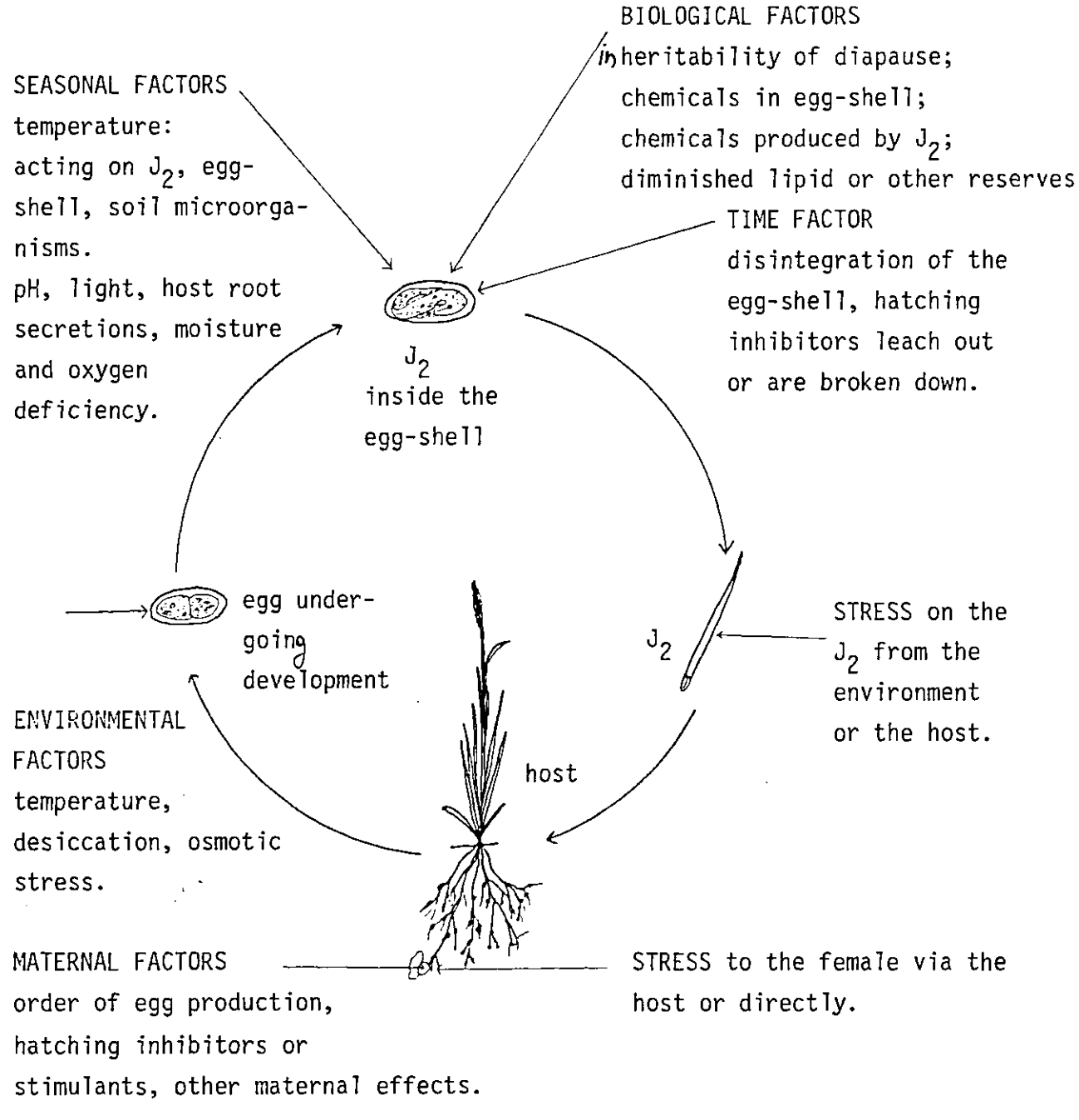


Fig. 49: Factors which might play a role in the induction or termination of diapause in *M. naasi*.

The appearence of new secondary galling, after only 10 weeks of culturing *M. naasi* in hydroponic cultures at 20° C, indicated that the life cycle under such optimal conditions of nutrient and water availability, might take a shorter time than in soil, with more stressful conditions. More detailed studies on this subject might reveal that the completion of the life cycle of *M. naasi* does not depend on temperature alone as it was suggested by Tyler (1933), and Franklin, *et al.*, (1971), but it might also depend on the relationship between the parasite and its host.

The effects of exogenous factors on the hatching behaviour of *M. naasi* have been studied during this work. As a next step however, the biochemical pathways which are involved, and which these factors interfere with, should be studied, to advance our understanding of the process. Only this way can we find ways to interfere in the life cycle of this and other such nematodes, in order to control them in the field and reduce crop losses.

Table 30 : Summary of factors affecting egg hatch in several isolates of *M. naasi*.

factors found to promote hatch.	factors found to inhibit hatch.	factors found to have no effect on hatch.
chilling (0° to 15°C) warm temperature incubations (25° to 15°C). chilling interrupted by short warm periods (up to 30 mins)	alternating cold and warmth chilling interrupted by periods of warmth (over 3 hours)	
Penicillin-G potassium (0.6 g/lt). Streptomycin sulphate (50 ppm)	Sodium dehydroacetate (500 ppm) distilled water	Xylene Benzene Absolute alcohol sterile tap water
squashed female body leaching (?)	eggs kept in sterile conditions away from the female (?)	
"young"eggmasses at time of chilling	rapid desiccation (lethal) osmotic stress stress on female directly or via host inhibits some egg hatch of progeny	eggs produced in hydroponic cultures

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Appendix 1 : The laboratory culturing history of the Belgian population of *M.naasi* during this project.

original soil stored at 5°C on 26- 9-80

F_0			F_1			F_2			F_3			F_4			F_5		
cp	date	exp	uprooted	cp	date	exp	uprooted	cp	date	exp	uprooted	cp	date	exp	uprooted	cp	date	exp	uprooted	date	exp
0	26- 9-80	1	19-12-80	7	6- 2-81	cu	1- 5-81	#													
				14	27- 3-81	cu	19- 6-81	0 to 20	19- 6-81 to 6-11-81	22 23*	11- 9-81 to #29- 1-82	-	8- 1-82	18	3- 4-82	13	3- 7-82	20	12- 9-82	12- 9-82	43#
				-	26-12-80	10	#					-	15- 1-82	21	#						
				-	20-12-80	2,4	#														
1	3-10-80	1	26-12-80	7	13-12-80	cu	8- 5-81	#													
				-	27-12-80	2,4	#														
2	10-10-80	1	2- 1-81	#																	
				-	3- 1-81	2,4	#														
3	17-10-80	1	9- 1-81	7	27- 2-81	cu	22- 5-81	#													
				11	27- 3-81	cu	19- 6-81	0 to 20	19- 6-81 to 6-11-81	22 23*	11- 9-81 to #29- 1-81	-	8- 1-82	18	3- 4-82	13	3- 7-82	20	12- 9-82	12 -9-82	43#
				-	10- 1-81	2,4	#					-	15- 1-81	21	#						
4	24-10-80	1	16- 1-81	#																	
				-	17- 1-81	2,4	#														
5	31-10-80	1	23- 1-81	#																	
				-	24- 1-81	2,4	#														
6	7-11-80	1	30- 1-81	7	20-3-81	cu	19- 6-81	0 to 20	19- 6-81 to 6-11-81	22 23*	11- 9-81 to #29- 1-82	-	8- 1-82	18	3- 4-82	13	3- 7-82	20	12- 9-82	12- 9-82	43#
				10	10- 4-81	cu	3- 7-81	#				-	15- 1-82	21	#						
				-	31- 1-81	2,4	#														
				-	8- 2-82	32	#														
7	14-11-80	1	6- 2-81	7	27- 3-81	cu	19- 6-81	22	20-11-81	19	#										
				-	7- 2-81	2,4	#														
				-	13- 2-81	10	#														

cp: chilling period, measured in weeks.

date: the date the eggmasses were first used for the experiment.

exp: number of the experiment.

uprooted: date the eggmasses were uprooted.

* : these eggmasses were mixed together for experiment 23.

\# : indicates that the cultures were killed at the end of the experiment.

\- : indicates that the eggmasses were chilled between the date they were uprooted and the date they were used.

Appendix 2 : Composition of Nutrient solution.

Phostrogen, of Phostrogen Ltd., Corwen, Clwyd, LL21 0EE.
In solution 1.5 g/lt of tap water.
The mixture contains :
Total Nitrogen (N) 10.0%
Phosphorus pentoxide (P_2O_5) soluble in Neutral Ammonium citrate and water 10.0% (P 4.4%)
Phosphorus pentoxide (P_2O_5) soluble in water 10.0% (P 4.4%)
Potassium oxide (K_2O) soluble in water 27.0% (K 22.4%)
Magnesium (Mg) 1.3%
Iron (Fe) 0.4%
Manganese (Mn) 200 mg/kg

Appendix 3 : Composition of Physiological Salt solution.

Physiological Salt solution (Ishibashi *et al*, 1964)
0.80 g NaCl
0.02 g $NaHCO_3$
0.001 g KCl
0.01 g $CaCl_2$
100 ml sterile water
Sodium dehydroacetate at 400 ppm (fungicide)

Appendix 4: An example of a 2 way analysis of variance of data, using Minitab Computer package at the Imperial College Computer Centre.

```
ANALYSIS OF VARIANCE

DUE TO            DF          SS        MS=SS/DF   F ratio
POP                1       27667.       27667.      2.479    nonsignificant
PATRE              5      351760.       70352.      6.304    significant
POP  * PATRE       5       64058.       12812.      1.148    nonsignificant
ERROR            132     1473162.       11160.
TOTAL            143     1916646.

CELL MEANS
ROWS ARE LEVELS OF POP    COLS ARE LEVELS OF PATRE

                1          2          3          4          5
    1         89.       262.       188.       177.       188.
    2         53.       161.       174.       173.       212.
COL.
MEANS         71.       211.       171.       175.       200.
                                    ROW
                6       MEANS
    1       140.       170.
    2        85.       143.
COL.
MEANS       113.       157.

POOLED ST. DEV. =       106.

INDIVIDUAL 95 PERCENT C. I. FOR LEVEL MEANS OF POP
(BASED ON POOLED STANDARD DEVIATION)
       +---------+---------+---------+---------+---------+---------+
1                          I*********I*********I
2         I*********I*********I
       +---------+---------+---------+---------+---------+---------+
     115.       130.       145.       160.       175.       190.       205.


INDIVIDUAL 95 PERCENT C. I. FOR LEVEL MEANS OF PATRE
(BASED ON POOLED STANDARD DEVIATION)
       +---------+---------+---------+---------+---------+---------+
1            I*******I********I
2                                        I*******I********I
3                                  I*******I********I
4                                  I********I*******I
5                                      I********I*******I
6                        I********I*******I
       +---------+---------+---------+---------+---------+---------+
       0.        50.       100.       150.       200.       250.       300.
```

Error bars are calculated by dividing pooled standard deviation by $\sqrt{\text{of the number of replicates.}}$

Appendix. 5: An example of a 3 way analysis of variance of data, using Genstat Computer package, at the University College Computer Centre.

***** ANALYSIS OF VARIANCE *****

VARIATE: HATCH

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	F ratio
EGGWA STRATUM						
PARTRE	5	11591.5	5.34	2318.4	6.226	*
TEMP	1	28291.7	13.04	28291.7	75.948	*
POP	1	1284.8	0.59	1284.8	3.449	-
PARTRE.TEMP	5	3430.5	1.58	687.0	1.847	-
PARTRE.POP	5	1883.3	0.87	376.7	1.011	-
TEMP.POP	1	130.9	0.06	130.9	0.351	-
PARTRE.TEMP.POP	5	1610.9	0.74	322.2	0.865	-
RESIDUAL	120	44701.5	20.60	372.5	3.195	-
TOTAL	143	92934.3	42.83	649.9	5.575	-
EGGWA.*UNITS* STRATUM						
TREFO	2	57985.1	26.72	28992.6	248.690	*
PARTRE.TREFO	10	7276.3	3.35	727.6	6.241	*
TEMP.TREFO	2	21565.2	9.94	10782.6	92.490	*
POP.TREFO	2	1107.6	0.51	553.8	5.134	-
PARTRE.TEMP.TREFO	10	4512.4	2.08	451.2	3.871	-
PARTRE.POP.TREFO	10	1902.9	0.88	190.3	1.632	*
TEMP.POP.TREFO	2	454.4	0.21	227.2	1.949	-
RESIDUAL	250	29145.3	13.43	116.6		-
TOTAL	288	123039.1	57.17	430.7		-
GRAND TOTAL	431	216973.4	100.00			

GRAND MEAN 17.07
TOTAL NUMBER OF OBSERVATIONS 432

* : significant
- : nonsignificant

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	PARTRE	TEMP	POP	TREFO	PARTRE TEMP	PARTRE POP	TEMP POP
REP	72	216	216	144	36	36	108
SED	3.217	1.857	1.857	1.272	4.549	4.549	2.626

TABLE	PARTRE TREFO	TEMP TREFO	POP TREFO	PARTRE TEMP POP	PARTRE TEMP TREFO	PARTRE POP TREFO	TEMP POP TREFO
REP	24	72	72	18	12	12	36
SED	4.102	2.368	2.368	6.434	5.801	5.801	3.349

EXCEPT WHEN COMPARING MEANS WITH SAME LEVEL(S) OF:

PARTRE	3.117						
TEMP		1.800					
POP			1.800				
PARTRE.TEMP					4.408		
PARTRE.POP						4.408	
TEMP.POP							2.545

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
EGGWA	120	11.143	62.0
EGGWA.*UNITS*	250	10.797	60.1

Error bars= $\frac{SED}{\sqrt{2}}$

Appendix. 6: Detailed data of the hatching behaviour of the 6 isolates of *M. naasi* after incubation at 0° C to 40° C for 0 to 20 weeks.

PERCENTAGE HATCHED POPULATION WEEKS	CHILLED AT 0 C						CHILLED AT 5 C						CHILLED AT 10 C					
	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA
0	0	0	0	0	0	0	.1	.1	0	0	0	.3	.2	2.6	0	.1	0	1.7
1	2.2	.9	.2	0	0	1.4	3.1	.2	.9	.3	.3	2.8	0	0	0	0	0	0
2	.4	.5	.3	0	0	0	15.0	.5	.6	.2	0	4.3	0	0	.2	0	0	0
3	.5	3.6	.3	.2	.2	.3	33.5	.2	.9	.1	.2	.7	2.5	2.5	2.4	.3	.2	.5
4	1.4	1.8	.5	2.1	1.2	.3	12.9	.1	.5	.7	1.2	3.1	7.0	0	2.6	1.2	.3	.5
5	3.5	2.4	1.1	.7	1.4	.4	19.2	6.3	1.4	2.6	1.0	8.5	49.0	0	32.3	1.9	.4	4.7
6	2.2	.4	1.2	8.0	1.1	2.2	4.4	1.0	.4	.4	1.0	8.4	26.2	.2	9.4	.3	.9	2.6
7	6.1	.1	7.3	.7	1.2	.7	12.0	3.7	.8	1.4	1.1	5.3	54.5	5.3	6.4	10.3	2.8	10.6
8	3.7	7.1	.8	.3	.9	.5	44.1	4.1	1.8	6.5	9.7	10.4	70.8	18.7	21.9	51.9	1.8	44.4
9	.6	4.5	.6	.1	.6	.3	55.5	9.4	4.0	7.5	10.4	26.0	58.4	14.4	6.6	8.6	11.3	11.3
10	.5	1.2	.7	.5	.5	.6	31.3	7.2	6.2	18.3	8.9	23.0	67.4	29.3	1.2	4.4	6.3	41.0
11	5.5	1.5	1.1	1.5	1.0	1.6	24.2	20.0	7.3	14.6	8.1	22.2	64.7	6.9	7.6	5.4	.6	16.8
12	5.8	.3	.1	1.2	.7	12.1	22.3	17.3	7.0	19.7	5.3	22.3	57.0	29.2	5.9	14.7	37.1	33.0
13	3.8	.3	.8	1.2	1.0	.3	27.4	11.1	16.0	23.7	45.3	36.5	36.8	34.0	24.8	45.4	3.4	21.7
14	9.8	.8	1.3	13.5	1.4	5.8	45.5	10.1	8.2	22.3	16.1	30.8	84.3	45.7	65.1	7.7	13.2	69.7
15	10.8	4.1	15.9	12.7	4.3	12.3	41.2	10.3	9.3	31.4	7.1	30.5	83.9	46.8	47.1	2.4	17.4	80.1
16	4.5	.9	14.9	6.2	2.9	3.1	39.8	18.8	25.7	29.8	30.0	33.9	89.2	41.6	62.3	17.5	3.8	52.1
17	14.6	2.6	13.1	6.9	2.2	16.8	21.0	33.8	16.9	43.4	45.3	63.1	87.4	60.8	73.1	52.0	3.4	61.7
18	16.7	2.4	11.5	26.5	2.6	19.7	19.9	26.9	32.6	20.5	26.0	43.7	89.0	72.7	71.3	35.6	4.0	62.7
19	21.0	1.3	6.5	11.4	1.2	6.1	46.4	14.5	12.7	6.8	7.1	72.0	86.3	52.5	55.7	72.3	2.7	56.5
20	32.7	.6	2.6	9.3	.6	2.3	44.4	8.7	11.6	13.6	9.5	69.2	64.5	53.2	48.9	67.4	1.1	54.8

Appendix. 6: cont.

PERCENTAGE HATCHED	CHILLED AT 15 C						CHILLED AT 20 C						CHILLED AT 25 C					
POPULATION WEEKS	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA
0	3.0	1.9	0	.1	0	.2	4.0	.7	1.4	29.0	1.0	.4	13.4	2.2	2.3	5.4	.3	8.7
1	.9	1.0	7.4	0	0	0	1.8	1.4	.6	8.6	0	0	2.7	0	0	0	0	0
2	1.1	0	.5	0	0	0	.8	0	0	5.4	0	0	3.3	.2	0	7.7	2.5	1.4
3	2.0	0	.4	.4	.3	.8	.3	0	0	1.8	0	0	2.4	2.8	2.6	2.9	1.1	1.0
4	.7	.7	.3	.9	1.3	.3	.2	.1	0	0	.2	0	5.1	10.1	.4	2.6	.8	.4
5	.3	.3	.5	16.2	2.2	1.0	0	0	0	0	0	0	3.8	5.4	.4	.8	.7	.4
6	.6	.3	1.7	1.8	.9	.5	0	0	.3	0	0	0	4.0	5.3	2.3	5.5	6.8	1.9
7	.2	.3	2.5	8.3	2.9	1.1	0	0	0	0	.2	0	2.2	.8	.2	.2	.6	.4
8	.4	.9	6.2	1.3	2.7	.1	1.8	.1	0	0	.1	0	3.9	3.2	18.1	.9	.5	.2
9	.6	.6	.4	.2	.4	.3	0	.3	0	0	.3	0	3.8	4.0	23.7	1.7	.8	.5
10	2.5	.8	1.3	14.9	.3	1.6	0	0	0	0	0	0	2.3	2.7	38.5	.2	.7	.3
11	4.0	.4	2.7	2.1	7.2	1.7	0	0	.1	0	0	.1	2.2	1.1	25.7	3.4	.1	.3
12	6.3	0	2.4	34.1	3.7	29.2	1.5	0	0	0	0	0	1.5	3.9	19.6	.4	1.0	1.2
13	9.2	.1	9.5	7.5	.5	20.7	0	.7	0	0	0	0	2.4	5.1	22.7	.5	2.9	1.9
14	12.3	.7	11.7	.9	.2	58.0	0	0	0	3.6	0	.3	2.2	4.4	36.1	3.3	1.0	.4
15	48.1	9.8	42.4	2.8	.7	22.5	0	0	.4	0	.6	0	7.6	4.9	21.2	.1	2.0	.6
16	59.5	3.1	34.6	11.8	.6	52.4	.2	0	0	0	0	0	3.4	10.0	46.0	1.1	1.2	2.2
17	2.0	1.0	7.9	1.0	1.3	33.0	0	0	0	0	0	.7	2.4	5.6	17.1	2.8	1.1	2.1
18	36.8	4.4	33.2	7.6	.6	67.0	0	0	0	0	0	0	5.0	3.8	8.2	3.6	1.3	4.7
19	39.0	3.1	53.1	2.0	.6	41.5	.1	0	0	0	0	0	3.8	2.6	4.2	1.3	.4	1.9
20	51.2	1.1	29.6	1.0	.3	18.0	0	0	.1	0	0	0	4.2	.7	1.6	2.5	.2	1.4

Appendix. 6: cont.

PERCENTAGE HATCHED POPULATION WEEKS	CHILLED AT 30 C						CHILLED AT 35 C						CHILLED AT 40 C					
	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA
0	.6	.3	.1	0	0	0	0	.3	0	0	0	0	0	0	0	0	0	0
1	12.5	0	.1	0	0	0	9.9	1.7	1.0	9.3	0	3.0	0	.3	0	0	0	0
2	1.4	1.6	2.2	.6	.7	1.5	8.9	10.0	6.7	33.8	3.5	6.4	0	0	0	0	0	0
3	20.1	3.8	3.9	2.0	1.5	.4	0	6.0	2.0	.5	.3	0	0	0	0	0	0	0
4	1.4	.9	4.8	1.1	1.3	4.1	0	0	0	0	0	0	0	0	0	0	0	0
5	.3	5.2	.4	2.8	1.7	1.7	0	.9	.2	.8	0	0	0	0	0	0	0	0
6	.9	1.4	1.2	2.1	1.1	3.0	0	0	0	0	0	0	0	0	0	0	0	0
7	1.3	3.8	2.1	1.3	1.4	.7	0	0	0	0	0	0	0	0	0	0	0	0
8	3.8	12.5	1.3	6.8	16.9	.4	0	0	0	0	0	0	0	0	0	0	0	0
9	3.9	7.2	.6	2.9	12.8	.1	0	0	0	0	0	0	0	0	0	0	0	0
10	4.0	20.7	1.9	1.6	3.2	4.0	0	0	0	0	0	0	0	0	0	0	0	0
11	5.2	16.1	1.1	5.5	0	4.5	0	0	0	0	0	0	0	0	0	0	0	0
12	1.0	12.8	.6	6.1	9.0	1.0	0	0	0	0	0	0	0	0	0	0	0	0
13	4.0	15.1	4.2	19.9	12.5	.5	0	0	0	0	0	0	0	0	0	0	0	0
14	2.8	6.5	1.9	6.8	20.2	.6	0	0	0	0	0	0	0	0	0	0	0	0
15	4.8	10.4	.2	5.0	23.5	2.6	0	0	0	0	0	0	0	0	0	0	0	0
16	2.3	1.7	0	26.2	24.8	.8	0	0	0	0	0	0	0	0	0	0	0	0
17	2.7	15.8	4.4	28.3	17.8	1.1	0	0	0	0	0	0	0	0	0	0	0	0
18	10.0	20.7	5.6	19.0	17.8	1.5	0	0	0	0	0	0	0	0	0	0	0	0
19	5.5	7.9	.5	12.9	11.0	.6	0	0	0	0	0	0	0	0	0	0	0	0
20	2.4	4.2	.5	6.0	5.7	.3	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 7 : Effect of host age at time of inoculation on the hatching behaviour of the French population of *M. naasi*

age of plants days	at 20°C total hatch %	H_1 %	H_2 %	H_4 %	at 5°C H_4 %
7	39.3	1.1	0.5	23.4	69.4
14	27.9	1.3	0.6	23.7	63.9
50	30.9	1.9	4.0	23.8	54.7
123	15.3	0.7	0	14.0	14.2

Appendix 8 : Percentage hatch during the various hatching periods for the Welsh and German populations of *M. naasi* for 0 to 7 weeks chilling of the parents.

population	chilling weeks	total hatch	H_1 %	H_3 %	H_4 %
WAL(1)	0	4.3	0.4	0.1	2.9
	1	26.2	1.9	0	22.6
	2	3.8	0	1.3	1.6
	3	44.8	0.6	0	42.4
	4	33.8	0.1	0.4	32.7
	7	36.7	0.1	8.2	13.8
GER	0	0.3	0	0	0.3
	1	6.3	0.1	0	6.0
	2	9.5	0.5	0.4	8.1
	3	5.0	0.4	0	3.8
	4	24.8	1.0	0.8	20.9
	7	20.2	0.1	0.7	18.2

Appendix. 9: Detailed data of egg mortality of the 6 isolates pf *M. naasi* after incubation at 0° to 40° C for 0 to 20 weeks.

Percentage dead / Incubation weeks	CHILLED AT 0 C						CHILLED AT 5 C						CHILLED AT 10 C					
	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA
0	.6	.6	.4	.5	.7	1.3	1.4	1.6	1.3	1.4	2.4	2.0	1.5	4.2	3.8	3.8	4.6	7.2
1	5.3	6.5	3.2	29.3	7.9	4.4	2.5	2.7	4.5	1.7	1.1	5.7	1.9	3.6	4.6	4.6	4.0	3.7
2	5.5	6.6	6.4	1.6	2.5	6.3	7.6	4.3	10.2	6.4	6.7	7.4	1.6	1.2	4.5	4.6	2.6	4.8
3	3.6	3.3	22.7	9.6	10.5	22.7	3.4	3.7	4.0	3.6	6.0	6.8	1.8	7.8	2.6	2.9	4.2	2.7
4	6.7	8.6	8.7	8.1	9.2	4.0	3.5	4.6	3.4	2.0	6.1	3.8	5.6	3.6	3.1	9.7	4.8	2.3
5	11.0	6.9	6.6	17.1	6.0	4.0	17.5	11.7	6.4	7.6	4.1	5.7	3.2	5.3	1.7	4.1	3.6	6.1
6	4.1	3.9	8.4	6.4	6.0	4.5	4.3	11.5	2.7	3.5	6.3	3.6	2.6	2.8	5.6	7.1	6.6	8.4
7	6.7	6.0	21.9	7.8	10.6	14.6	11.7	7.1	9.1	4.2	4.4	3.6	1.5	4.5	6.6	9.0	4.2	4.7
8	11.5	10.1	15.6	13.6	9.6	12.4	2.1	11.1	3.1	4.5	6.4	6.6	2.9	12.1	6.6	9.7	7.1	7.6
9	12.1	12.5	12.3	14.2	16.5	9.6	8.9	7.5	6.1	4.5	2.4	6.4	4.6	9.7	7.0	18.5	42.7	17.1
10	5.4	10.1	12.1	19.4	10.2	21.1	5.1	6.3	5.1	3.8	8.2	6.6	1.7	5.9	7.6	9.1	9.5	7.9
11	9.7	30.7	15.6	21.5	15.8	15.1	5.0	7.9	2.6	5.0	5.1	7.9	2.5	3.0	7.3	24.7	11.2	6.7
12	7.9	18.5	13.5	45.6	23.8	12.3	6.3	4.6	7.1	3.7	9.9	5.3	3.4	7.1	7.7	24.9	18.3	3.1
13	10.1	15.7	18.2	21.2	20.5	6.2	4.4	4.2	7.3	7.7	22.5	5.2	1.7	7.6	9.6	6.9	20.4	6.9
14	20.1	13.7	15.7	23.3	9.0	24.0	3.9	3.9	7.4	5.6	6.0	6.4	4.3	2.6	3.7	3.0	14.1	2.9
15	10.0	19.7	23.6	7.4	12.2	12.5	4.6	6.0	7.1	4.1	5.9	5.8	2.5	4.0	5.9	11.9	28.5	3.9
16	7.2	33.6	18.3	34.8	18.7	9.6	9.5	5.1	6.3	3.8	6.5	8.4	5.0	10.8	4.6	14.9	10.1	6.1
17	11.4	14.0	15.5	26.9	21.1	7.8	10.2	4.5	12.4	6.9	5.9	6.4	3.2	14.4	7.9	5.1	11.7	4.6
18	11.0	20.8	13.5	10.0	28.6	9.6	10.4	6.9	8.5	7.0	6.5	9.8	6.1	10.0	10.0	8.9	17.3	3.9
19	13.3	20.1	17.0	18.0	22.9	13.6	5.1	8.6	6.2	6.6	8.2	5.5	1.3	21.5	19.5	3.9	12.8	9.7
20	8.1	17.7	21.6	16.4	33.7	18.6	5.8	9.1	9.3	10.7	10.6	3.7	3.0	19.0	20.9	7.2	20.1	14.9

Appendix. 9: cont.

PERCENTAGE DEAD POPULATION WEEKS	CHILLED AT 15 C						CHILLED AT 20 C						CHILLED AT 25 C					
	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA	NZ	WL	FR	BG	GE	CA
0	2.1	1.6	1.1	1.9	1.2	1.3	7.6	11.0	7.8	2.3	11.0	10.2	5.3	2.6	0.1	3.2	2.7	3.8
1	9.2	4.6	3.4	6.5	3.4	6.7	5.2	7.7	5.3	5.0	5.8	6.5	6.3	3.6	4.2	90.6	2.3	66.4
2	7.2	4.9	4.9	6.1	4.3	7.1	6.3	6.2	5.6	6.6	6.4	7.6	3.0	3.6	8.6	10.4	3.5	2.9
3	2.1	1.1	2.3	12.4	5.5	2.5	6.1	9.5	6.5	6.3	7.3	8.5	3.1	2.4	2.5	4.5	3.1	4.2
4	1.5	3.2	2.7	2.8	4.0	3.2	6.9	11.9	7.8	8.6	7.4	7.9	1.7	5.6	5.5	7.6	5.9	2.8
5	2.3	1.8	1.4	3.8	9.6	2.0	6.7	12.6	10.4	9.2	9.0	8.5	5.8	4.8	2.5	3.2	12.8	6.2
6	1.5	3.3	0.1	5.1	6.9	9.4	8.0	12.3	9.0	12.1	9.1	9.7	5.3	4.8	7.3	5.3	6.4	6.5
7	3.5	12.9	10.8	12.6	29.7	10.7	9.2	13.0	7.8	9.9	10.0	10.7	1.0	2.3	6.0	5.0	6.1	4.8
8	3.6	5.7	7.7	7.5	9.6	9.8	11.1	14.7	10.7	13.8	11.5	11.4	10.9	6.9	8.4	17.1	14.2	8.4
9	9.0	10.9	3.5	14.6	10.3	7.3	12.0	15.5	10.3	14.3	12.0	10.8	6.9	7.1	9.4	16.0	15.8	10.1
10	4.4	9.1	0.1	9.6	5.4	9.3	11.5	17.1	11.4	16.6	12.7	11.0	6.6	11.0	13.0	16.1	25.4	16.3
11	6.5	13.5	6.3	10.4	6.2	11.0	12.1	16.3	12.0	17.1	14.7	12.6	16.4	12.8	16.5	17.2	42.2	13.3
12	5.7	7.9	29.2	11.9	5.9	3.9	12.5	17.3	11.1	15.9	13.8	12.4	5.9	12.1	10.5	9.7	18.7	6.0
13	6.0	13.0	11.2	12.4	13.6	6.0	11.7	17.8	12.9	16.4	14.1	12.4	8.0	16.3	13.9	12.0	20.6	9.5
14	6.8	6.2	0.7	13.8	9.7	3.3	12.4	20.2	12.9	14.7	15.9	14.4	9.1	19.9	14.3	22.4	7.5	16.4
15	5.0	8.3	10.4	24.6	18.8	9.4	14.4	17.7	12.9	16.3	14.3	12.1	5.8	10.6	17.2	18.4	18.0	14.9
16	8.0	18.7	10.9	11.8	12.2	2.8	16.3	20.6	15.1	20.2	17.0	15.3	12.6	15.0	17.5	24.0	15.3	8.9
17	10.0	7.8	9.1	10.8	12.9	4.2	15.6	19.5	16.4	17.8	17.7	14.4	10.2	16.5	33.7	22.1	18.2	10.6
18	7.2	10.4	6.0	16.0	14.9	7.1	18.3	22.0	14.7	22.0	14.3	15.2	9.0	14.7	12.7	12.3	15.8	8.0
19	4.4	15.8	3.0	22.6	15.8	16.3	15.1	16.9	15.6	19.3	17.3	15.7	11.9	16.3	15.3	12.7	14.2	11.3
20	3.5	24.2	10.1	28.5	17.1	21.4	17.1	22.8	16.7	21.3	19.4	16.5	12.2	27.5	20.5	19.6	23.5	21.5

Appendix. 9: cont.

PERCENTAGE DEAD POPULATION	CHILLED AT 30 C						CHILLED AT 35 C						CHILLED AT 40 C					
HOURS	BZ	BL	FR	BG	GE	CA	BZ	BL	FR	BG	GE	CA	BZ	BL	FR	BG	GL	CA
0	4.0	4.8	10.5	6.8	5.0	13.6	21.0	11.0	22.3	23.7	18.9	23.7	33.9	25.7	52.2	10.9	20.6	34.7
1	3.1	1.8	3.4	75.2	6.9	46.5	10.4	15.1	7.5	57.6	6.8	8.2	99.7	84.5	100.0	97.7	100.0	100.0
2	6.6	17.5	11.5	6.5	4.0	3.7	70.1	21.6	19.4	9.2	16.8	6.6	81.3	57.9	99.6	77.6	87.5	88.6
3	69.4	13.3	18.6	17.3	17.4	1.2	86.9	74.3	71.2	76.1	71.5	64.5	99.1	93.9	84.6	89.6	93.2	89.6
4	19.6	12.2	13.0	6.9	7.9	7.0	66.9	76.6	66.5	65.8	61.8	76.3	96.9	93.3	94.1	97.8	96.1	95.2
5	21.9	17.8	13.9	17.9	6.9	7.3	93.5	76.3	86.4	87.4	83.7	90.1	99.1	95.5	99.1	98.2	96.9	98.6
6	19.1	12.5	5.9	9.6	6.0	11.2	99.3	99.3	98.8	99.5	98.8	99.6	100.0	98.8	98.4	99.2	99.3	84.4
7	7.7	23.3	26.2	35.2	24.3	20.9	99.6	99.5	100.0	100.0	99.6	99.9	99.7	100.0	100.0	100.0	100.0	100.0
8	20.0	25.2	11.9	55.3	39.2	30.9	100.0	99.8	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.6
9	9.3	14.8	10.9	23.0	32.5	23.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10	17.5	19.6	14.9	46.3	18.1	21.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.3
11	13.6	37.1	20.0	19.5	53.7	15.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
12	15.1	37.7	9.8	16.7	30.6	20.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
13	29.7	39.8	23.2	21.9	27.6	35.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
14	27.8	39.6	39.4	34.3	20.8	20.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
15	17.1	34.6	19.1	40.7	22.5	27.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
16	26.7	39.3	37.7	14.1	26.7	44.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
17	31.4	51.4	21.4	32.3	25.2	13.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
18	35.1	55.7	35.2	39.6	23.0	15.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
19	13.7	62.5	35.4	14.1	20.3	23.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20	16.1	53.7	37.3	24.0	28.4	25.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Appendix 10 : Percentage egg mortality during interrupted chilling by short periods of warmth.

interruption time, daily	5 min	10 min	15 min	30 min	3 hrs
% mortality	6.2	3.0	4.8	3.4	4.3

Appendix 11 : Mortality of *M. naasi* eggs after different chemical treatments (population WAL(2)).

chemical	tap water	Xylene	Benzene	Absolute alcohol
% mortality	8.4	4.8	4.2	6.7

Appendix 12 : Egg mortality during alternating and constant temperature treatments (see section 3 B).

population	BEL	FRA	GER	WAL(1)	WAL(2)
average % egg mortality A_1+A_2	46.7	24.1	41.6	41.8	38.5

parent treatm. weeks chilling		0	1	2	3	4	5	6	7
average	A_1	56.5	65.2	45.0	43.3	14.1	11.9	17.2	38.2
percent	A_2	49.2	45.0	89.4	38.9	23.2	15.0	40.5	31.6
egg	A_1+A_2	52.9	55.2	69.7	41.1	18.5	13.4	28.1	34.9
mort/ty	BEL A+B	-	47.4	-	24.1	18.4	14.2	42.2	26.7