INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON THE DEVELOPMENT OF THE TICK *Haemaphysalis longicornis* NEUMANN (ACARINA:IXODIDAE) WITH PARTICULAR REFERENCE TO ITS COLONISATION IN THE LABORATORY

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ABSTRACT

This project presented the principal biological records and findings obtained from various experiments and observations which had been designed for the accumulative influences of tick-population dynamics on the feeding performance, and for the particular influences of ambient temperature and relative humidity under controlled conditions on the development of the Weonseong strain of the tick *Haemaphysalis longicornis* Neumann 1901, which is of bisexual race originating from cattle in the central part of the Korean Peninsula. It also described techniques for colonisation and maintenance of the stock-line in the laboratory. Quantitative data on the several developmental processes, such as, feeding and growth, oviposition, egg incubation and hatchability, metamorphoses in each stage, and reproductive pattern, were given in this detailed report.

The one or two-year life-cycle in nature was possibly reduced to a minimum of two months in the laboratory. Reducing the generation interval was the major benefit of the colonisation. Also it provided a great quantity of specimens of known history and homogeneous quality at any developmental stage.

High population density of the tick caused the mean feeding periods of each instar to be delayed. But there was no significant difference for the mean engorged weights within the treatment groups of different tick-population.

Temperature was the major factor controlling the duration of the developmental processes. The developmental velocity was accelerated by raising the temperature and retarded by lowering it. However, humidity influenced the successful hatching rates and metamorphoses of the immature instars. It had also been noted that the developmental velocity in the nymph-to-female metamorhposis was lower than that in the nymph-to-male metamorphosis.

There was a very highly significant correlation between the oviposition potential and the engorged weight of the female tick regardless of the effects of temperature or humidity. Females fed without males showed imperfect body-weight increase and poor oviposition ability and no larva emerged from the eggs laid by the single females. No sporadic parthenogenesis was observed.

CHAPTER ONE : INTRODUCTION

1.1 GENERAL CONSIDERATION

FACTORS CONTROLLING ECOLOGY AND PHYSIOLOGY OF THE TICK;

In general the life histories of ticks consist of the feeding periods on the host animal, developmental periods at each stage and fasting periods between feedings. Therefore, with the exception of one-host type ticks, most of the life histories of ticks is spent between blood meals, with only a small fraction occurring while the parasite is actually feeding and living on the host. Hoogstraal (1956)'s mention of the requirement of two or three hosts, often with divergent habitats, limits the tick distribution to certain faunal areas. It should be noted that the ecological properties, such as life-cycles, distribution, population dynamics, development and survival of ticks in the natural environment, are closely concerned with the physiological significance of the ticks, and *vice-versa*.

According to many references available up to date, the factors controlling the ecological properties of the tick like almost all economic arthropods having an epidemiological and/or epizootological vector role are summarised as follows:

A. Biological Factors:

a. Somatic physiological status for individual growth, metamorphosis and reproduction,

b. Heredity and evolutionary characteristics including behaviour,

c. Morphological and anatomical significance of the tick,

d. Vegetation and other biological environmental habitats,

e. Availability of suitable hosts, host specificity and immune response of hosts to tick parasitism, and

f. Relationship with predators and role as a reservoir or a vector of disease agents in animal society.

B. Physical Factors:

a. Ambient temperatre - Hot and cold air temperature including soil temperature and its annual average and daily/seasonal variations,

b. Relative humidity - Amount and distribution of precipitation throughout the year, the duration of intervening dry periods, and the moisture content in the microhabitats,

c. Insolation and photoperiod - Intensity and duration of sunlight, the significance of light-dark cycle,

d. Soil status - Topography and the physio-chemical properties of the soil including the residue of pesticides used,

e. Air movement - Velocity, direction and frequency of the wind, and

f. Altitude - Height above sea level, and the true density of the air for each altitudinal increment.

Numerous studies both in laboratory and in field have been conducted in order to understand the relationship between the two factors, biological and physical, in several species of ticks, and it has been suggested that ticks would be regulated to a great extent by such factors as ambient temperature and relative humidity including

soil moisture (Bodenheimer 1934, MacLeod 1934, 1935a and 1935b, Lees 1946, Feldman-Muhsam 1947 and 1949, Milne 1950a and 1950b, Arthur 1951, Kitaoka and Yajima 1958c, Sonenshine and Levy 1971, Sonenshine and Ziv 1971, Semtner, Barker and Hair 1971, and Newson and Punyua 1978). Therefore, knowledge of the effects or counter-effects of these two parameters should be of value in trying to establish the laboratory colonisation technique of ticks for quantity production and to assess the ecological and physiological properties and the relevance to the epidemiological and or epizootological vector role of ticks. A master plan for the eradication of ticks and tick-borne diseases should be based on such principal information.

Ambient temperature means all thermal fluctuations which are closely related to relative humidity both in nature and in micro-habitats. Campbell (1946) reported that the prevalence of abnormalities in *Ixodes ricinus* larval ticks was temperature dependent. Observing the life history of the ticks under field conditions and under controlled laboratory conditions, there was evidence indicating that temperature was the principal factor determining the duration of the developmental phases and the rate of development and that the temperature relations in developing ticks varied according to the previous temperature history with regard to a process of pre-conditioning phenomenon (Campbell 1948). With increased temperature the oviposition periods of *Hyalomma anatolicum anatolicum* were decreased (Snow and Arthur 1966), and in *Rhipicephalus appendiculatus* the rate of development at all stages was accelerated by raising the temperature and retarded by lowering it (Branagan 1973a). The evidence collected by Hoogstraal (1956) and Arthur (1961) indicated the probability that the rate of development of any other ixodid ticks would be governed

by air temperature. Concerning the effect of temperature, diapause, which is an alternative event for survival of individuals under the unfavourable environmental conditions should be carefully considered as an additional factor which may cause certain unexpected bias in biological experiments.

There are some experiments shown that relative humidity had no or little significance upon the development of certain species of ixodid ticks, whereas there are some contrasting reports on the influence of relative humidity. It, therefore, appears worthwhile to study this problem further.

In relation to relative humidity, water balance and water vapour exchange kinetics are the most interesting fields for understanding the ecological significance of ticks and for assessing the effect of saturation deficits which are the predominant factor in microclimate circumstances. The activity of various arthropods to absorb water from saturated or even from unsaturated air has been extensively studied. Mellanby (1935) suggested that water absorption and loss in *Ornithodoros moubata* occurred through the tracheal system, and then Hefnawy (1970) confirmed that in *Hyalomma dromedarii* and *O. savingnyi*. However, these could not solve satisfactorily the problem in the larval stage, because of the absence of the spiracles in the ixodid larva. Another historical theory of water balance mechanism in ticks comes from the work of Lees (1946 and 1947) who suggested that the excess fluid of the blood-meal might be excreted by evaporation from the integument, and later, several reports have shown that certain arthropods have the ability to absorb water from atmospheres of high relative humidity through the integument (Browning 1954, Wharton and Kanungo 1962, Beament 1964, Knülle 1966 and 1967,

Hafez, El-Ziady and Hefnawy 1970a, Sauer and Hair 1971, Bassal and Hefnawy 1972, Hair, Sauer and Durham 1975, and Osman 1975).

It has also been reported that certain species are able to convert dry weight reserves to metabolic water to replace water lost via transpiration during desiccation (Edney 1966, and Noble-Nesbitt 1969). The implications of these phenomena are that arthropods have a well developed pumping system in the integument for maintaining water balance under unfavourable conditions. On the other hand, Gregson (1957) and McMullen, Sauer and Burton (1976) have proposed that the salivary glands may function in osmoregulation instead of the integument. Kaufman and Phillips (1973a, 1973b and 1973c) have confirmed the relative importance of the salivary glands and anus in the adult female of *Dermacentor andersoni* and Shih, Sauer, Eikenbary and Hair (1973) have described the effects of desiccation and rehydration on the water content, hemolymph volume, osmorality and concentrations of Na, K, Mg, and Ca in the hemolymph of *Amblyomma americanum*. In the recent review on vapour exchange kinetics in insects and acarines, Wharton and Richards (1978) have indicated that the earlier emphasis on the integument as the site of active uptake of vapour was erroneous. Therefore, it became apparent that the best understood vapour uptake mechanisms involves formation of hygroscopic materials, absorption of water and solutes by the gut, and recycling of the solutes by concentrating them in the hygroscopic material. Schuntner and Tatchell (1970) have reported the direct uptake of water - drinking-in *Boophilus microplus* larvae, and Wharton and Richards (1978) have explained that low humidities can be overcome by ingestion of moist food in insects and acarines.

In addition to the effects of temperature and humidity, the existence of rhythms concerned with photoperiod has been recognised in the development and activity of certain arthropods, and light-dark cycle has been proved to be an important phase setter which controls the ecological and physiological significance especially in terrestrial arthropods in the changing environment where daily and seasonal climatic changes accompanied by the various fluctuations of temperature and humidity may occur. Since rhythmic responses of the basis of sensory physiology in ticks have been noted by several workers (Lees 1948, Lees and Milne 1951, Wilkinson 1953, and Dethier 1957), photoperiodism as an environmental stimulus has been demonstrated to be an important clue for the understanding of drop-off rhythm (Kitaoka 1962, Hadani and Rechav 1969, Amin 1970, and George 1971), activity and behaviour (Atwood and Sonenshine 1967, and Howell 1976), and the mechanism of induction and termination of tick diapause (Wright 1969a). It has been also proved that the ovipositional rhythm of ticks is influenced by photoperiods. Snow and Arthur (1966) have reported that in *Hyalomma a. anatolicum* oviposition proceeds more rapidly in constant darkness than in continuous light. Also, ovipositing females of *Anocentor nitens* were shown to be sensitive to photoperiod, in that their peak of daily oviposition was significantly affected by different photoperiodical regimens (Wright 1969b). Also the analysis of variance showed a significant difference between photoperiods in the daily oviposition of *Amblyomma maculatum* females (Wright 1971). Fujisaki, Kitaoka and Morii (1973) have reported that the ovipositional rhythm in *Haemaphysalis longicornis* and *Ixodes persulcatus* was affected by photoperiods between treatment groups but no ovipositional rhythm was observed in continuous light or dark conditions in both species of ticks.

LABORATORY COLONISATION AND ITS IMPORTANCE;

Laboratory tick colonisation implies the quantity production of the ticks by rearing and maintenance of free-living stages in the laboratory. And it has been recognised as the first essential step to the study of the biology of the ticks and any other related applied sciences, such as, toxicology and pesticides, epidemiology/epizootology of tick-borne disease, and so forth. Numerous techniques have been experimented and used.

The merit and importance of laboratory colonisation and the material colonised in the laboratory could be itemised and described as follows:

A. Modulation of Generation Interval - In ixodid ticks there are three parasitic stages in their life-cycle, i.e. the larval, nymphal, and adult stage. The life-cycle type is divided into one-, two-, and three-host type according to the number of hosts required for feeding. Therefore, most of the life-span of ticks is spent on waiting for the host in field. Even when they met the host animal easily and engorged sufficiently, a long-delayed developmental period for the fed instar is still expected. With some exceptions, therefore, the life-cycle of most ixodid ticks in nature requires one or two years. But, under laboratory conditions it can be reduced to a minimum, and this means time-economy. On the other hand, artifical delay of the generation interval is, conversely, also possible by means of temperature control in the laboratory. It is convenient to the time schedule of the experimental work.

B. Sufficient Quantity of Specimens - Needless to say, most of biological experiments require large number of specimens at the same time. For the analysis of variances it is extremely important to provide sufficient number of samples or replicates. The purpose of statistical analysis is to extract from the sample worthwhile data about the population, and as the number of replications increases, estimates of population means (the observed treatment means) become more precise. Sample errors may be reduced by increasing the number of replications and increasing the size of counts. On the other hand, mass production of ticks is greatly needed in the researches of tick-borne diseases, in which it is critical for the transmission experiments and maintenance of the pathogens.

C. High Quality Specimens - Laboratory material provides not only good clean specimens but also a good series of known history. It is essential for an assessment of the morphological variations which may occur in deciding the taxonomic significance for identification of the tick. We cannot expect to conduct the precise and complex research involved in studies of the various physiological processes in ticks unless the ticks under study can be colonised and investigated in the controlled laboratory conditions because field-collected ticks are too various in age, nutritional condition, general health, experience of pesticides and history of pre-conditioning to environmental factors, to be employed for critical physiological studies. In addition, we can avoid or reduce bias by the use of the same brood specimens presenting higher homogeneity than field specimens.

D. Easy Maintenance of the Stock-line - Maintenance of the line is necessary to achieve several types of serial experiments, such as comparative physiology, resistance to pesticides, formation of immunity and genetical researches. Maintenance in the laboratory is much easier than in the field, indeed, the trial in the field to maintain the stock-line is impossible or laborious. In the laboratory, the free-living stage of the tick can be expanded effectively, safely and easily for a long time by the means of the temperature and relative humidity control operations. On the other hand, the use of small inexpensive laboratory animals instead of large-sized domestic animals, may be an additional benefit from an economic point of view. Although artifical feeding of the argasid ticks has been accomplished by placing ticks on an artifical membrane over blood food at appropriate temperature condition (Tarshis 1958), it has not yet been practicable in the colonisation of the slow-feeding ixodid ticks. Recently, Howarth and Hokama (1978) have indicated that there are four necessary factors as stimuli in the artificial feeding of argasid ticks; i.e. hair on the feeding surface, acceptable material used as a substitute for skin, the temperature of the feeding surface and food, and the substance offered as food. It seems possible to feed certain ixodid tick species in this manner at certain stages. Then it will be the greatest advantage in laboratory tick colonisation.

1.2 ON THE TICK *Haemaphysalis longicornis* NEUMANN 1901

CLASSIFICATION, IDENTIFICATION AND GEOGRAPHICAL DISTRIBUTION;

The tick species *Haemaphysalis longicornis* used in this project belongs to the subgenus *Kaiseriana*, and one of *H. (K.) bispinosa* group of Asian haemaphysalids (Hoogstraal, Roberts, Kohls and Tipton

1968, Hoogstraal, Dhanda and Bhat 1970, Hoogstraal and Yamaguti 1970, and Saito and Hoogstraal 1972). The devices of taxonomy have always been subject to individual opinions and the International Code of Zoological Nomenclature allows complete freedom of choice of the family names. With these reservations in mind, the classification of the tick species is arranged as follows:

Phylum	ARTHROPODA
Class	ARACHNIDA
Order	ACARINA or ACARI
Suborder	IXODIDES (Leach, 1815)
Superfamily	IXODOIDEA (Banks, 1894)
Family	IXODIDAE (Murray, 1877)
Subfamily	AMBLYOMMINAE (Neumann, 1907)
Genus	*Haemaphysalis* (Koch, 1844)
Subgenus	*H. Kaiseriana* (Santos Dias, 1958)
Species	*H.(K.) longicornis* (Neumann 1901)

Morphologically, this species is very close to *H. bispinosa* Neumann 1897, which is a tropical species occurring in southeast Asia. Therefore, for many years it has been incorrectly referred to as *H. bispinosa* in the nomenclature of the species in Australia, New Zealand, Japan, Korea and the U.S.S.R. Meanwhile, the name of *H. neumanni* Dönitz 1905, has also been used by Nikol'sky and Meshcheryakova (1964) and Belikova and Somov (1967) for the Russian species and by Hoogstraal and Trapido (1966) for the Japanese one. However, Hoogstraal *et al* (1968) have reviewed the references and proved that the taxon *H. bispinosa* used for the temperate parthenogenetic species in Australia, New

Zealand, New Caledonia, Fiji, northern Japan, and the U.S.S.R. should be replaced by *H. longicornis* and that the taxon *H. neumanni* is a synonym of *H. longicornis*. Also they suggested that the bisexual population of the spcecies in southern Japan, Korea, and the U.S.S.R. should be tentatively referred to the taxon *H. longicornis*. Oliver, Tanaka and Sawada (1973 and 1974) have mentioned that there should be further cytogenetical experiments and discussions on the taxonomical status of various geographical races of the species.

Roberts (1963 as *H. bispinosa*, and 1970) has described the detail of the species and presented a key for the identification of the Australian haemaphysalids, while Yamaguti, Tipton, Keegan and Toshioka (1971) have reviewed the ticks in Japan and Korea. This species may be distinguished from *H. bispinosa* on the basis of anatomical, distributional, and biological significances;

	H. logicornis	*H. bispinosa*
Hypostome dentition	5/5 in adults	4/4 in adults
	3/3 in nymphs	2/2 in nymphs
Nymphal palpal article III	Without dorsal spur	With bulging posterial margin
Distribution	Australia, New Zealand, New Caledonia, Fiji, New Hebrides, Tonga, China, Japan, Korea and the U.S.S.R. (Primorye)	India, Sri Lanka, Pakistan, Thailand, Burma, Nepal, and the Malay Peninsula
Reproductive Type	Parthenogenetic, Bisexual or both.	Bisexual only.

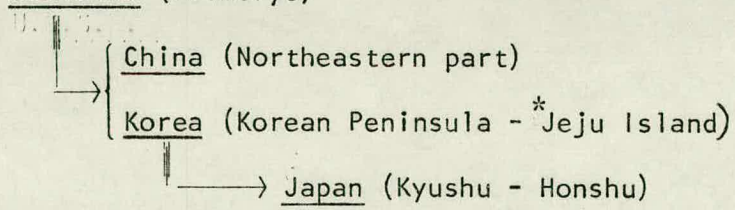
Hoogstraal *et al* (1968) have presented an assumption of the introductory routes of the parthenogenetic race. It is quite agreed, however, that a supplementary assumption may be suggested as follows; (refer to Figure 1).

A. Geographical Origin of the Tick Species:

The geographical origin of the both parthenogenetic and bisexual races is assumed to be the southeast part of the U.S.S.R. (the Primorye Region) or nearby elsewhere in the far northeastern part of the Asian Continent.

B. Introductory Route I (for the parthenogenetic race):

C. Introductory Route II (for the bisexual race):



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* Jeju = New spelling for Cheju or Che-ju.

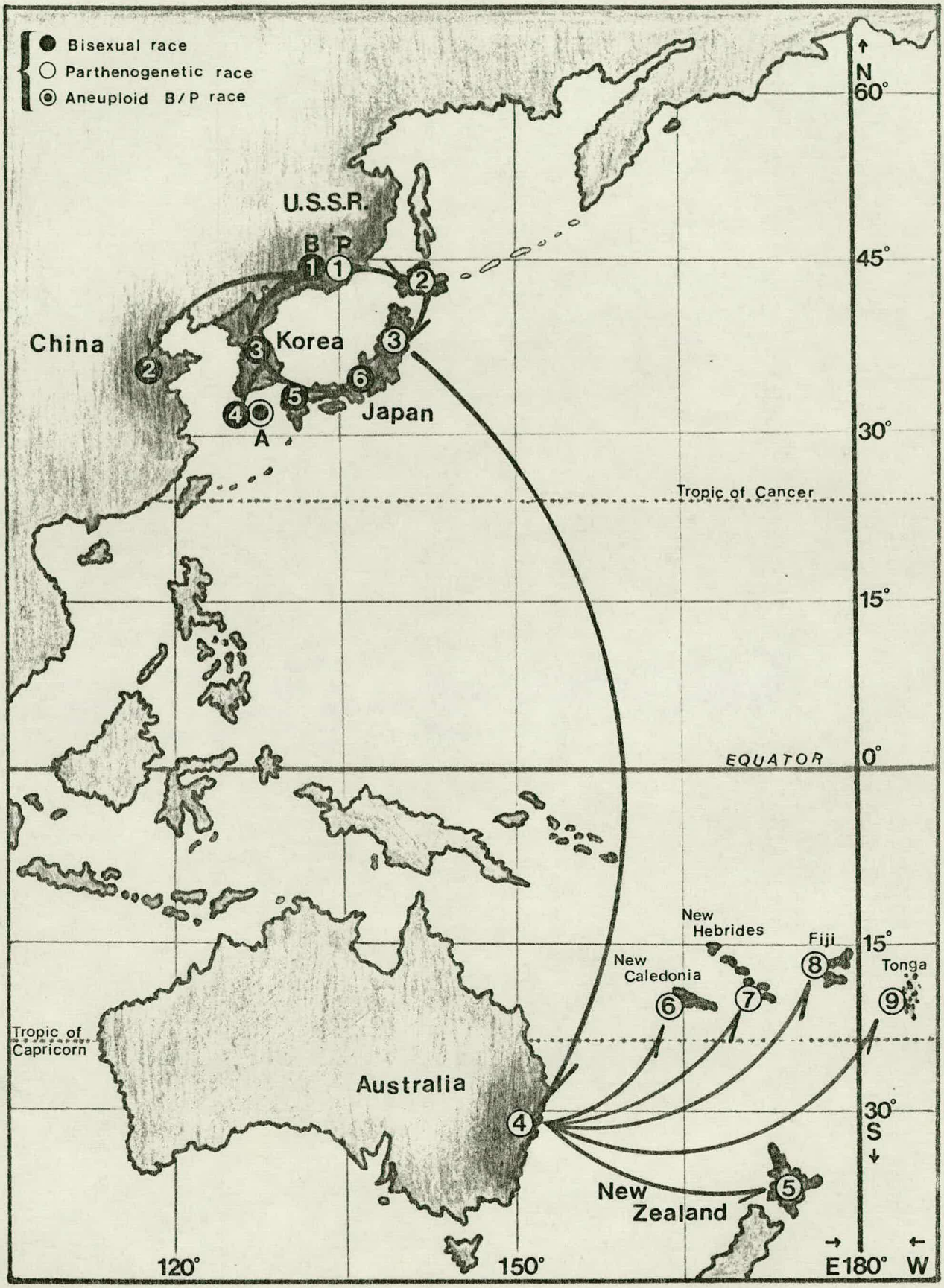

Fig. 1. A map showing the known distribution and assumed introductory routes of the tick H. longicornis.

D. Distribution of the Aneuploid Race:

Jeju Island (Korea) is the only place where the aneuploid race has been reported yet, however, it is uncertain whether this race is naturally parthenogenetic, bisexual or both.

EPIDEMIOLOGICAL/EPIZOOTOLOGICAL VECTOR ROLE;

H. longicornis have been shown to be the vectors of *Coxiella burneti*, the agent of Q fever (Smith 1942, as *Rickettsia burneti* and as *H. Bispinosa*) and *Theileria mutans*, the agent of Australian bovine theileriosis (Riek 1966, as *H. bispinosa)* in Australia.

In the U.S.S.R., the ticks have been recognised as the proven vectors of *Theileria sergenti*, the agent of Russian bovine theileriosis in Primorye (Mal'tsev 1960, as *H. bispinosa*, and Nikol'sky and Meshcheryakova 1964, as *H. neumanni)*, the virus causing Russian spring-summer encephalitis (RSSE) in the same region (Hoogstraal 1966, as *H. neumanni*), and the Powassan virus and the Khasan virus belonging to the families Togaviridae and Bunyaviridae, respectively (L'vov 1978) in the south of the Far East.* However, Belikova and Somov (1967) were unable to prove the presence of transstadial and transovarial transmission of *Rickettsia siberica* (as *Dermacentroxenus sibericus*) in the ticks (as *H. neumanni*) in Primorye.

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* The "Far East" indicates here the Far East area of the U.S.S.R., and it should not be confused with the same spelling "Far East" which is usually used to call part of Asia excluding the Middle East, Asia Minor and Central Asia.

In Japan, the ticks (or as *H. bispinosa/H. neumanni)* have been believed to be the vector of *T. sergenti* (as *Gonderia mutans/T. mutans/Theileria* sp. (Japanese strain)/Small-type Piroplasma*) causing bovine theileriosis (or as small-type piroplasmosis**) (Ishihara and Ishii 1958, Namba 1963, Ishihara 1968 and 1971, and Kitaoka 1970), with the same in Korea (Han and Kim 1965 and 1967, and Han 1968a and 1968b) as in Japan.

When Ishihara (1968) had compared the Japanese *Theileria* sp. strain with *T. sergenti* strain from the U.S.S.R. and *T. mutans* strains from Australia and South Africa, he came into an embarrassing confusion; however, he eventually considered that the Japanese strain resembled the Russian *T. sergenti* strain in relationship to the vectors. Later, the serological diagnosis (Takahashi, Yamashita, Isayama and Shimizu 1976) with the application of the indirect fluorescent antibody (IFA) test has confirmed the identical nature of the Japanese strain and the Russian *T. Sergenti* strain.

On the other hand, in spite of that most Western researchers have been suspicious about the validity of the species name *T. sergenti,* Barnett (1968) and Uilenberg (1976) admitted that *T. sergenti* is also one of the valid species. Conversely, Uilenberg (1976) and Uilenberg, McGregor, Mpangalla, Callow and de Vos (1978) have been suspciious about the validity of the European *T. mutans* strain which is quite distinct from the original East African *T. mutans* strain. About this point, Brocklesby (1978) has mentioned that; "We were

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* "Small-type piroplasma" (misused name) indicates here *Theileria* sp. and it should not be confused with small-form piroplasma indicating *Babesia bovis (=argentina)* and *B. divergens.*

** Same principle to the foot-note (*).

extremely surprised that there was no cross-reaction at all between the British theilerial parasite and any of the five available African *T. mutans* isolates. .... It therefore seems likely that the British, American and Australian parasites are really strains of *T. sergenti*". However, in a recent Newsletter*, the Centre for Tropical Veterinary Medicine in collaboration with Dr. R.E. Purnell of the Institute for Research on Animal Diseases, Berkshire, U.K., has reported that 73% of the Korean bovine sera reacted significantly to the British *T. mutans* (?) antigen at levels equivalent to or higher than the homologous positive control.

BIOLOGICAL/GENETICAL SIGNIFICANCE;

According to the various references, this tick is associated with mainly the domestic animals (cattle, sheep, goats and horses) on which heavy infestations may occur. Specimens have also been reported from dogs, pigs, cats, some kind of wild animals, birds and man.

In Korea, this tick species was reported long ago by some Japanese workers, such as Ogura and Takada (1927), Kishida (1936) and Itagaki, Noda and Yamaguchi (1944 = 1955), as the names of *H. bispinosa* or *H. neumanni*. Later, Korean workers, Han, Kim and Jeon (1966) surveyed the ticks on cattle in Korea (South) and reported that *H. longicornis* (as *H. bispinosa*) was more abundant than *Boophilus microplus*, the rival tick on cattle in the area in spring and early summer, and the latter was a great deal more prevalent in the late summer and autumn. And this has been confirmed

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* Newsletter, No 29 (December 1979) p. 15., The Centre for Tropical Veterinary Medicine, University of Edinburgh, Midlothian, U.K.

by the Australian co-operators McCulloch and Hall (1970) who had visited Korea to survey the problem of cattle ticks in Korea. McCulloch and Hall (1970) also recognised that the Korean theilerial strain (as *T. mutans*) was more virulent than that in Australia. To date, no successful researches on ticks and tick-borne diseases in Korea have been reported although ticks had long been recognised as an important factor in animal industry as well as in public health.

On the other hand, Kitaoka and Yajima (1958a and 1958b), Namba (1958, 1963a and 1963b), and Kitaoka (1961 and 1962) studied the ecological and physiological significance of the Japanese species (as *H. bispinosa*), however, observing the life-cycle of the species, Hoogstraal *et al.* (1968) indicated that factors influencing the differences in feeding and resting periods of each developmental stage of those two populations, parthenogenetic and bisexual, would be especially worthy of more detailed study.

On the cytological significance of this species, some noticeable references, such as Bremner (1959), Oliver and Bremner (1968) Khalil (1972), Oliver, Tanaka and Sawada (1973 and 1974), and Oliver (1977) are available. Bremner (1959) has reported a ratio of 1 male tick to every 400 females in the Australian population (as *H. bispinosa*), while Oliver *et al.* (1973) have found 1 male to every 1500 females in the triploid parthenogenetic race of Japan. Oliver *et al.* (1973 and 1974) have mentioned that it is uncertain whether the three distinct reproductive and chromosome types, i.e. diploid bisexual (20 + XX♀: 20 + X♂), triploid parthenogenetic (30 - 35 chromosomes), and aneuploid parthenogenetic/bisexual (22 - 28 chromosomes) races, should be considered one species. Oliver (1977) has reviewed extensively the

cytogenetics of mites and ticks, and added that *H. longicornis* is the most interesting tick yet studied cytogenetically and reproductively.

1.3 ON THE PRESENT RESEARCH PROJECT

PURPOSE OF THE RESEARCH PROJECT;

As reviewed above, *H. longicornis* is reckoned to be the most valuable and interesting tick in various types of biological experiments. Especially as this tick is an important vector of a bovine theileriosis, a comparative experiment* with any other vector ticks, such as *Hyalomma* spp., *Rhipicephalus* spp., and *Amblyomma* spp., should be noteworthy not only of the epizootological significance but also of conclusive understanding of the ecological and physiological properties of the ticks of different geographical origin.

The primary objective of this project is to present the principal biological data on the colonisation of the tick, including an observation of the life-cycle under controlled conditions. This has been attempted with reference to: a. feeding of each stage, b. population ecology, and c. reproduction pattern.

The secondary objective is to provide the results obtained from the experiments on the development of the ticks relating to

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* A series of comparative experiments on the ticks, *H. longicornis* (Korea), *Hyalomma a. anatolicum* (India), *Hy. a. excavatum* (Iran) and *Rhipicephalus appendiculatus* (Kenyan and South African strains) has been accomplished by the author (not published)

the influences of temperature and humidity under various controlled laboratory conditions.

This project therefore, is not intended as a complete work for the biology or vector role of the tick, but rather as a corner-stone providing the fundamental biological information of the tick. It is the hope of the author that this project will be of value to workers in many important research fields of applied biology associated with the ticks and in the epidemiology/epizootology of the tick-borne diseases which they transmit.

CHAPTER TWO : MATERIALS AND METHODS

The work was carried out in a series of several experiments under the various laboratory conditions. However, unless otherwise described and discussed in the appropriate pages of the main text, the general materials and methods used in these experiments are defined as follows.

2.1 MATERIALS

TICKS AS PARASITE;

Engorged adults and nymphs were readily obtained during the active season (in May and June, late spring or early summer) by collection from cattle in fields in Munmag Village, a local centre of intensive farming for rice production and live-stock industry in Weonseong District, Gangweon Province, Korea. The village is located in the central part of the Korean Penisula and in a distance of approximately 90 Km east from Seoul, the capital of the nation (Figure 2).

Large numbers of replete females were often gathered from the ground in pens where cattle had been gathered. The replete females were kept individually in plastic tubes (18 mm in diameter and 70 mm in height) plugged with cotton wool and gauze, while the unfed or partly fed females, all males and nymphs were confined in groups of 5 to 20 individuals in a tube. The tubes containing ticks were stored vertically in a plastic container (100 mm in diameter, 250 mm

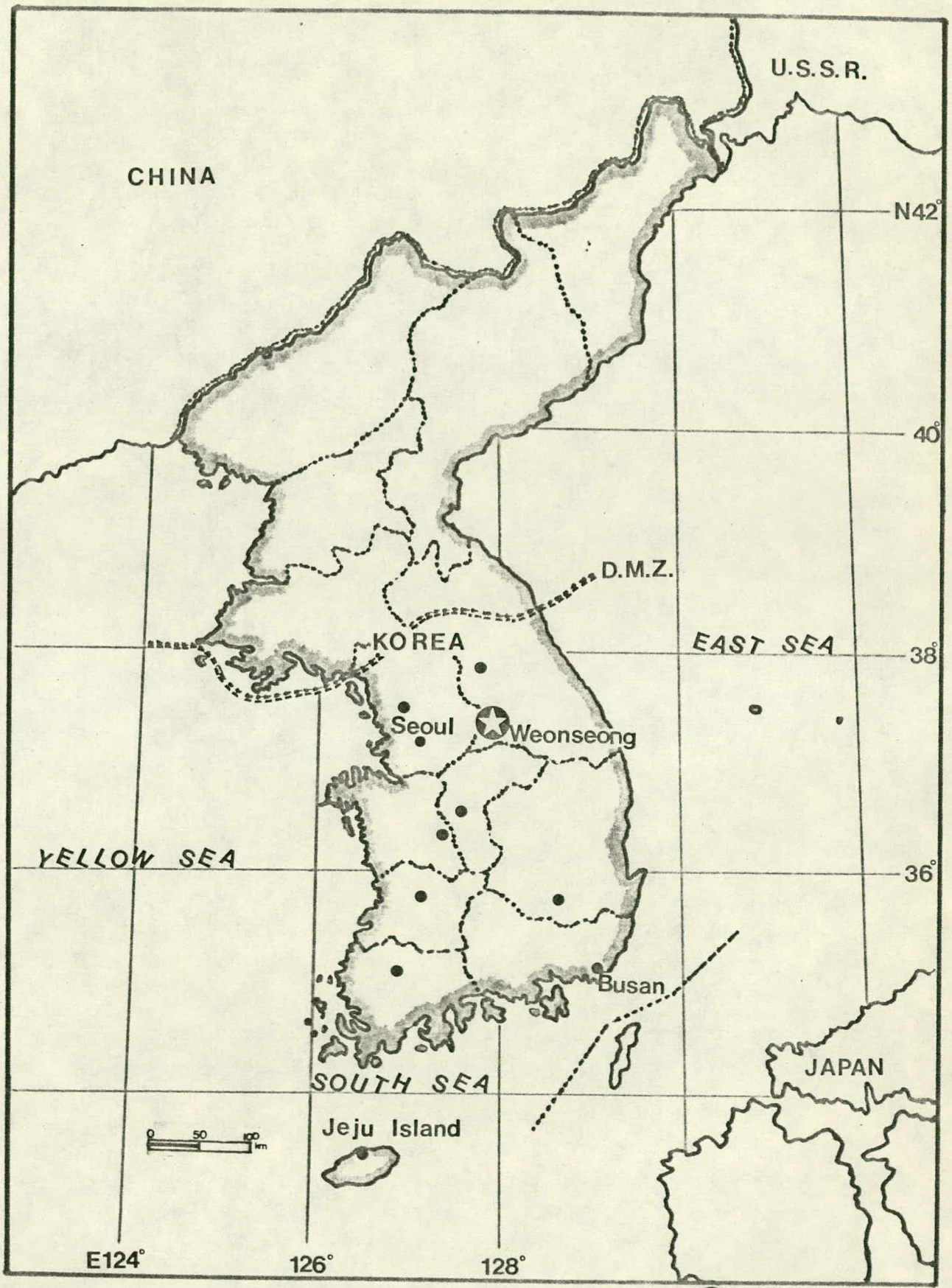

Fig. 2. A map showing the collecting area (★) of the tick H. longicornis used in this project.

in height) with moistened cotton wool at the bottom, then the containers were packed and transported to the laboratory by air as immediately as was convenient. Although a small number of females had already started oviposition during the period of air-transportation, the ticks were transferred to and maintained at a temperature of 25°C and at a relative humidity of 85% under the controlled laboratory condition.

Identification was mainly based on the morphological characteristics of the unfed ticks, and especially *Haemaphysalis longicornis* species were identified according to the views of Hoogstraal *et al.* (1968), Roberts (1970) and Yamaguti *et al.* (1971). The identified unfed-adult ticks were fed on rabbits' ears in the method described below, and the larvae hatching from the egg batches laid by the engorged female ticks were used as the main stock-line of the strain in these experiments. The strain was named "*Haemaphysalis longicornis* Weongseong strain" after the geographical origin "Weonseong District". The methods of maintaining this colony are described later on.

For the comparisons and contrasts, the standard colonies of the ticks, *Hyalomma anatolicum anatolicum* Punjab strain (India), *Hy. a. excavatum* Tehran strain (Iran), and *Rhipicephalus appendiculatus* Muguga strain (Kenya) and Ondestepoort strain (South Africa) were provided by the Centre for Tropical Veterinary Medicine, the University of Edinburgh.

RABBITS AS HOST IN EXPERIMENTAL LABORATORY FEEDING;

The rabbits used were of the conventional breed, and were supplied by the Centre for Laboratory Animals, Bush Estate, and

reared at the Animal Rooms of the Department in separate standard cages and fed on a standard diet (SG1, Oxoid) provided by the University. They were all males aged four to seven months and were inexperienced to any tick-feeding experiments. They were used only once for tick colonisation to avoid the possible immunological interference but were used in feeding of different tick species.

2.2 METHODS

EXPERIMENTAL FEEDING OF TICKS IN LABORATORY;

All tick instars such as larvae, nymphs and adults, were fed on experimental young rabbits in laboratory animal rooms. All ticks applied to rabbits' ears were confined in nylon ear-envelopes (Figure 3) which were fixed to the base of the ears by zinc-oxide adhesive tape and were closed at the upper ends of the envelopes by means of the elastic rubber bands. This made the regular inspections and the collection of the engorged ticks practicable and convenient. Neck-collars made from plastic 2 mm in thickness were used in tick-feeding on rabbits throughout the experiments to prevent the rabbits scratching off the ear-envelopes which contained the feeding ticks. The details of the neck-collar are shown in Figure 4.

Before fitting the collar to the rabbit's neck, the long hair around the base of the ear was cut short, but it was not necessary to shave the ears. A piece of cotton wool was placed inside the ear to prevent ticks dropping into the ear since they made the host rabbit nervous and restless. Then the cut hole of

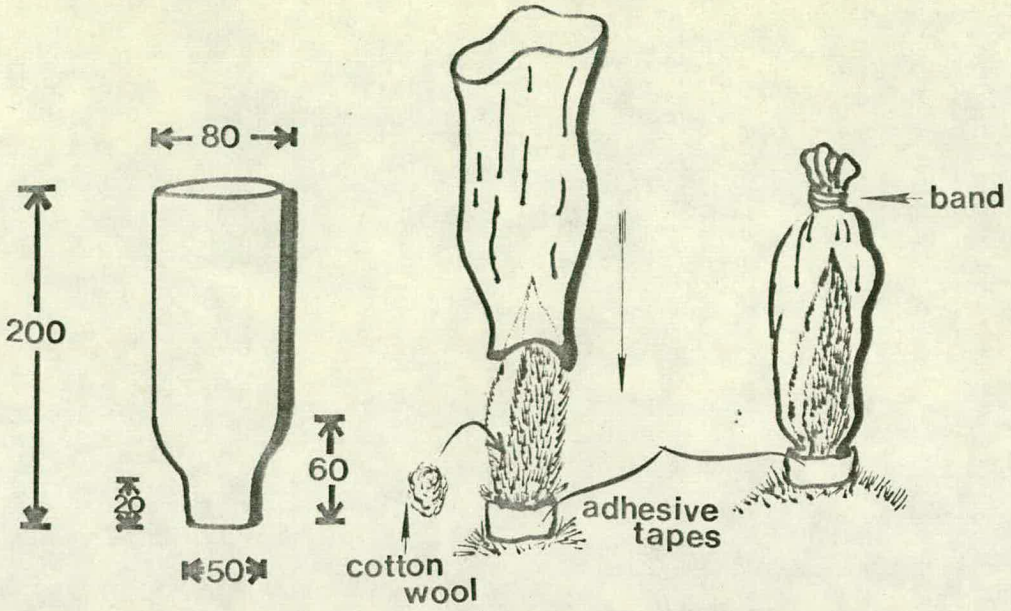

Fig. 3. A nylon ear-envelope designed for tick feeding on rabbit in the laboratory. (Unit; mm)

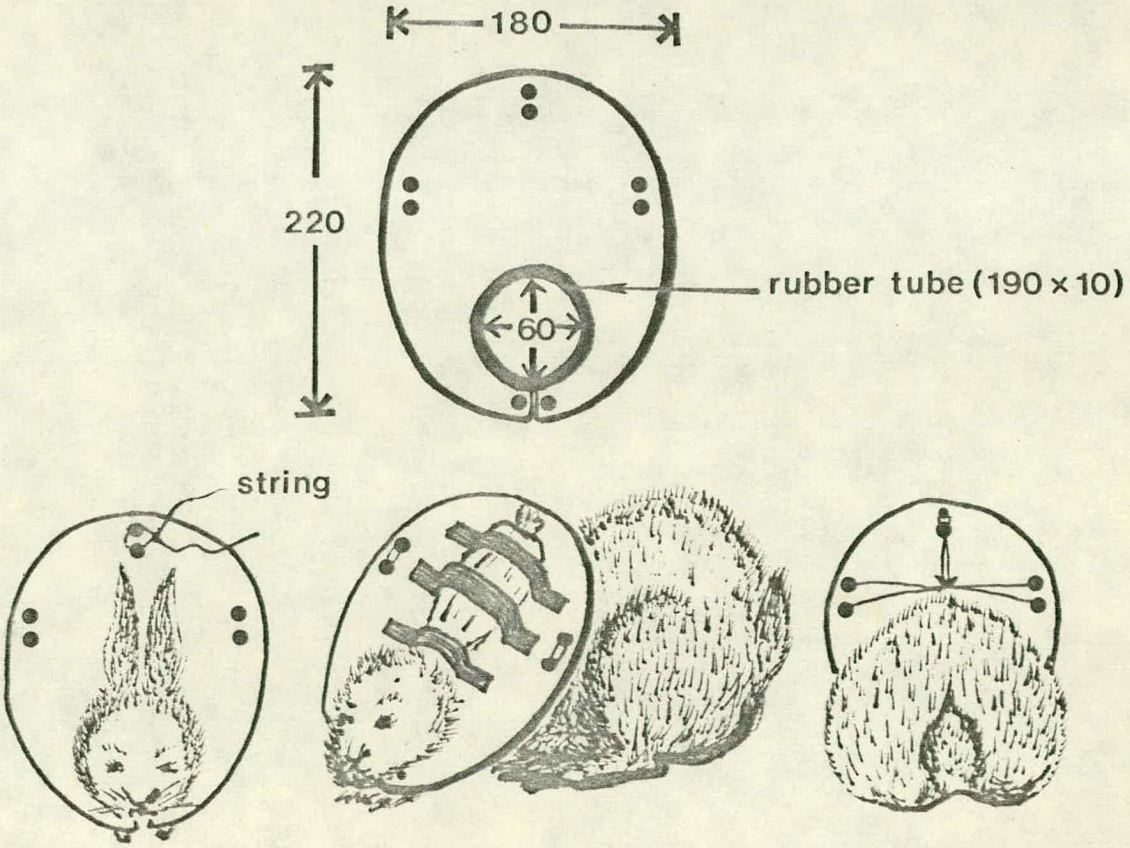

Fig. 4. A plastic neck-collar and its application to the rabbit-neck. (Unit; mm)

the collar was placed around the rabbit's neck, and a length of 2 meter cotton string was threaded from the front through the bottom holes and the ends taken back under the forelimbs of the rabbit and threaded across through the side holes of the collar and tied together on its back, so that the base of the collar was thus pulled up against the rabbit's chest holding the collar upright and preventing the collar from flopping over sideways. No sores caused by the collar or the strings were found in any parts of the neck and the axillae of the rabbit. It was apparent that the plastic collars were more useful than the leather collars designed by Bailey (1960). The biggest advantage of the plastic ones was the lighter weight compared to the leather ones so that the plastic collars stayed in a good position and they gave the rabbits less trouble. Another advantage was that the clean surface of the plastic collar permitted the adhesive tape to adhere quite strongly on it and that it was cheap, washable and usable repeatedly as many times as needed.

In the principal colonisation for mass production, numbers of approximately 500 to 4500 unfed larvae, 100 to 900 nymphs, and 4 to 20 pairs of adults were applied to each rabbit ear. In adult feeding, becuase ticks mate on the host during feeding, equal numbers of females and males were applied at the same time unless the experiment was designed for sporadic parthenogenesis in which case only females were applied to the host. Unfed ticks were introduced to the mid-part of the outer surface of the ear through the opened upper end of the ear-envelope, and closed by an elastic rubber band, then the two envelopes of each ear were tied together to the apex of the neck-collar. Finally, the ear-envelopes were fixed to the collar by means of zinc-oxide adhesive tapes in two to three places. The rabbits were

examined at least once a day throughout the tick-feeding period. Collection of the engorged ticks was done every morning and the engorged ticks were counted and weighed in the afternoons as a rule. For collection of the engorged ticks, the upper end of the ear-envelope was untied and the ticks allowed to fall onto a white enamel tray. Ticks were dropped naturally when fully engorged, although males tended to stay on the host for a long period and it was often necessary to remove them from the host by force with a pair of fine forceps. For the application of the unfed ticks, the regular inspection of the ears and the collection of the engorged ticks, a piece of cotton cloth (1 meter x 1.5 meter) was used for restraining the rabbit. Using this wrapping cloth was more convenient than using a conventional rabbit-box to restrain the animal in the laboratory for tick colonisation.

MAINTENANCE OF THE STOCK-LINE AND HANDLING OF THE TICKS;

Throughout the course of the experiments on the tick colonisation for mass production and on the general life-cycle studies in the laboratory, tick strains were maintained under the strictly controlled conditions of 25°C constant temperature and 85% relative humidity, whereas the resultant instars derived from the laboratory colonised stock were subjected to the several kinds of experiments on the effects of physical environmental factors on the development of the ticks under the various conditions of temperatures, humidities, or temperature-humidity combinations. The details of the experimental conditions are described in the appropriate places of the main text.

The handling of the active and vulnerable, immature larvae was inpracticable, and it was preferable to apportion the preceeding egg

before they hatched-out. Skill was required in the handling of ticks. All nymphs both unfed or engorged, all males and unfed females were handled with the aid of very small hair-brushes or a pair of fine opthalmic-forceps. Engorged female ticks were manipulated by means of ordinary water-colour painting brushes.

Eggs were regularly collected and weighed (or the number of the eggs was counted in certain experiments) at 24 hour intervals (or 48 hour intervals in certain cases), then the eggs were transferred to clean glass-tubes (13 mm in diamater and 50 mm in height). Special care was paid to avoid or to minimise the possible damage by desiccation. Engorged ticks were counted and weighted as soon as convenient, and then confined in clean glass-tubes (18 mm in diameter, 50 mm in height) plugged with non-absorbent coloured-cotton-wool in groups of 200, 50, 5 to 10, or individuals for larvae, nymphs, adult males, and adult females, respectively. Then the tubes were labelled both inside and outside. It was recognised that the double-labelling was remarkably practical especially in the cases of high humidity or low temperature treatments. Although some engorged ticks were often contaminated with blood, pus or excreta, the ticks were never washed with water, but they were then gently brushed on dried blotting-paper. Severely contaminated ticks were never used. During the experimental period the ticks kept at high relative humidity conditions were sometimes affected by the growth of fungi. Treatment with anti-fungal drugs was avoided, but any tubes affected by fungi were replaced by clean ones. It was confirmed that the regular inspection and tube-replacement at fortnightly intervals was quite effective to avoid or to reduce the fungal infections in long-term experimentations.

Weighing was done on an electric substitution balance (Oertling, Release-O-Matic, Model H03 5908) accurate to the nearest 10^{-2}mg. Because of the small size of the ticks used, the mean weights of each instar were determined for the groups of appropriate numbers of the ticks, for example, as many as possible groups of 100 engorged larvae, 10 engorged nymphs, 5 fed males, and each individual of engorged females. The details of the sampling sizes and replicate numbers are described elswhere in the text.

EXPERIMENTAL PHYSICAL FACTOR CONTROL;

For the sake of reference and uniformity it was desirable to maintain all stages of ticks in closed containers of known relative humidity levels at certain constant temperatures. In these experiments, the ticks were subjected to five different temperatures ($10^{o}C$, $20^{o}C$, $25^{o}C$, $30^{o}C$ and $35^{o}C$) and various relative humidities.

Constant temperatures were provided by electric thermostatic incubators kept in the laboratory, and the temperature of each thermostat was checked regularly with a maximum-minimum thermometer and a recording thermograph. The temperature range did not vary more than $\pm 1^{o}C$. Constant relative humidities were maintained at each temperature within glass screw-capped jars (100 mm x 100 mm x 180 mm) containing approximately 200 ml of the appropriate saturated salt solution for the humidity required and as recommened by Buxton (1931), Buxton and Mellanby (1934), Solomon (1951) and Winston and Bates (1960). The salt solutions used in these experiments were as follows:

	TEMPERATURE			
	20°C	25°C	30°C	35°C
R.H. Range (%)	*Chemical Agents and Relative Humidities			
11.5 - 12.5	LiCl 12.5%	LiCl 12.0%	LiCl 11.5%	LiCl 11.5%
32.5 - 33.0	$MgCl_2$ 33.0%	$MgCl_2$ 32.5%	$MgCl_2$ 32.5%	$MgCl_2$ 32.5%
55.0 - 56.0	Glucose 55.0%	Glucose 55.0%	NaBr 56.0%	Glucose 55.0%
75.5 - 76.0	NaCl 76.0%	NaCl 75.5%	NaCl 75.5%	NaCl 75.5%
83.0 - 85.0	KCl 85.0%	KCl 85.0%	KCl 84.5%	KCl 83.0%
96.0 - 98.0	K_2SO_4 98.0%	K_2SO_4 97.5%	K_2SO_4 96.5%	K_2SO_4 96.0%

* Each chemical agent represents its super saturated salt solution.

In addition, the remainder of the ticks were kept at 88% relative humidity maintained by means of super-saturated KCl solution within a 10°C incubator kept in cold-room. At this temperature the developmental processes of the ticks were much prolonged or nearly negligible.

The tubes or the weighing-bags confining the appropriate numbers of the ticks were placed vertically in an inner circular container (80 mm in diameter and 110 mm in height) approximately 50 mm above the salt solution in the humidity controlling jar.

CHAPTER THREE : RESULTS

PART I. TICK FEEDING UNDER CONTROLLED LABORATORY CONDITIONS AND THE EFFECTS OF THE NUMBER OF TICKS APPLIED

In these experiments, each instar of *H. longicornis* was applied on rabbits' ears in the laboratory. The feeding performance, such as the successful feeding number, the feeding period and the mean engorged weight, were observed at each developmental stage of the ticks. Table 1 showed the summarised data for the feeding experiments. However, the data showed the overall results of the feeding experiments with different numbers of ticks applied to each rabbit ear. The range of the initial numbers of ticks applied was 500 to 4500 larvae, 100 to 900 nymphs, and 4 to 20 pairs of adult ticks per ear, and the detailed results for the feeding of each instar were shown in the following Sections 1, 2 and 3. Additionally, an experiment was designed and carried out for the feeding of adult females in the absence of male ticks, and the results were shown in Section 4.

The feeding rates, defined as the percentages of the number of ticks fed successfully (engorged and dropped naturally) out of the total numbers of ticks applied initially were 89.89%, 92.57% and 86.11% for larvae, nymphs and adult females, respectively. The feeding period was defined here as the interval which elapses between the application of the tick to the host and the dropping of the engorged tick. The feeding periods of each instar varied greatly, i.e. 3 to 9 days for larvae (the overall mean = 5.24 days,

SD = 1.314, SE = 0.008, N = 26968; Velocity = 0.1908), 3 to 8 days for nymphs (the overall mean = 4.75 days, SD = 1.267, SE = 0.017, N = 5554; Velocity = 0.2105), and 7 to 14 days for adult females (the overall mean = 9.73 days, SD = 1.848, SE = 0.166, N = 124; Velocity = 0.1028). Adult males usually tended to stay on the host for a long period and it was often necessary to remove them from th host by force with a pair of forceps, so that the feeding periods of the males were not recorded in these experiments. Figure 5 showed the percentage frequency distribution for the days to engorge and the overall mean feeding periods of each instar.

The engorged weigths were observed in groups of sample specimens in each trial, and the overall mean engorged weights were 0.536 mg, 3.463 mg, and 251.950 mg, for larvae, nymphs and adult females, respectively. For fed males the mean fed weight was recorded 1.854 mg. On the other hand, the rate of body weight increase was defined here as the feeding efficiency (%), i.e.

$$\text{F.E. (\%)} = \frac{\text{Engorged weight - Unfed weight}}{\text{Unfed weight}} \times 100$$

The F.E§ (%) for larvae, nymphs, adult females and adult males were, respectively around 1389, 1343, 13660 and 63.

1. EXPERIMENT FOR LARVAL FEEDING

An experiment was designed as shown in Table 2 in order to assess the effect of the number of larvae applied on a host at the same time. Detailed data for the larval feeding experiment were recorded in Appendix I-1, 2, 3, 4 and 5.

	Developmental Stages		
	Larva	Nymph	Adult Female
No. of ticks applied per rabbit ear	500* - 4500*	100 - 900	4** - 20**
No. of trials (ears) observed	12	12	12
Total No. of ticks (collected/applied)	26968/30000 *	5554/6000	124/144
Overall mean feeding periods (days) ±SE	5.24±0.008	4.75±0.017	9.73±0.166
Mean unfed weight (mg)	0.036	0.240	1.831
Mean fed weight (mg)	0.536	3.463	251.950
Mean F.E. (%)	1389	1343	13660

* Approximate numbers ** With same number of male ticks

Table 1: Summarised Data for the Feeding of *H. longicornis* at Each Developmental Stage on the Rabbit Host

	Treatment Groups				
	I	II	III	IV	V
Approximate No. of larvae applied per ear	500	1500	2500	3500	4500
Actual weight (mg) of eggs used	25	75	125	175	225
Observations					
Replicate 1.	K12-L	K13-L	K4-L	K13-R	K12-R
2.	K15-R	K14-R	K4-R	K14-L	K15-L
3.			K3-L		
4.			K3-R		

Table 2: Experimental Design for *H. longicornis* Larval Feeding in the Laboratory

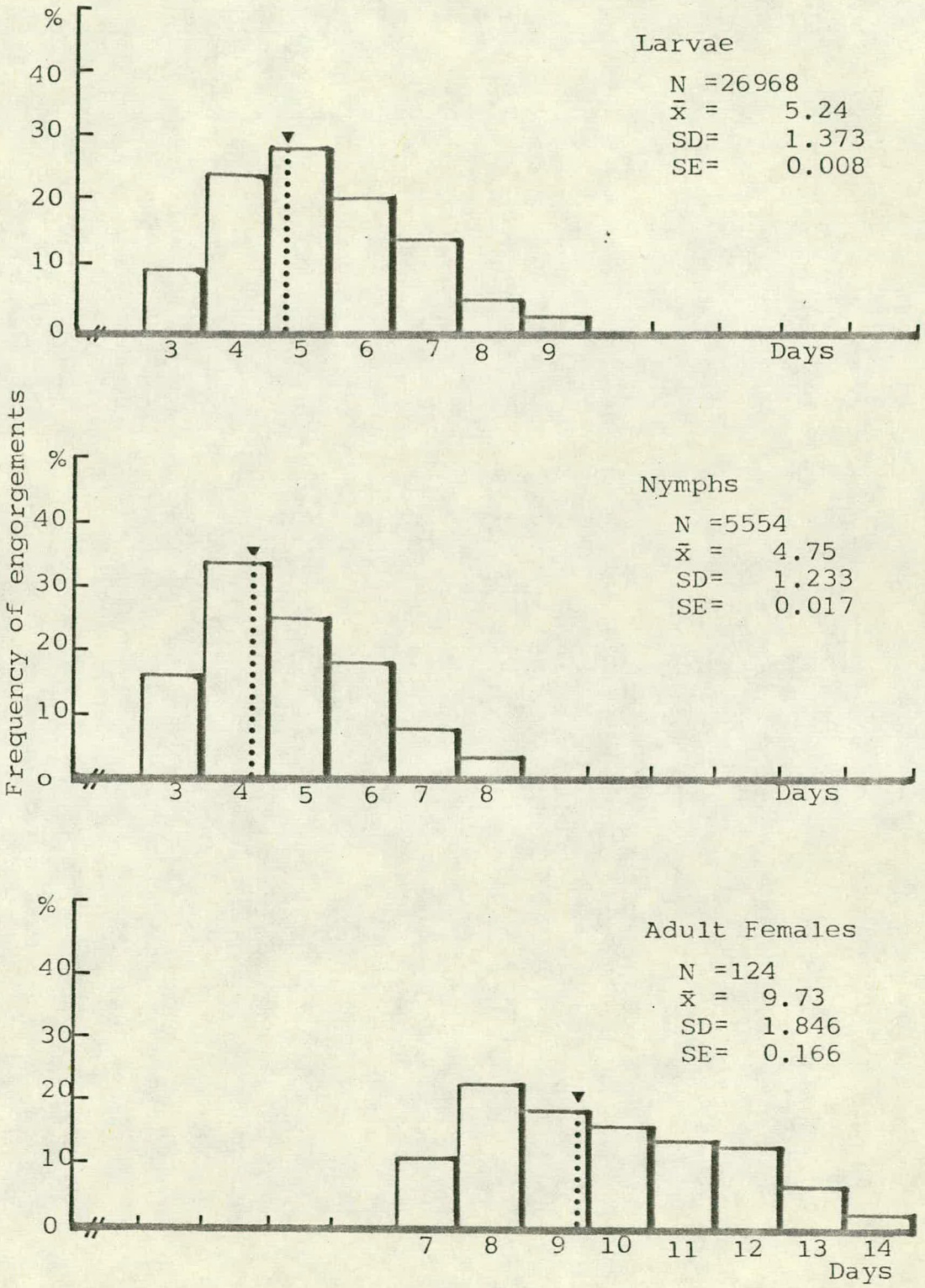

Fig. 5. The frequency(%) distribution of the engorgement periods in days and the overall mean feeding periods at each developmental stage of the tick H. longicornis fed on the rabbit hosts.

The Successful Feeding Rates;

The mean successful feeding rates (%) of *H. longicornis* larvae in each treatment group with different numbers of larvae applied initially were 96.20% for Group I, 90.83% for Group II, 93.45% for Group III, 88.27% for Group IV, and 85.08% for Group V. In general, the successful feeding rate (%) decreased as the number of larvae applied initially increased. However, the statistical analysis of variance for the larval feeding experiment (Table 3) showed that the computed F value (= 2.712) within treatments did not exceed the tabulated value $F.95(4, 7) = 4.12$, therefore the variance among treatment groups with different numbers of larvae was not significant ($p > 0.05$).

Sources of Variation	Sums-of-squares	Degrees of Freedom	Mean Squares	F
Within treatments	162.73	4	40.6825	2.712(NS)
Error	105.02	7	15.0028	-
Total	267.75	11	-	-

Table 3: Analysis of Variance for the Successful Feeding Rates (%) of *H. longicornis* Larvae Fed in Different Numbers on Each Rabbit Ear

The Mean Feeding Periods;

The mean feeding period of the larvae in each treatment group in different numbers of larvae applied were 4.64 days for Group I, 4.74 days for Group II, 5.12 days for Group III, 5.33 days for Group IV,

and 5.58 days for Group V. It was shown that an increase in the number of larvae applied resulted in an overall extension in the length of the engorgement period of the larvae. The statistical analysis of variance (Table 4) showed that the computed F value (= 291.722) within treatments greatly exceeded the tabulated value of $F.999(4,\infty)= 4.62$, so it was concluded that the variance among treatments was very highly significant.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	2100.04	4	525.1	291.722***
Error	48736.3	26963	1.8	-
Total	50836.7	26967	-	-

Table 4: Analysis of Variance for the Feeding Periods (in days) of *H. longicornis* Larvae Fed in Different Numbers on Each Rabbit Ear

The velocity defined as the reciprocal value of the actual period in the larval feeding was retarded as the number of larvae increased. On the other hand, the actual feeding periods varied individually, and the percentage frequency distribution of the engorgement days of the larval *H. longicornis* was shown in Figure 6.

The Mean Engorged Weights;

The mean engorged weights of 100 larvae x 15 sample specimens in each trial for each treatment group in the larval feeding exper-

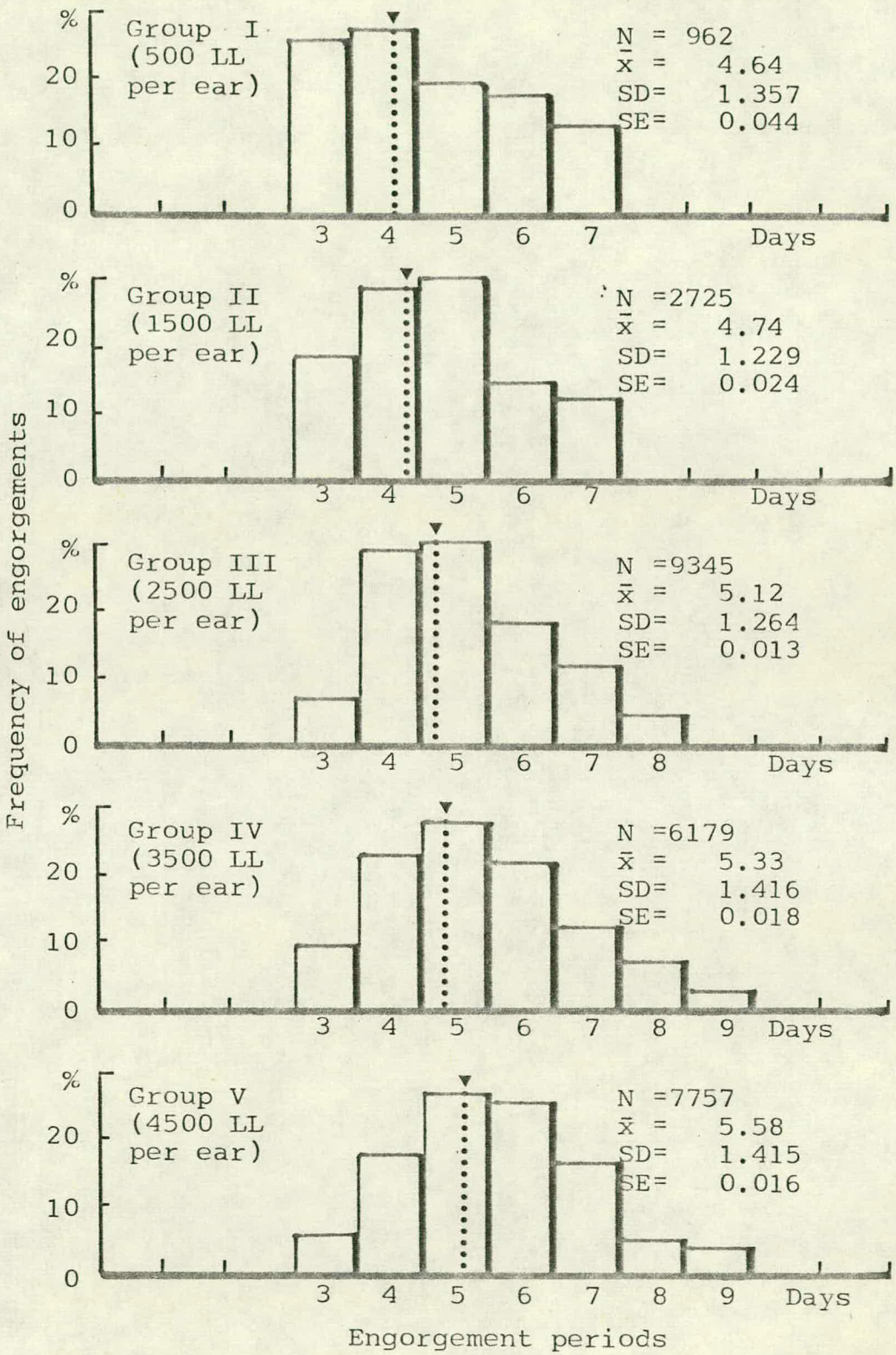

Fig. 6. The frequency(%) distribution of the engorgement periods in days of each treatment group applied with different numbers of H. longicornis larvae.

iment were 54.28 mg for Group I, 53.47 mg for Group II, 53.51 mg for Group III, 53.48 mg for Group IV, and 53.43 mg for Group V. However, the statistical analysis of variance (Table 5) showed that the computed value of F (= 0.329) did not exceed the tabulated value of $F_{.95}(4,\infty) = 2.37$, therefore, it was concluded that the large or small number which ranged from c 500 larvae to c 4500 larvae applied per ear did not affect the mean engorged weight of the larval group fed simultaneously.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	1.597	4	0.399	0.329(NS)
Error	212.1	175	1.212	-
Total	213.697	179	-	-

Table 5: Analysis of Variance for the Mean Engorged Weights (in mg unit) of *H. longicornis* Larvae Fed in Different Numbers on Each Rabbit Ear

Figure 7 showed the comparisons of the mean successful feeding rates (%) (A), the mean feeding periods in days and their reciprocal value, velocities (B), and the mean engorged weights in mg unit (C), in the larval *H. longicornis* feeding experiment with different treatment levels.

2. EXPERIMENT FOR NYMPHAL FEEDING

Another experiment was set up as shown in Table 6 in order to assess the effect of the number of nymphs applied on the feeding

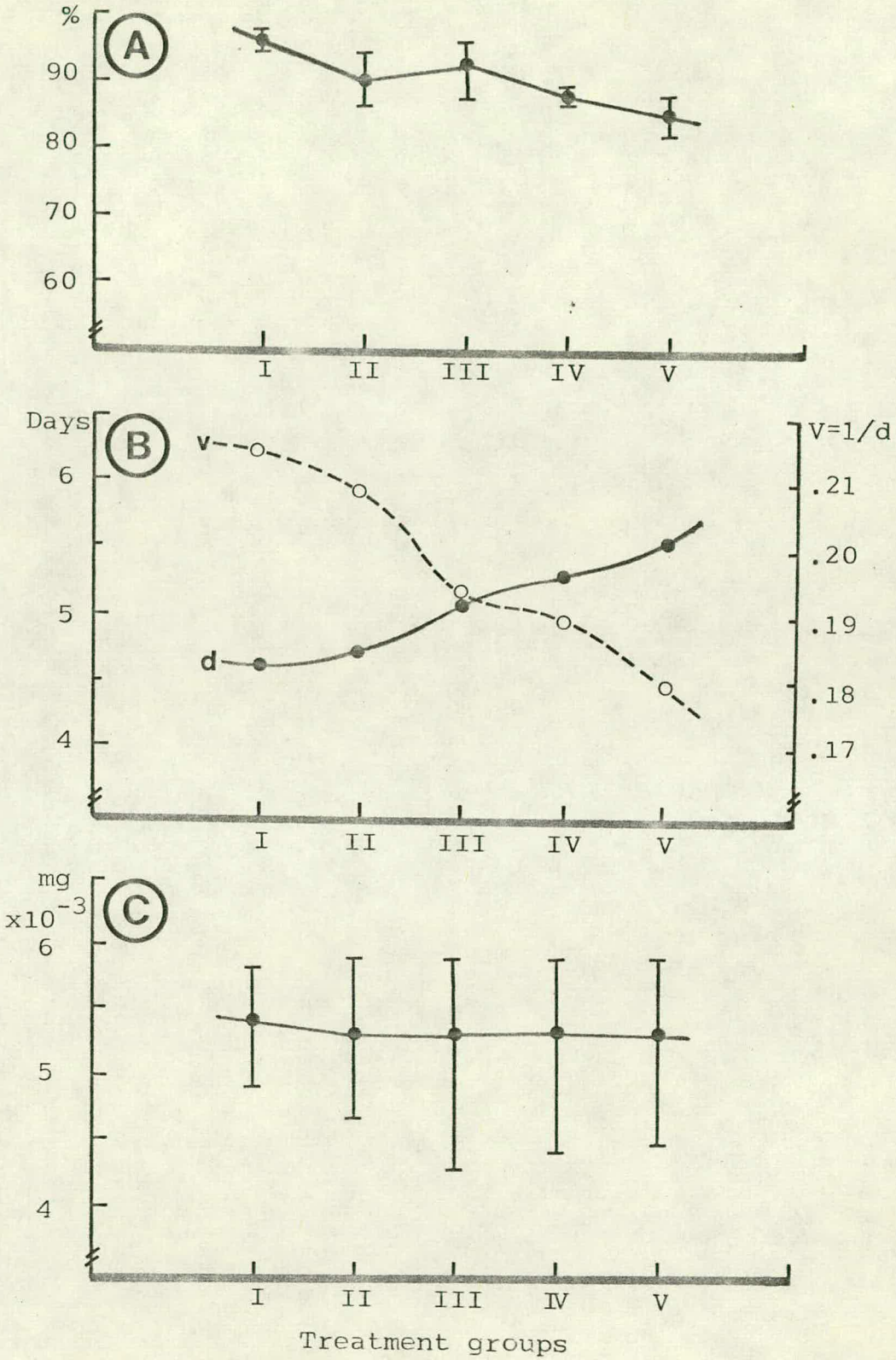

Fig. 7. The successful feeding rates (Means, Minima & Maxima) in % (A), the mean feeding periods in days and their velocities (B), and the engorged weights in mg (C) of H. longicornis larvae applied with different numbers. (I 500, II 1500, III 2500, IV 3500, V 4500, per ear)

performance of the nymphs. Detailed data for the nymphal feeding experiment were recorded in Appendix II-1, 2, 3, 4 and 5.

	Treatment Groups				
	I	II	III	IV	V
No. of nymphs applied per ear	100	300	500	700	900
Observations					
1.	K5-L	K10-L	K1-L	K1-R	K10-R
2.	K6-R	K11-R	K2-R	K2-L	K11-L
3.			K5-R		
4.			K6-L		

Table 6: Experimental Design for *H. longicornis* Nymphal Feeding in the Laboratory

The Successful Feeding Rates;

It was shown that an increase in the number of nymphs applied resulted in an overall reduction in the successful feeding numbers, and the percentage rates were 98.00% for Group I, 97.50% for Group II, 96.10% for Group III, 92.00% for Group IV, and 86.84% for Group V. The statistical analysis of variance (Table 7) showed that the computed F value (= 14.438) within treatments greatly exceeded the tabulated value of F.99(4, 7) 7.85, therefore it was concluded that the variance among treatment groups in different numbers of nymphs applied was highly significant for the successful feeding of the nymphs.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	182.67	4	45.667	14.438***
Error	22.14	7	3.163	-
Total	204.81	11	-	-

Table 7: Analysis of Variance for the Successful Feeding Rates (%) of *H. longicornis* Nymphs Fed in Different Numbers on Each Rabbit Ear

The Mean Feeding Periods;

It was shown that an increase in the number of nymphs applied resulted in an overall extension in the length of the engorgement periods of the nymphs. The mean feeding periods were 4.33 days for Group I, 4.35 days for Group II, 4.46 days for Group III, 4.83 days for Group IV, and 5.26 days for Group V. The statistical analysis of variance (Table 8) showed that the computed value of F (= 9.566) within treatments greatly exceeded the tabulated value of $F_{.999(4,\infty)}$ = 4.62, so it was concluded that the variance among treatments was very highly significant.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	606.14	4	151.535	9.566***
Error	8697.01	549	15.841	-
Total	9303.15	553	-	-

Table 8: Analysis of Variance for the Feeding Periods (in days) of *H. longicornis* Nymphs Fed in Different Numbers on Each Rabbit Ear

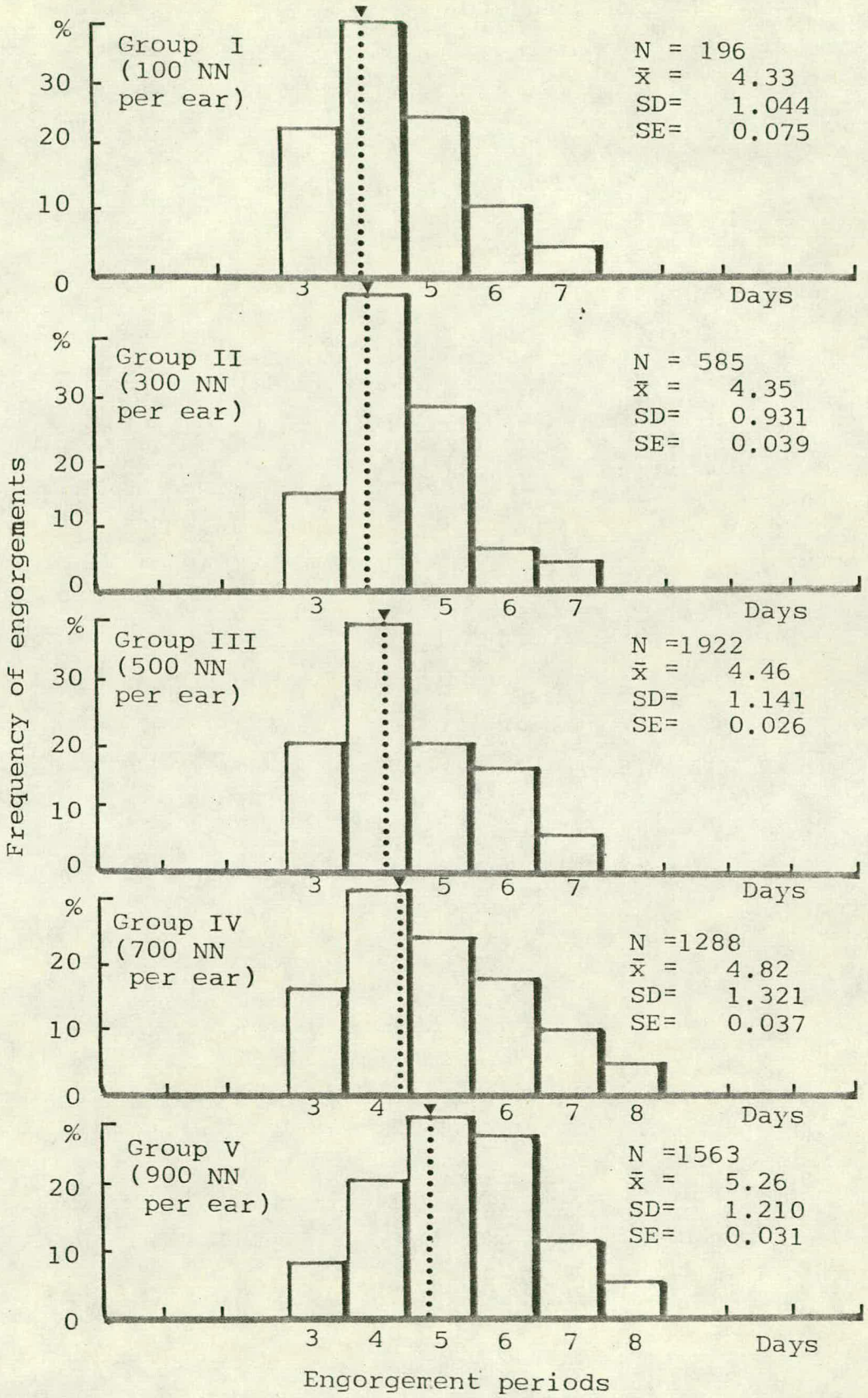

Fig. 8. The frequency(%) distribution of the engorgement periods in days of each treatment group applied with different numbers of H. longicornis nymphs.

On the other hand, the velocity in the nymphal feeding was retarded as the number of nymphs increased. The percentage frequency distribution of the engorgement period of the nymphal *H. longicornis* was shown in Figure 8.

The Mean Engorged Weights;

The mean engorged weights of 10 nymphs x 15 sample specimens in each trial for each treatment group with the exception of Group I for which 8 sample specimens were used, showed no significant difference between the treatment groups. The mean engorged weights of each treatment group were 34.91 mg for Group I, 35.03 mg for Group II, 34.82 mg for Group III, 34.42 mg for Group IV, and 33.90 mg for Group V. It was confirmed by the statistical analysis of variance (Table 9) that the computed F value (= 1.836) was smaller than the tabulated F value with $4/\infty$ degrees of freedom at 5% level (= 4.12).

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	2.555	4	0.639	1.836 (NS)
Error	56.106	161	0.348	-
Total	58.661	165	-	-

Table 9: Analysis of Variance for the Mean Engorged Weights (in mg unit) of *H. longicornis* Nymphs Fed in Different Numbers on Each Rabbit Ear

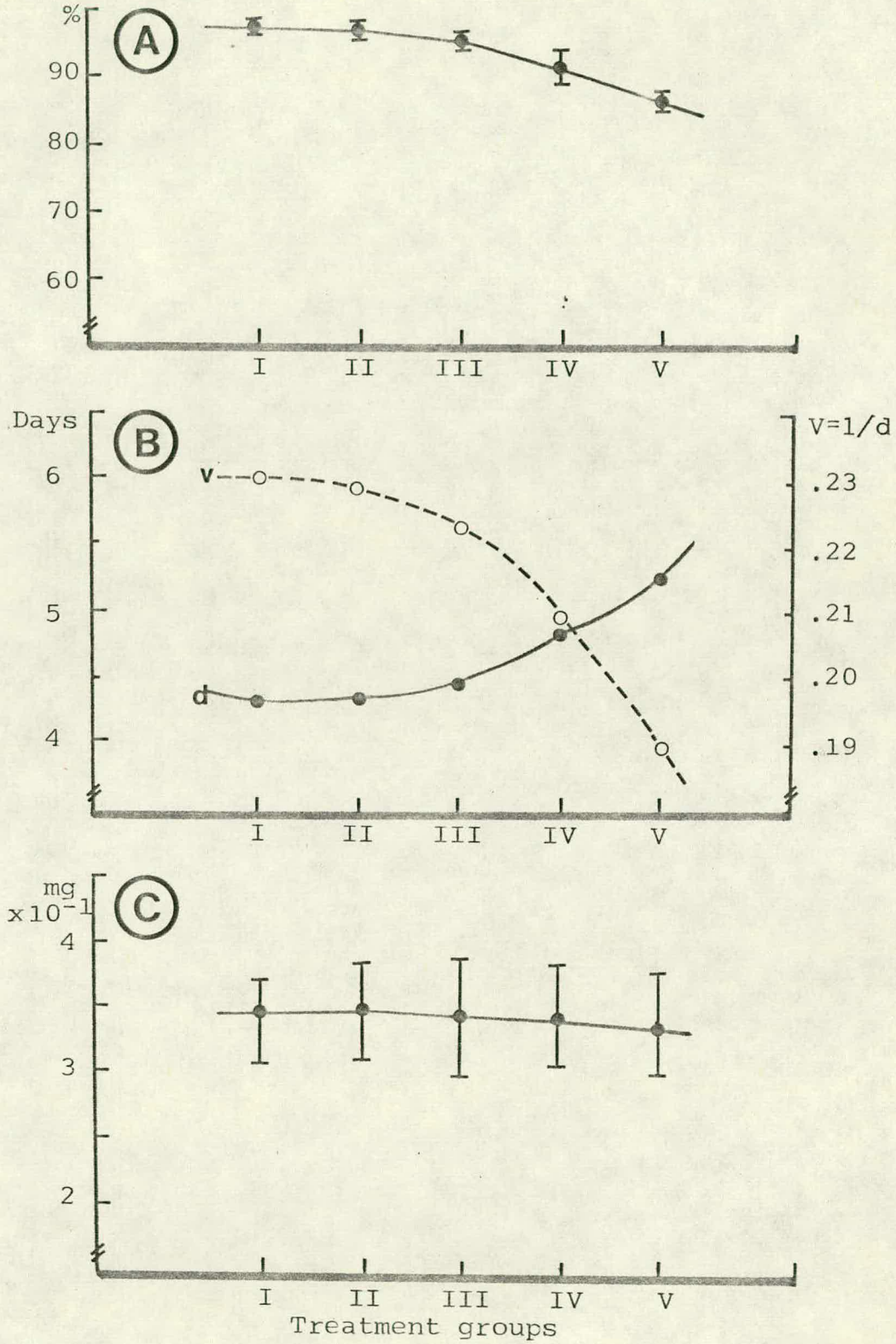

Fig. 9. The successful feeding rates (Means, Minima & Maxima) in % (A), the mean feeding periods in days and their velocities (B), and the engorged weights in mg (C) of H. longicornis nymphs applied with different numbers. (I 100, II 300, III 500, IV 700, V 900, per ear)

Figure 9 showed the comparisons of the mean successful feeding rates (%) (A), the mean feeding periods in days and their reciprocal value, velocities (B), and the mean engorged weights in mg unit (C), in the feeding experiment for the nymphs fed in different numbers at same time on the host.

3. EXPERIMENT FOR ADULT TICK FEEDING

To assess the effect of population density in adult tick feeding, an experiment was designed (Table 10), and the ticks were applied in equal numbers of both sexes but different numbers of pairs according to the treatment levels. Detailed data for the adult feeding experiment were recorded in Appendix III-1, 2, 3, 4 and 5.

	Treatment Groups				
	I	II	III	IV	V
No. of pairs of adults applied per ear	4	8	12	16	20
Observations					
1.	K46-L	K47-L	R40-L	K46-R	K47-R
2.	K48-R	K49-R	R40-R	K48-L	K49-L
3.			R41-L		
4.			R41-R		

Table 10: Experimental Design for *H. longicornis* Adult Tick Feeding in the Laboratory

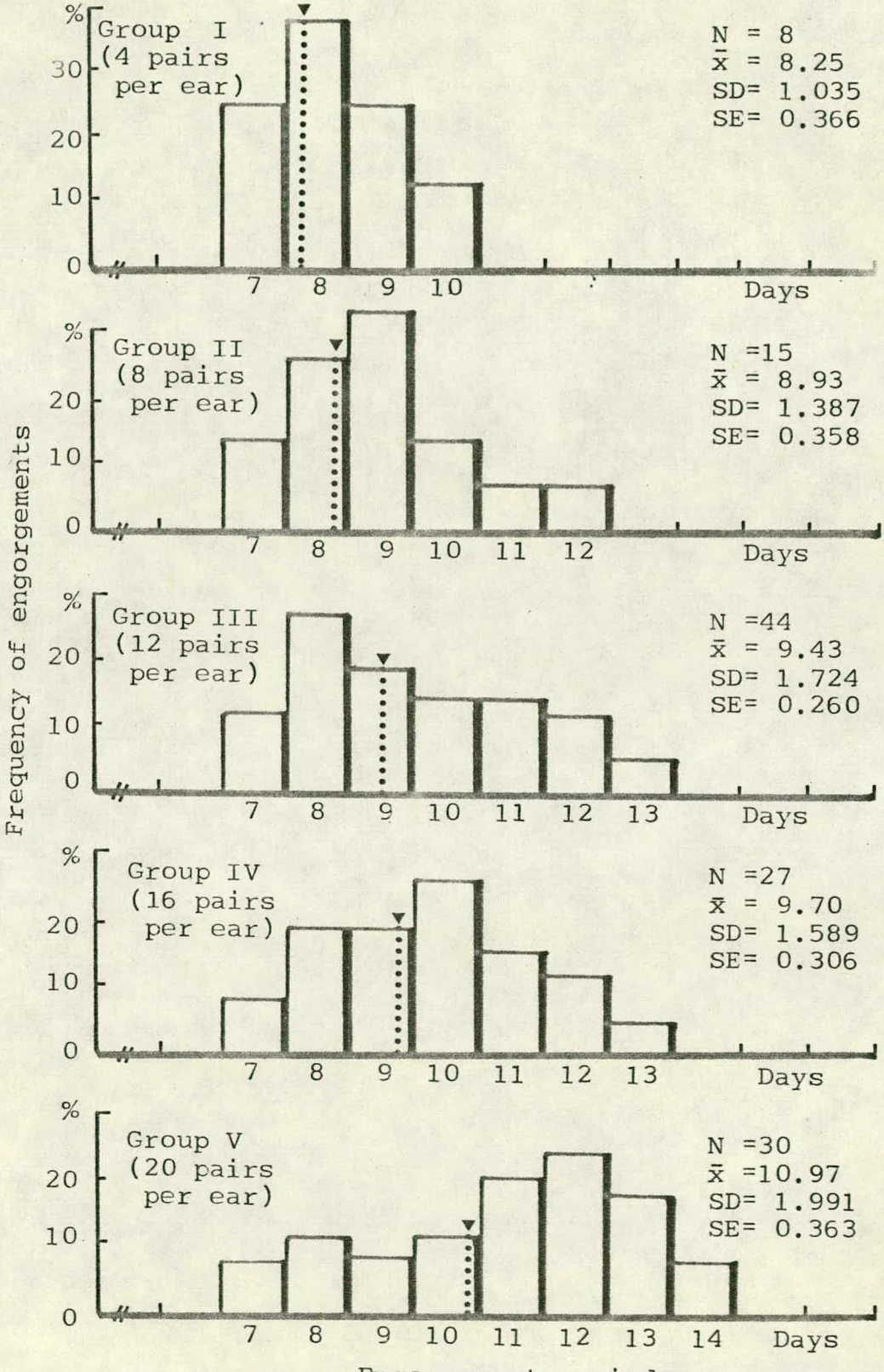

Fig. 10. The frequency(%) distribution of the engorgement periods in days of the females of each treatment group applied with different numbers of pairs of adult H. longicornis.

The Successful Feeding Rates;

In the Treatment Group I, all female ticks were engorged. However, as the number of pairs of ticks applied increased, the successful feeding rate (%) decreased progressively, such as, 100% for Group I, 93.75% for Group II, 91.67% for Group III, 84.38% for Group IV, and 75.0% for Group V. The statistical analysis of variance (Table 11) showed that the computed F value (= 4.569) slightly exceeded the tabulated value of $F.95(4, 7) = 4.12$, so the variance among treatment groups was significant at 5% confidence level.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	748.351	4	187.088	4.569*
Error	286.600	7	40.943	-
Total	1034.951	11	-	-

Table 11: Analysis of Variance for the Successful Feeding Rates (%) of *H. longicornis* Adult Females Fed in Different Numbers of Pairs on Each Rabbit Ear

The Mean Feeding Periods;

The mean feeding period of the adult female was affected by the numbers of pairs of ticks applied initially. As the tick population density increased the main feeding period of the group was delayed, i.e. 8.25 days for Group I, 8.89 days for Group II, 9.43 days for Group III, 9.72 days for Group IV, and 10.92 days for Group V.

The statistical analysis of variance (Table 12) showed that the computed value of F (= 6.611) within treatments exceeded the tabulated F value with 4/119 degrees of freedom and 0.1% confidence limit (F.999(4,119)= 4.96), so it was concluded that population density was a factor controlling the feeding period and its reciprocal value velocity.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	76.852	4	19.213	6.611***
Error	345.825	119	2.906	-
Total	422.677	123	-	-

Total 12: Analysis of Variance for the Feeding Periods (in days) of *H. longicornis* Adult Females Fed in Different Numbers of Pairs on Each Rabbit Ear

The percentage frequency distribution of the engorgement periods of *H. longicornis* adult females was shown in Figure 10.

The Mean Engorged Weights;

The engorged weights of each individual female tick in each trial for each treatment group were observed and the mean engorged weights of each treatment group were 255.70 mg for Group I, 252,79 mg for Group II, 257.93 mg for Group III, 244.24 mg for Group IV, and 248.08 mg for Group V. They were compared with each other, and the statistical analysis of variance (Table 13) showed that

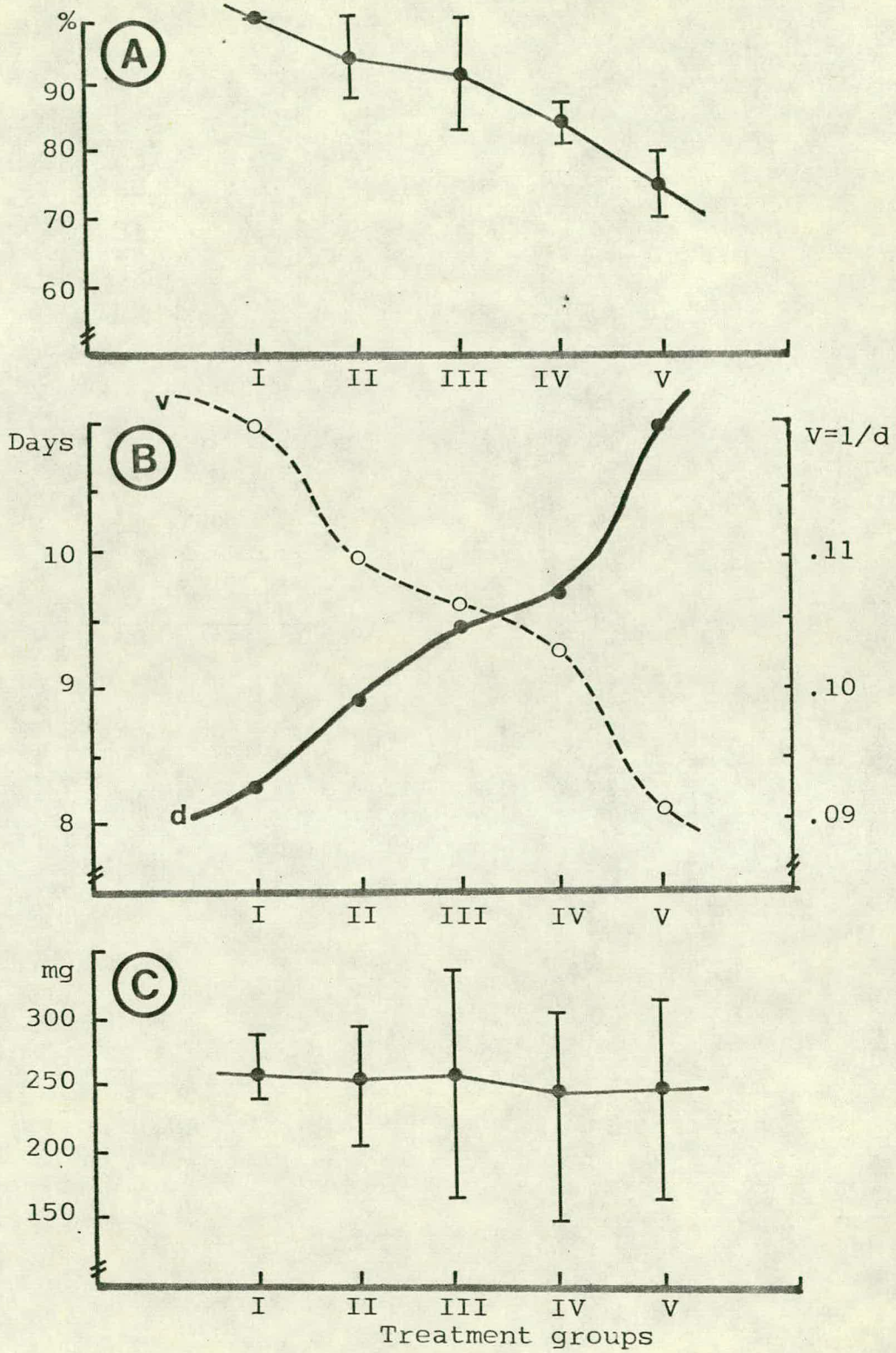

Fig. 11. The successful feeding rates (Means, Minima & Maxima) in % (A), the mean feeding periods in days and their velocities (B), and the engorged weights in mg (C) of H. longicornis females applied with different numbers of pairs. (I 4, II 8, III 12, IV 16, V 20, per ear)

there was no significant difference between treatment groups. In other words the mean engorged weight of female ticks was almost constant with little variation in individuals, and the population density of ticks applied did not influence the mean engorged weight of the female tick group.

Sources of Variations	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	3707.4	4	926.85	1.266(NS)
Error	87084.5	119	731.80	-
Total	90791.9	123	-	-

Table 13: Analysis of Variance for the Engorged Weights (in mg unit) of *H. longicornis* Adult Females Fed in Different Numbers of Pairs on Each Rabbit Ear

The comparisons of the mean successful feeding rates (%) (A), the mean feeding periods in days and their reciprocal value, velocities (B), and the mean engorged weights in mg unit (C), of the adult females fed in different numbers of pairs of ticks were shown in Figure 11.

4. EXPERIMENT FOR FEEDING OF FEMALE TICKS WITHOUT MALES

As reviewed in Section 2, Chapter I (Introduction), it has been proved that there are three distinct reproductive types, i.e., the diploid bisexual, triploid parthenogenetic, and aneuploid bisexual/parthenogenetic races in *H. longicornis* species. The distributional

characteristics of each type are peculiar and no parthenogenetic race of the ticks has yet been reported in the Korean Peninsula, although Jeju (Cheju) Island situated in the southern extreme of the nation has been reckoned to be the only place where the aneuploid race has been reported.

There were two major objects of observation in this introductory experiment on the feeding of female ticks in the absence of males. These were: (1) to observe the feeding characteristics of the female ticks fed without males, such as the percentage rate of feeding, the feeding period and velocity including the course of feeding, and the fed weight, and (2) to observe a possible sporadic parthenogenesis in the ticks, because that may happen occasionally in certain insect species normally reproducing bisexually. It was hoped that this experiment would be the first step in the cytogenetical study of the curious tick *H. longicornis*, Korean strain bisexual race, collected from the mainland, and for the comparative study of different geographical strains of the ticks in hybridisation experiments and taxonomical speciation.

The results of the observation of the feeding characteristics of the laboratory colonised population were reported in this section, whereas the results of the reproductive characateristics were reported in PartII of this Chapter, and the mode of reproduction of the ticks was discussed later (Section 1, Chapter IV).

As the results shown in Table 14, it was concluded that when females were applied to the host in absence of male ticks, the mean feeding rate (%) was lowered, and the mean feeding period of the

females was much prolonged, and the mean fed weight (here, 'fed' does not imply the meaning of 'fully engorged' but 'fed and dropped naturally') did not reach the average of the females fed normally with male ticks. The overall mean percentage rate of feeding was 52.08% (25 females fed out of 48 females applied, range 41.67% to 66.67%), the overall mean feeding period was 15.32 ±0.718 days (range 10 to 20 days), and the overall mean fed weight was 71.84 ±6.53 mg (range 20.5 to 142.6 mg).

	Trials (Observations as Replicates)				Overall
	I	II	III	IV	
Host & site	K5-L	K9-R	K49-L	K49-R	
No. of females applied initially	12	12	12	12	48
No. of females collected	7	5	5	8	25
Percentage rate of females fed	58.33	41.67	41.67	66.67	52.08
Mean feeding period in days	15.43	15.00	14.60	15.87	15.32
Mean velocity for feeding	0.065	0.067	0.068	0.063	0.065
Mean fed weight (mg)	70.44	78.12	65.20	73.30	71.84

Table 14: Summarised Data for the Feeding of *H. longicornis* Female Ticks (Bisexual Race, Weonseong Strain) in the Absence of Males

The percentage frequency distribution of the feeding periods was very irregular (Figure 12) and the fed weights of the females were scattered over a wide range (Table 15).

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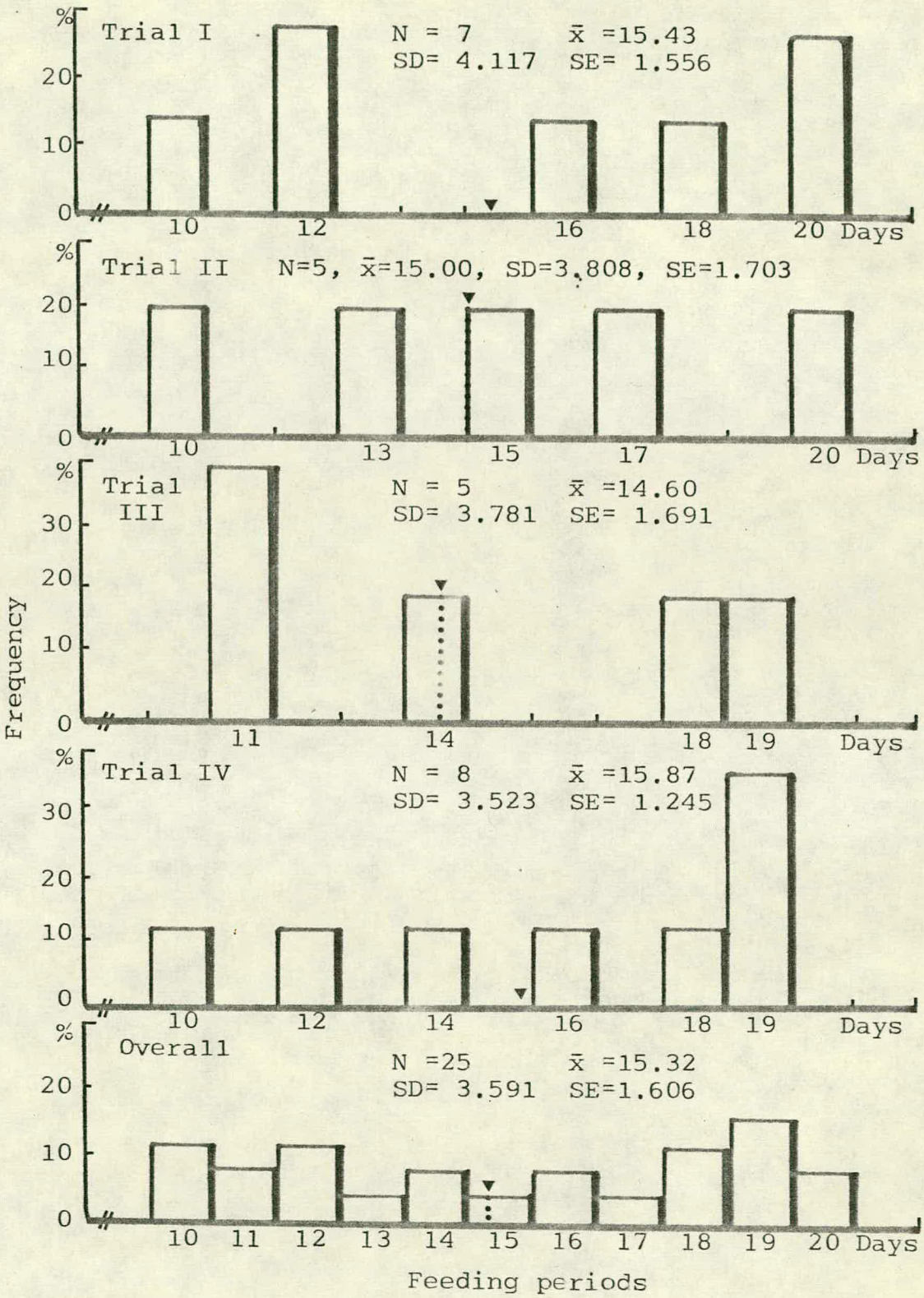

Fig. 12. The frequency(%) distribution of the feeding periods in days of the females applied without males.

	Trials (Observations as Replicates)			
	I	II	III	IV
Days to Drop				
10	82.9	53.6	-	74.0
11	-	-	37.9 58.4	-
12	98.7 56.5	-	-	68.3
13	-	75.7	-	-
14	-	-	64.3	43.5
15	-	60.3	-	-
16	122.4	-	-	94.0
17	-	131.2	-	-
18	84.9	-	115.7	89.5
19	-	-	49.7	142.6 24.2 50.3
20	27.2 20.5	69.8	-	-
Total	493.1	390.6	326.0	586.4
No. collected	7	5	5	8
Mean weight	70.44	78.12	65.20	73.30
S.D.	37.463	30.868	29.928	36.541
S.E.	14.160	13.804	13.384	12.919
Overall Mean ± SE		71.84 ± 6.533		

Table 15: Records for the Fed Weights and Droppings of *H. longicornis* Female Ticks (Bisexual Race, Weonseong Strain) Fed Without Male Ticks (Weight Unit; mg)

CHAPTER THREE : RESULTS

PART II. INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON THE REPRODUCTION OF THE TICKS

That part of the laboratory colonisation and the life-cycle observation of the tick, *H. longicornis*, which was dealt with in Part II, fell into four distinct events: ovarian or gonad development, oviposition, egg development or incubation, and hatch or eclosion; these four were collectively referred to as reproduction, so that the former two events were dealt with in Section 1, whereas the latter two were in Section 2.

1. INFLUENCE ON THE OVARIAN DEVELOPMENT AND OVIPOSITION OF THE ENGORGED FEMALES

The limited supply of host rabbits often restricted the number of engorged female ticks available. It was therefore necessary to arrange a modified experimental design so that the experiment on the influence of differing levels of temperature and humidity upon the ovarian development and oviposition was designed on the one hand to expose groups of engorged females to five different relative humidities at 25°C. (Series A), and on the other hand to expose other groups of engorged females to four different treatment remperatures at 83-85% relative humidity (Series B), making a total of 9 different temperature-humidity combinations (Tables 16 and 17). Additionally, an experimental design was made for a total of 25 females fed without male ticks, under

the controlled conditions of 25°C and 85% relative humidity combination.

		Treatment Groups				
		I	II	III	IV	V
R.H. Treatment		12.0%	32.5%	55.0%	75.5%	97.5%
Replicates	1.	R40L-1 232.8	R40L-2 280.5	R40R-3 160.1	R41R-2 301.4	R46R-6 278.1
(Tick No. and engorged weight (mg))	2.	R40R-1 338.1	R40L-3 255.5	R40R-4 297.6	R41R-3 266.5	R40R-7 208.3
	3.	R41L-1 292.8	R40L-4 288.3	R41L-3 206.5	R40L-6 253.7	R41R-4 213.7
	4.	R41L-2 232.7	R40L-5 215.5	R41L-4 245.0	R40L-7 227.6	R41R-5 288.6
	5.	R41R-1 176.2	R40R-2 260.4	R41L-5 214.1	R40R-5 215.4	R41R-6 206.5

Table 16: Experimental Design for the Influence of Relative Humidity on the Ovarian Development and Oviposition of *H. longicornis* Females at 25°C (Series A)

All females were maintained under almost completely dark conditions. Bias from the differences in the individual engorged weights was unavoidable, however, effort was made to minimise the possible bias by using as many replicates as were available. A total of 44 engorged females was used in this experiment for the normal reproduction with additional 25 females for the parthenogenetic reproduction. As this experimental design was not set up as a completely randomised block design, the statistical analysis was based on the single-factor analysis of variance for the normal variables.

		Treatment Groups			
		I	II	III	IV
Temperature Treatment		20°C	25°C	30°C	35°C
Replicates	1.	R40L-8 292.8	R4iR-8 185.6	R41R-9 236.0	R41L-10 310.6
(Tick No. and engorged weight (mg))	2.	R40R-8 210.1	R40L-9 358.7	R41R-10 270.0	R41R-11 265.3
	3.	R41L-6 275.4	R40L-10 226.2	R40L-11 219.4	R40R-10 279.4
	4.	R41L-7 246.8	R41L-8 213.9	R40L-12 214.0	R41L-11 279.4
	5.	R41R-7 329.5	R41L-9 275.5	R40R-9 316.2	Not tested

Table 17: Experimental Design for the Influence of Temperature on the Ovarian Development and Oviposition of *H. longicornis* Females at 83-85% Relative Humidity (Series B)

Pre-Oviposition Period;

It was defined here as the time interval which elapsed between the detachment of an engorged female from the host and the commencement of oviposition. It is often called the duration of the ovarian (or gonad) developmental period as the ovaries (or gonads) develop during this period.

The results (Figures 13 and 14) showed the mean pre-oviposition period to range from 7.6 days at 12.0% relative humidity to 8.2 days, 7.8 days, 8.6 days, and 8.4 days at 32.5% relative humidity, 55.0% relative humidity, 75.5% relative humidity and 97.5% relative humidity

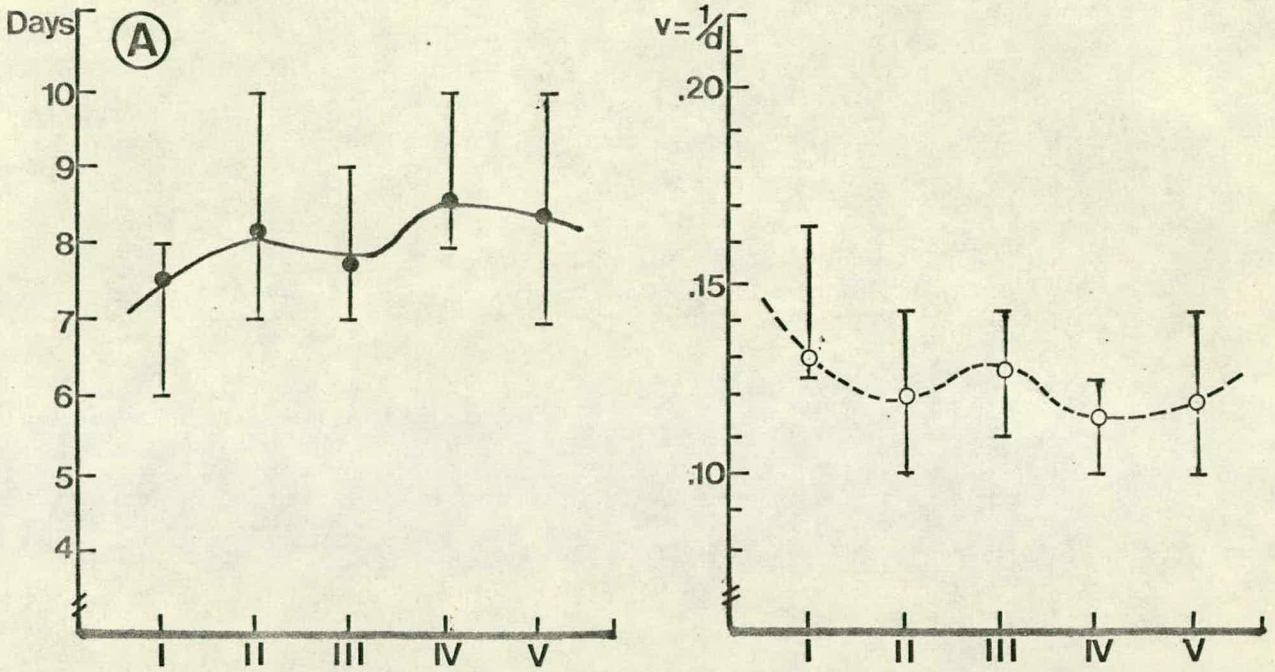

Fig. 13. The pre-oviposition periods (Means, Minima & Maxima) and their velocities of H. longicornis females maintained at 25°C and various relative humidities (Series A).
(I 12.0% RH, II 32.5% RH, III 55.0% RH, IV 75.5% RH, V 97.5% RH)

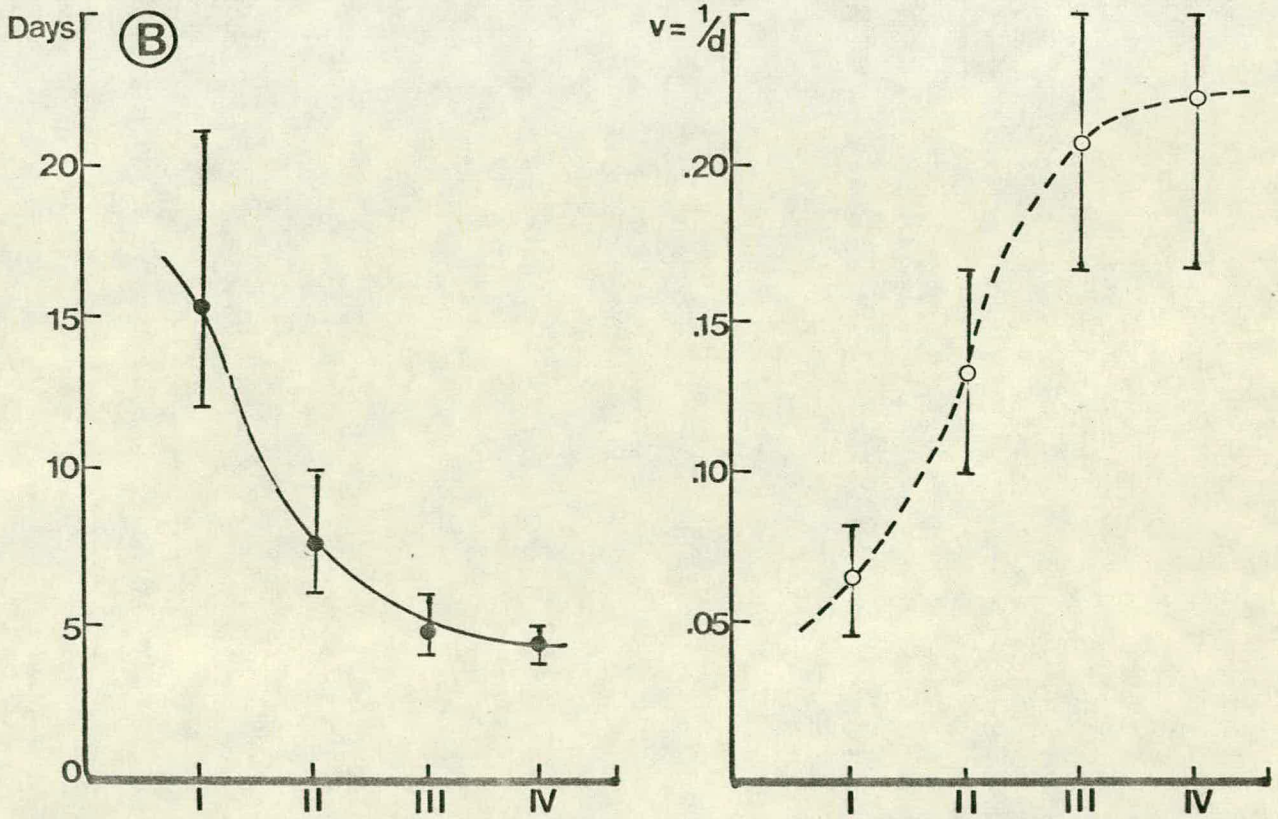

Fig. 14. The pre-oviposition periods (Means, Minima & Maxima) and their velocities of H. longicornis females maintained at 85.0% relative humidity and various temperatures (Series B).
(I 20°C, II 25°C, III 30°C, IV 35°C)

conditions, respectively, in Series A (at 25°C), while in Series B (at 83-85% relative humidity), from 15.2 days at 20°C to 7.6 days, 4.8 days, and 4.5 days at 25°C, 30°C and 35°C respectively. However, the statistical analysis of variance for the per-oviposition periods showed that there was no significant difference ($F = 0.811$ with 4/20 degrees of freedom $p > 0.05$) within relative humidity treatment groups at 25°C (Table 18), while there was a very highly significant difference ($F = 27.305$ with 3/15 degrees of freedom, $p < 0.001$) within temperature treatment groups at 83-85% relative humidity (Table 19).

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatment	3.44	4	0.86	0.811(NS)
Error	21.20	20	1.06	-
Total	24.64	24	-	-

Table 18: Analysis of Variance for the Pre-Oviposition Periods (Days) of *H. longicornis* Females at 25°C and Various Relative Humidities (Series A)

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatment	359.358	3	119.768	27.305***
Error	65.8	15	4.387	-
Total	425.158	18	-	-

Table 19: Analysis of Variance for the Pre-Oviposition Periods (Days) of *H. longicornis* Females at 83-85% Relative Humidity and Various Temperatures (Series B)

Therefore, it was concluded that the pre-oviposition period was not influenced by humidity at 25°C but was greatly influenced by temperature (ranging from 20°C to 35°C) at a constant 83-85% relative humidity. The ovarian developmental velocities computed from the developmental periods were also shown in Figures 13 and 14. The velocity for the ovarian development of the ticks was accelerated by raising the temperature and retarded by lowering it.

On the other hand, the mean pre-oviposition period of the female fed without males and maintained at 25°C and 85.0% relative humidity was 10.83 days (N = 12, SD = 2.480, SE = 0.716, Velocity = 0.092) Figure 22 showed the percentage frequency distribution of the pre-oviposition periods of the females fed without males. It was clear that the mean pre-oviposition period of the females fed without male ticks was much delayed regardless of the effects of temperature or relative humidity.

Oviposition Period;

The influencesof temperature and humidity on the oviposition period were also examined. The general trend of the results as shown in Figures 15 and 16 was very similar to the results of the above observation for the pre-oviposition period. At 25°C, the mean oviposition periods (ranging from 15.6 days to 19.0 days) were not influenced by the humidity treatments. Within the humidity treatment groups there was no significant difference ($F = 1.713$ with 4/20 degrees of freedom, $p > 0.05$) (Table 20), whereas at 83-85% relative humidity the period decreased as treatment temperature increased, from 21.4 days at 20°C to 15.5 days at 35°C, and it was clarified that the

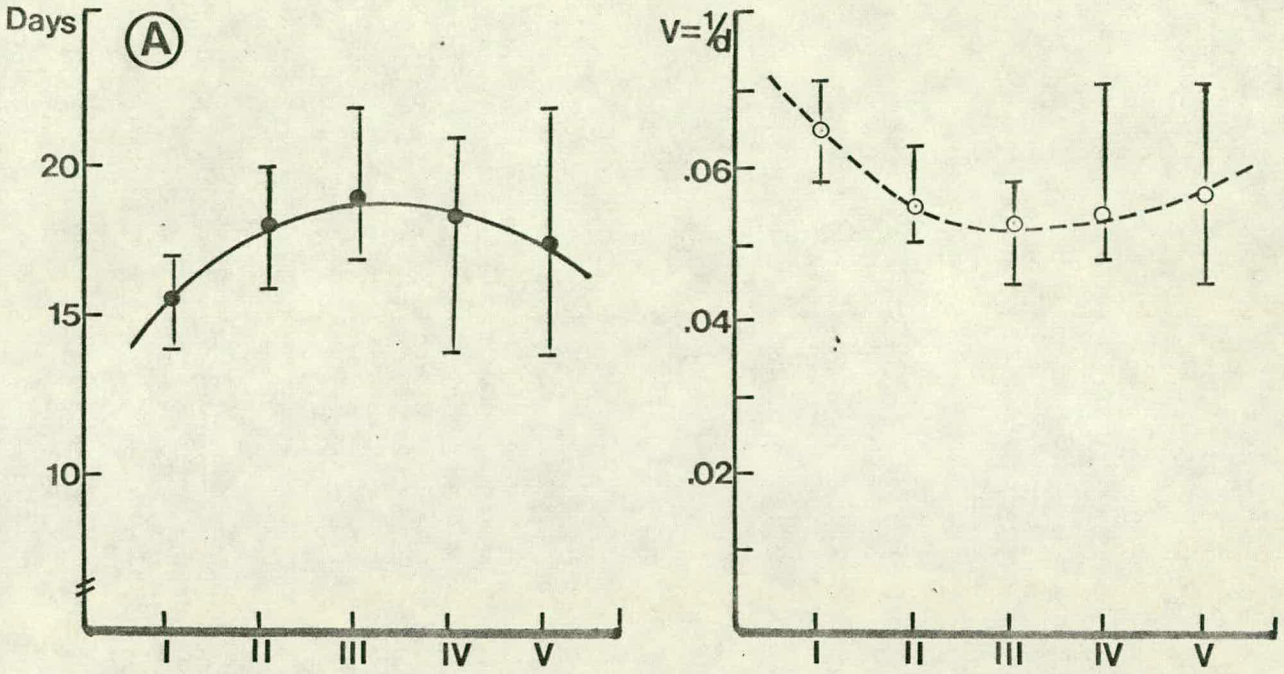

Fig. 15. The oviposition periods (Means, Minima & Maxima) and their velocities of H. longicornis females maintained at 25°C and various relative humidities (Series A).
(I 12.0% RH, II 32.5% RH, III 55.0% RH, IV 75.5% RH, V 97.5% RH)

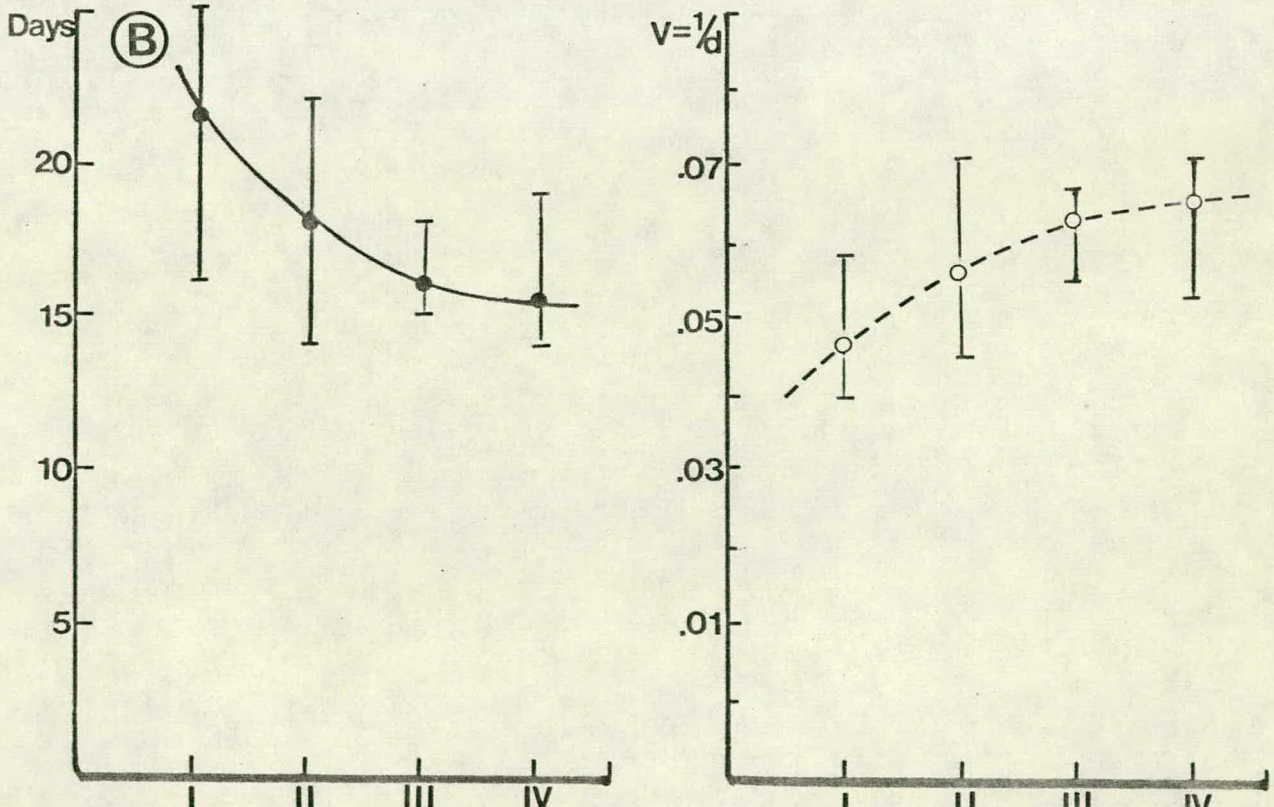

Fig. 16. The oviposition periods (Means, Minima & Maxima) and their velocities of H. longicornis females maintained at 85.0% relative humidity and various temperatures (Series B).
(I 20°C, II 25°C, III 30°C, IV 35°C)

velocity for oviposition was accelerated by raising the treatment temperature and retarded by lowering it. Also the statistical analysis of variance proved the significant difference ($F = 5.266$ with 3/15 degrees of freedom, $p < 0.05$) within treatment-temperature groups (Table 21).

However, the mean oviposition period (Figure 23) of the females fed without males and maintained at 25°C and 85.0% relative humidity was 11.16 days ($N = 12$, $SD = 2.725$, $SE = 0.787$, and Velocity = 0.060), whereas it was clear that the mean oviposition period of the females fed without males was shorter than that of those fed normally with males, regardless of the effects of temperature or humidity. The short mean oviposition period of the single-fed females was a contrast to the quite delayed mean pre-oviposition period in the same females.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	33.44	4	8.36	1.713 (NS)
Error	97.60	20	4.88	-
Total	131.04	24	-	-

Table 20: Analysis of Variance for the Oviposition Periods (Days) of *H. longicornis* Females at 25°C and Various Relative Humidities (Series A)

Oviposition Ability;

The oviposition ability was examined as a percentage of the total weight of eggs laid by individual female compared to the engorged

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	102.158	3	34.053	5.266*
Error	97	15	6.467	-
Total	199.158	18	-	-

Table 21: Analysis of Variance for the Oviposition Periods (Days) of *H. longicornis* Females at 83-85% Relative Humidity and Various Temperatures (Series B)

weight of the female. The overall mean level of the total weight of eggs laid per female was 47.45% of the engorged weights and it ranged individually from 41.63% to 52.59% (Appendix IV-1, and 2). However, the statistical analysis of variance (Tables 22 and 23) showed that there was no significant differences within the relative humidity treatment groups ($F = 1.146$ with 4/20 degrees of freedom, $p > 0.05$) or within the temperature treatment froups ($F = 0.487$ with 3/15 degrees of freedom, $p > 0.05$). Figure 17 showed the comparison.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	45.568	4	11.392	1.146 (NS)
Error	198.844	20	9.942	-
Total	244.412	24	-	-

Table 22: Analysis of Variance for the Percentage Total Egg Weight/ Engorged Weight of *H. longicornis* Females at 25°C and Various Relative Humidities (Series A)

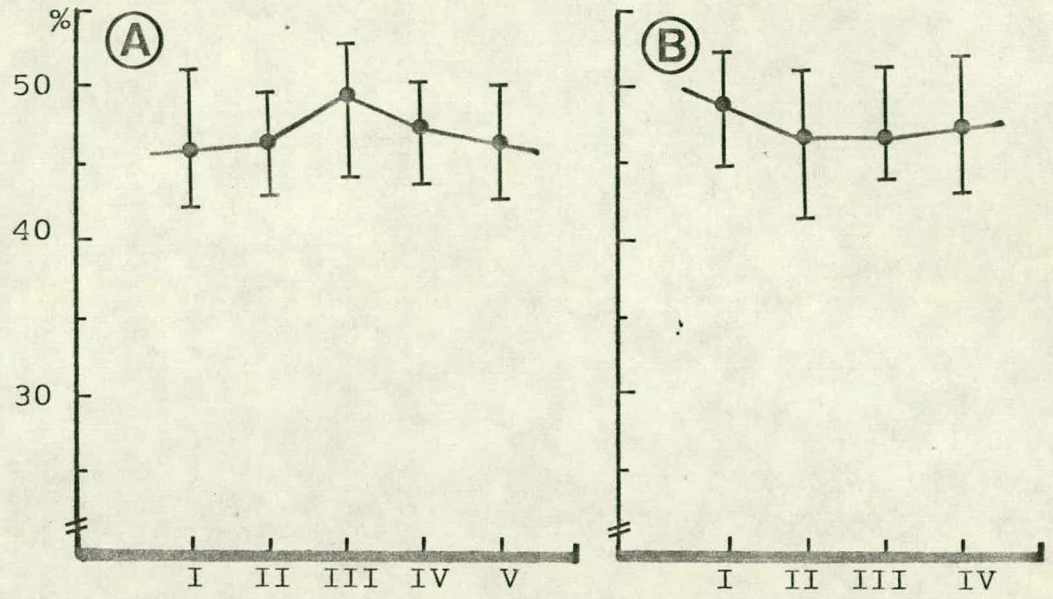

Fig. 17. The oviposition ability (% of the total weight of eggs laid by individual/the engorged weight of the female) maintained at various conditions.
Series A(25°C) I 12% RH,II 32.5% RH,III 55% RH, IV 75.5% RH, V 97.5% RH.
Series B(85%RH) I 20°C, II 25°C, III 30°C, IV 35°C.
Statistical analysis showed no significant difference.

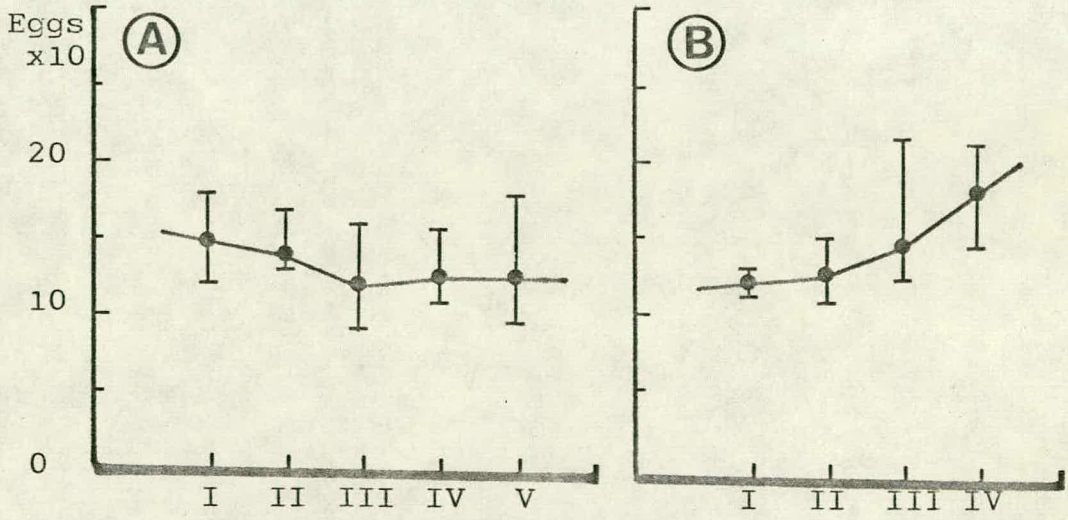

Fig. 18. The oviposition ability (mean daily egg-output of females maintained at various conditions.
Series A ; 25°C and various relative humidities.
Series B ; 85.0% relative humidity and various temperatures.
Statistical analysis showed no significant difference.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	15.551	3	5.184	0.487 (NS)
Error	159.785	15	10.652	-
Total	175.336	18	-	-

Table 23: Analysis of Variance for the Percentage Total Egg Weight/ Engorged Weight of *H. longicornis* Females at 83-85% Relative Humidity and Various Temperatures (Series B)

On the other hand, the overall mean daily output of eggs per female was 139.94 eggs/day/tick and it ranged individually from 93.55 eggs to 217.47 eggs per day per female. Figure 18 showed the comparison of these results. The statistical analysis of variance showed no significant difference ($F = 0.723$ with 4/20 degrees of freedom, $p > 0.05$) within relative humidity treatment groups (Table 24) but it showed a significant difference ($F = 4.031$ with 3/15 degrees of freedom, $p < 0.05$) within temperature treatment groups (Table 25). It was therefore concluded that under certain favourable constant conditions the oviposition ability of the ticks was not influenced or little influenced by whole range of relative humidities or temperatures ($20^{o}C$ to $35^{o}C$).

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	1699.8	4	424.95	0.723(NS)
Error	11761.03	20	588.515	-
Total	13460.83	24	-	-

Table 24: Analysis of Variance for the Mean Daily Output of Eggs by *H. longicornis* Females at $25^{o}C$ and Various Relative Humidites (Series A)

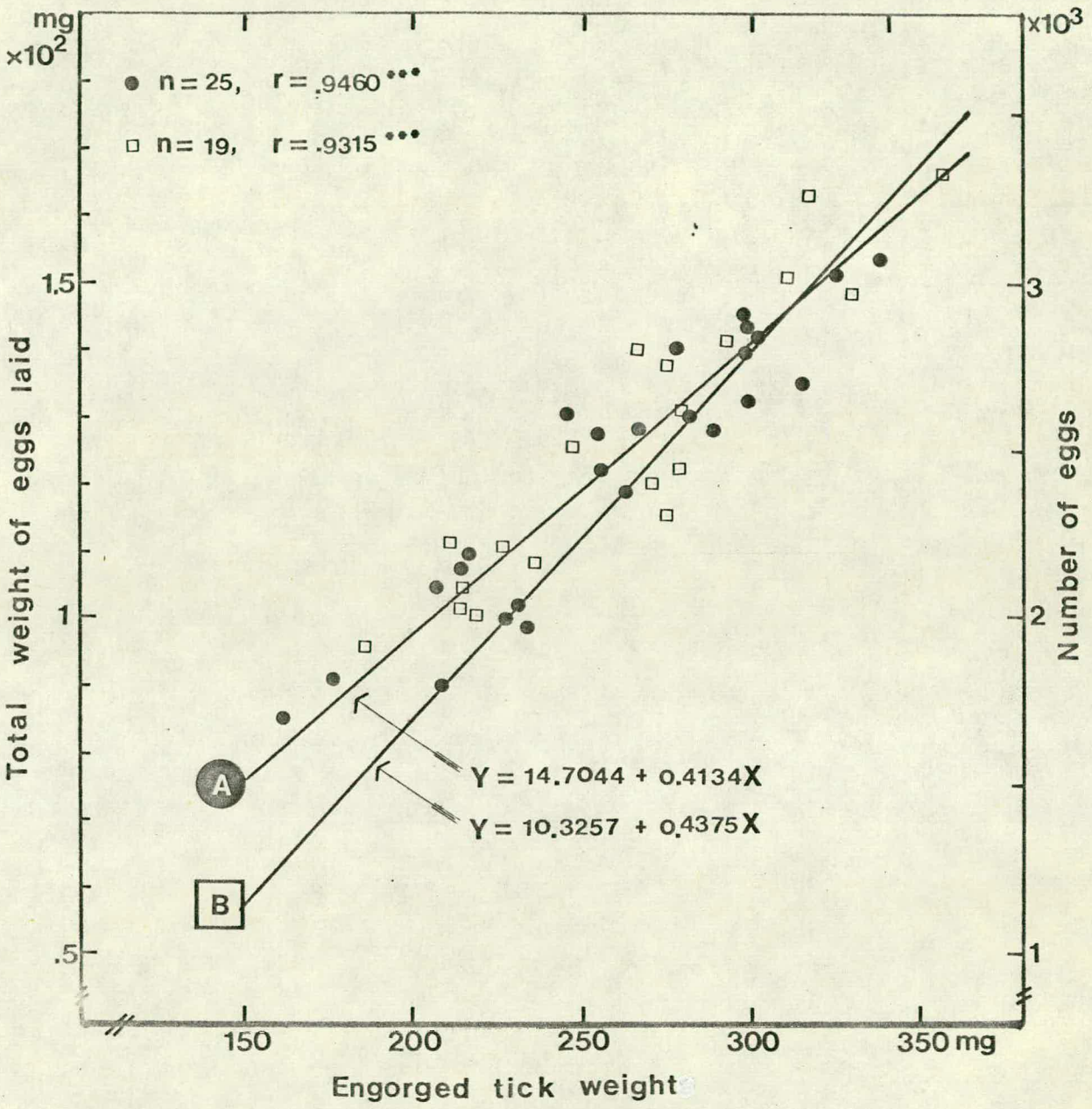

Fig. 19. Relationship between the oviposition ability and the engorged weights of each female of H. longicornis maintained at various temerature-humidity conditions.

Series A; 25°C and various relative humidities.

Series B; 85.0% relative humidity and various temperatures.

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	7368.31	3	2456.103	4.031*
Error	9140.17	15	609.345	-
Total	16508.48	18	-	-

Table 25: Analysis of Variance for the Mean Daily Output of Eggs by *H. longicornis* Females at 83-85% Relative Humidity and Various Temperatures (Series B)

Besides these physical factors, the correlation between the oviposition ability and the engorged weight of the female tick was also examined. There was a very highly significant correlation between the total weight of eggs laid and the engorged weight of the female tick (Figure 19) regardless of the effects of relative humidity. ($Y = 14.7044 + 0.4134X$, $N = 25$, $r = 0.9460^{***}$), or of temperature ($Y = 10.3257 + 0.4375X$, $N = 19$, $r = 0.91315^{***}$). The overall mean number calculated from the total weight of eggs laid was 9.49 eggs per mg of the engorged body weight of the tick.

Oviposition Pattern;

The observed oviposition pattern varied greatly in individuals, but there was a common trend that the peak of this oviposition curve appeared within two to five days after the onset of egg-laying,with a successive gradual decline thereafter (Figures 20 and 21). Therefore, it was possible to divide the entire oviposition period arbitrarily into three phases, i.e. the 1st phase (inductive phase,

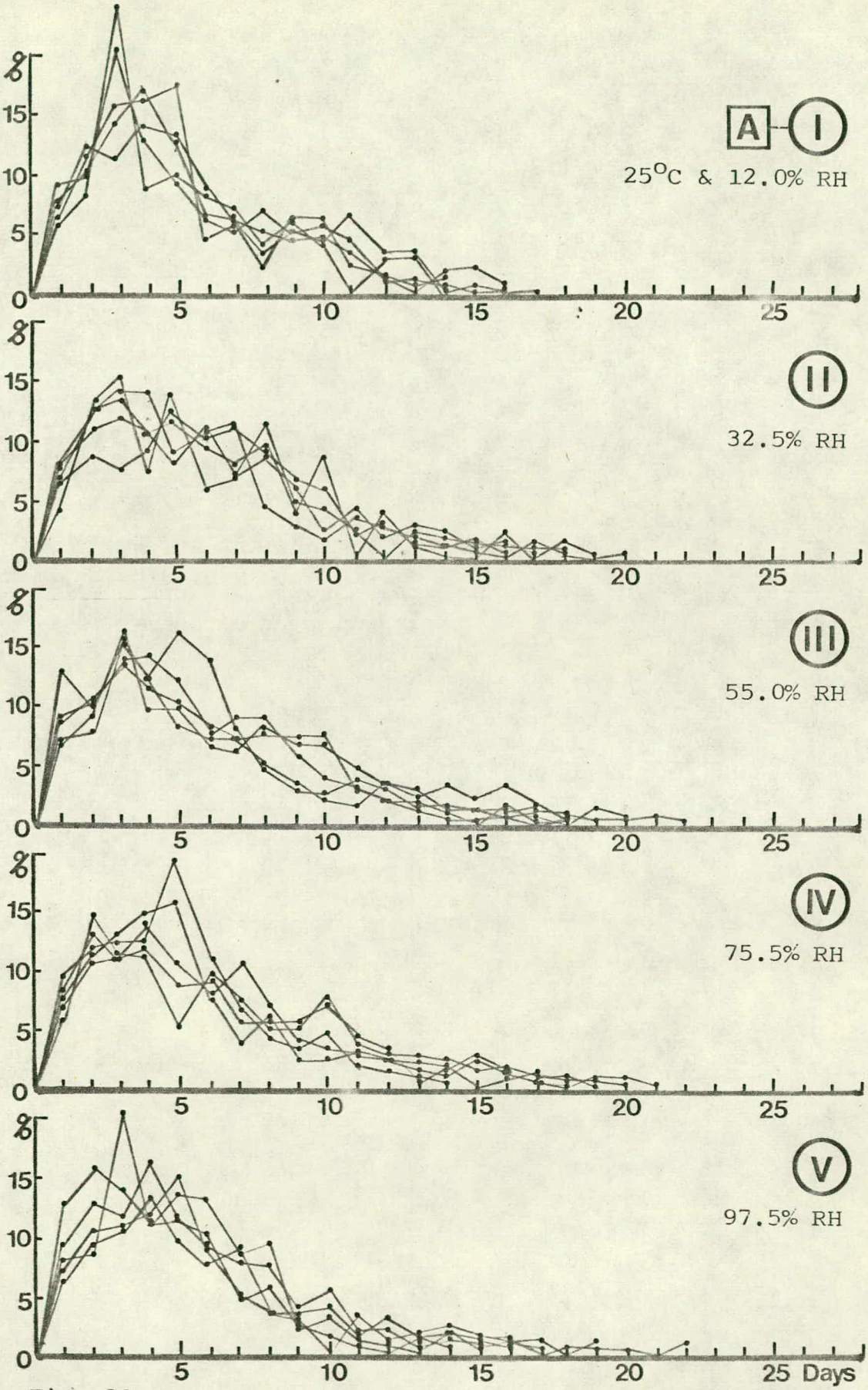

Fig. 20. Oviposition records in individuals(Series A).

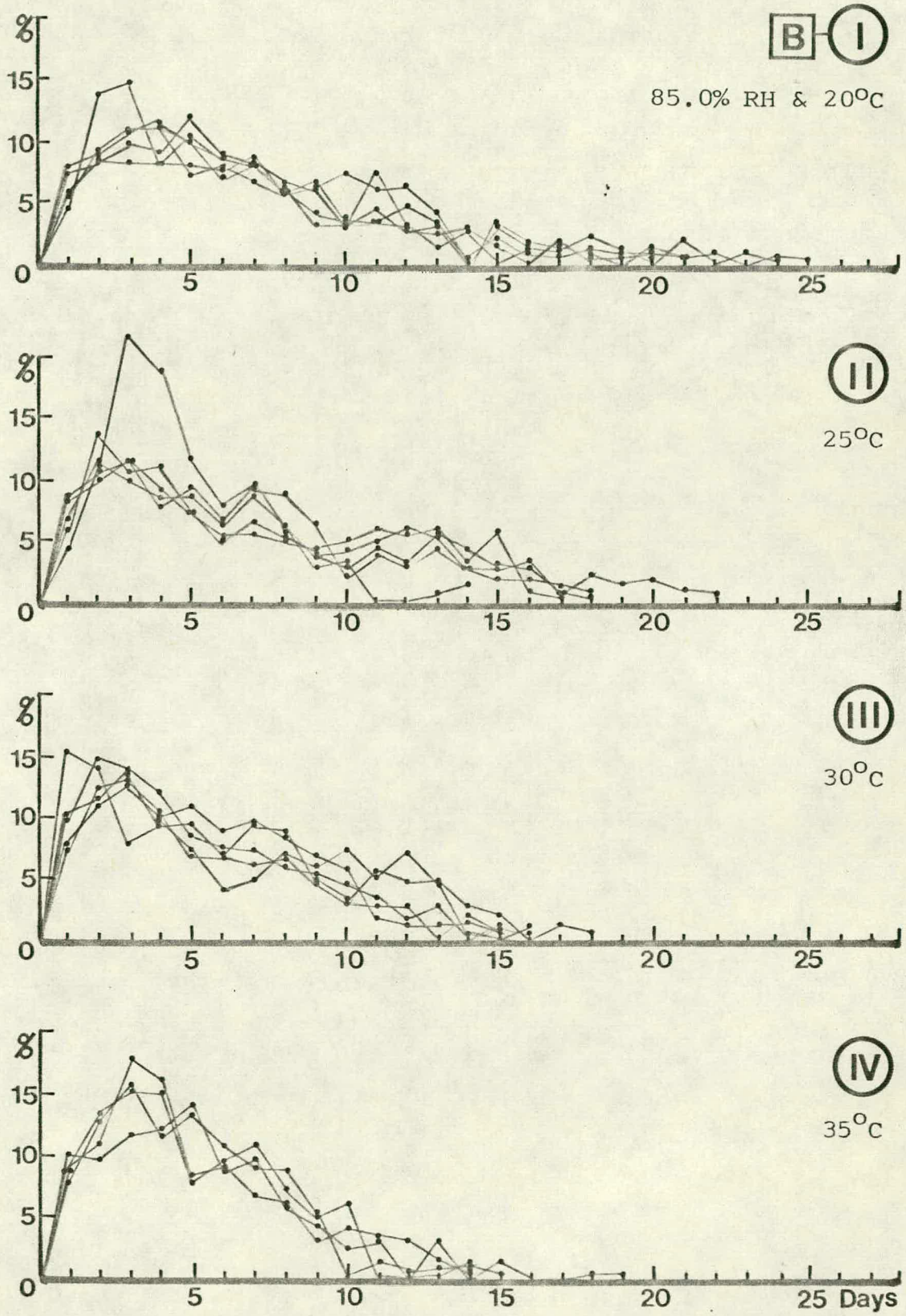

Fig. 21. Oviposition records in individuals(Series B).

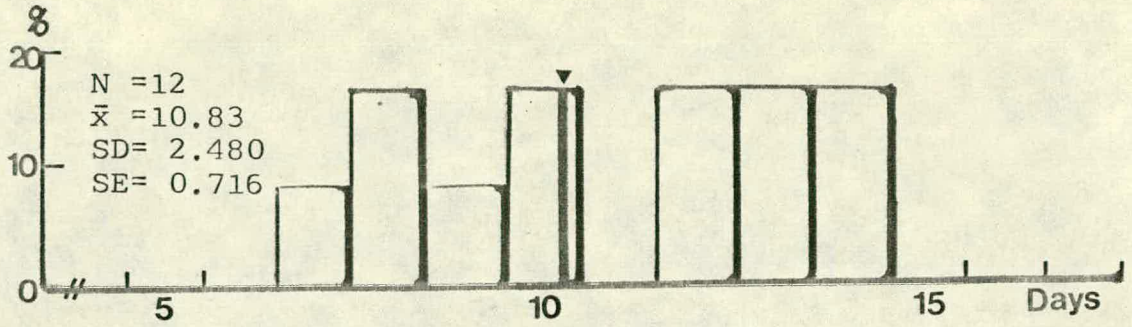

Fig. 22. The frequency(%) distribution of the pre-oviposition period in days of H. longicornis females fed without males.

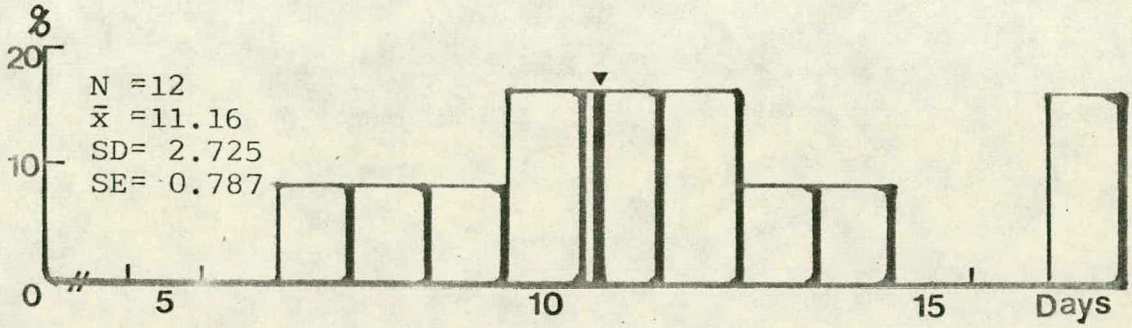

Fig. 23. The frequency(%) distribution of the oviposition period in days of H. longicornis females fed without males.

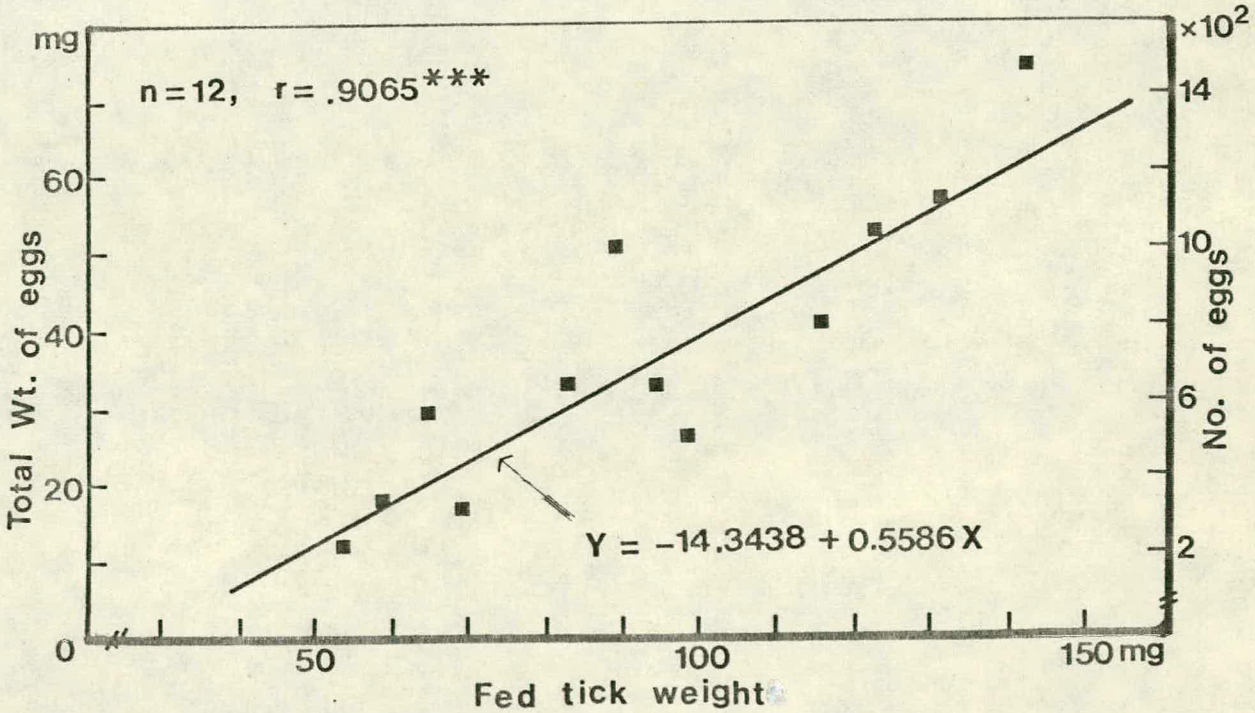

Fig. 24. Relationship between the oviposition ability and the fed weights of females fed without males.

usually the very short period of the beginning, day 1 to day 2, of the oviposition); the 2nd phase (prosperous phase, usually from day 2 to day 10); and the 3rd phase (debilitated phase, residual period of the oviposition). However, no significantly different oviposition pattern influenced by the treatments of temperature or humidity was observed throughout the experimental period.

On the other hand, the females fed without males showed poor oviposition ability. The mean percentage of the total amount of eggs laid to the engorged weight was 38.91%, the mean daily egg output 66.28 eggs, and the mean number of eggs per mg of body weight was 7.8 eggs (Appendix V). However, there was also a very high significant correlation between the total amount of eggs laid and the fed weight of the female ($Y = -14.3438 + 0.5586X$, $N = 12$, $r = 0.9065^{***}$) (Figure 24).

2. INFLUENCE ON THE INCUBATION PERIOD AND HATCHABILITY OF THE EGGS

The design for this experiment was the same as in Section 1 overleaf, with the exception of the origin of the sample specimens and the sample-size. To increase the homogeneity of the sample and to reduce the possible bias, the specimen eggs were collected on the same day from the egg batches laid by a group of females kept at the same physical conditions of 25°C and 85.0% relative humidity since engorgement. The collected eggs were pooled, counted and divided into groups of 200 eggs per test-tube (13 mm x 50 mm), and then a total of 108 tubes was incubated in each experimental condition, so that each experimental group held 12 replicates containing 200 eggs, each having the same pre-conditioning history. Skill

was required in the handling of these vulnerable eggs. Although this experiment was done under almost completely dark conditions, special care was taken to avoid the side-effects of light during the examination time and to minimise the exposure time.

Incubation Period;

This period is often called 'egg developmental period', 'hatching period' or 'pre-eclosion period'. It was defined here as the minimum interval which elapsed between the day of egg-laying and the commencement of larval emergence from the egg-cluster. The mean incubation period was defined here as the arithmetic mean of the incubation periods (minima) of the groups used as replicates under the same experimental conditions, with the result that it did not indicate the mean period of the same (one) egg-cluster.

Firstly, the influence of relative humidity on the mean incubation period and successful hatching rate (%) (of the clusters) of the eggs at 25°C and various relative humidities (Series A) was examined. As shown in Table 26 no larva emerged from the eggs maintained at or below 32.5% relative humidity, while at 55.0% relative humidity the larvae emerged from only three egg-clusters out of a total of 12(25%). But at the higher relative humidities the larvae emerged from more egg-clusters, i.e. from 9 egg-clusters (75%) at 75.5% relative humidity and from 10 egg-clusters (83.33%) at 97.5% relative humidity. The mean incubation periods for Groups III (55.0% relative humidity) IV (75.5% relative humidity) and (V, relative humidity were, respectively, 28.33 days, 27.11 days and 26.70 days (Figure 25).

The overall mean incubation period in the Series A was 27.09 days (N = 22, SD = 1.087, SE = 0.232, and Velocity = 0.037). There was, however, no significant difference within relative humidity treatment groups (Group III, IV and V) in the statistical analysis of variance ($F = 3.138$ with 2/19 degrees of freedom, $p > 0.05$) (Table 27). Therefore, it was concluded that relative humidity did not influence the incubation period of the eggs but influenced the successful hatching rate (%) of the egg-clusters.

On the other hand, as shown in Table 28, at constant condition of 83.85% realtive humidity, temperature greatly influenced the incubation period of the eggs (Series B). In the experimental Group I (20°C), the mean incubation period was 46.82 days, and it was shortened as temperature increased, i.e. 26.89 days, 21.75 days, and 21.17 days in the Groups of II (25°C), III (30°C), and IV (35°C), respectively. The velocities of each group were 0.021, 0.037, 0.046, and 0.047, respectively. At low temperature, the velocity was retarded apparently (Figure 26).

The statistical analysis of variance (Table 29) showed that there was a very highly significant difference ($F = 116.818$ with 3/30 degrees of freedom, $p < 0.001$) within temperature treatment groups. It was of interest however that as temperature increased the successful hatching rate (%) of the egg-clusters in the group decreased, i.e. from 91.67% at 20°C to 75%, 66.67%, and 50%, at 25°C, 30°C and 35°C, respectively.

		Treatment Groups				
		I	II	III	IV	V
Replicates	1.	-	-	-	27	29
	2.	-	-	-	24	26
	3.	-	-	-	26	25
	4.	-	-	28	-	30
	5.	-	-	-	29	26
	6.	-	-	-	27	-
	7.	-	-	-	-	27
	8.	-	-	31	-	25
	9.	-	-	26	26	26
	10.	-	-	-	27	-
	11.	-	-	-	30	27
	12.	-	-	-	28	25
No. of clusters hatched		0	0	3	9	10
%		0	0	25	75	83.33
Mean incubation period		-	-	28.33	27.11	26.70

Table 26: The Incubation Period (Days) of *H. longicornis* Eggs Maintained at 25°C and Various Relative Humidities (Series A)

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	6.163	2	3.0815	3.138 (NS)
Error	18.656	19	0.19819	-
Total	24.819	21	-	-

Table 27: Analysis of Variance for the Incubation Period (Days) of *H. longicornis* Eggs Maintained at 25°C and Various Relative Humidities (55.0% Relative Humidity, 75.5% Relative Humidity and 97.5% Relative Humidity) (Series A)

		Treatment Groups			
		I	II	III	IV
Replicates	1.	49	26	27	-
	2.	-	31	-	-
	3.	51	-	24	21
	4.	46	-	20	-
	5.	43	27	-	18
	6.	57	24	21	-
	7.	44	30	21	18
	8.	50	25	-	26
	9.	45	25	-	19
	10.	43	-	20	-
	11.	44	29	21	25
	12.	43	25	20	-
No. of clusters hatched		11	9	8	6
%		91.67	75	66.67	50
Mean incubation period (days)		46.82	26.89	21.75	21.17

Table 28: The Incubation Periods (Days) of *H. longicornis* Eggs Maintained at 83-85% Relative Humidity and Various Temperatures (Series B)

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	4168.67	3	1389.557	116.818***
Error	356.86	30	11.895	-
Total	4525.53	33	-	-

Table 29: Analysis of Variance for the Incubation Periods (Days) of *H. longicornis* Eggs Maintained at 83-85% Relative Humidity and Various Temperatures (Series B)

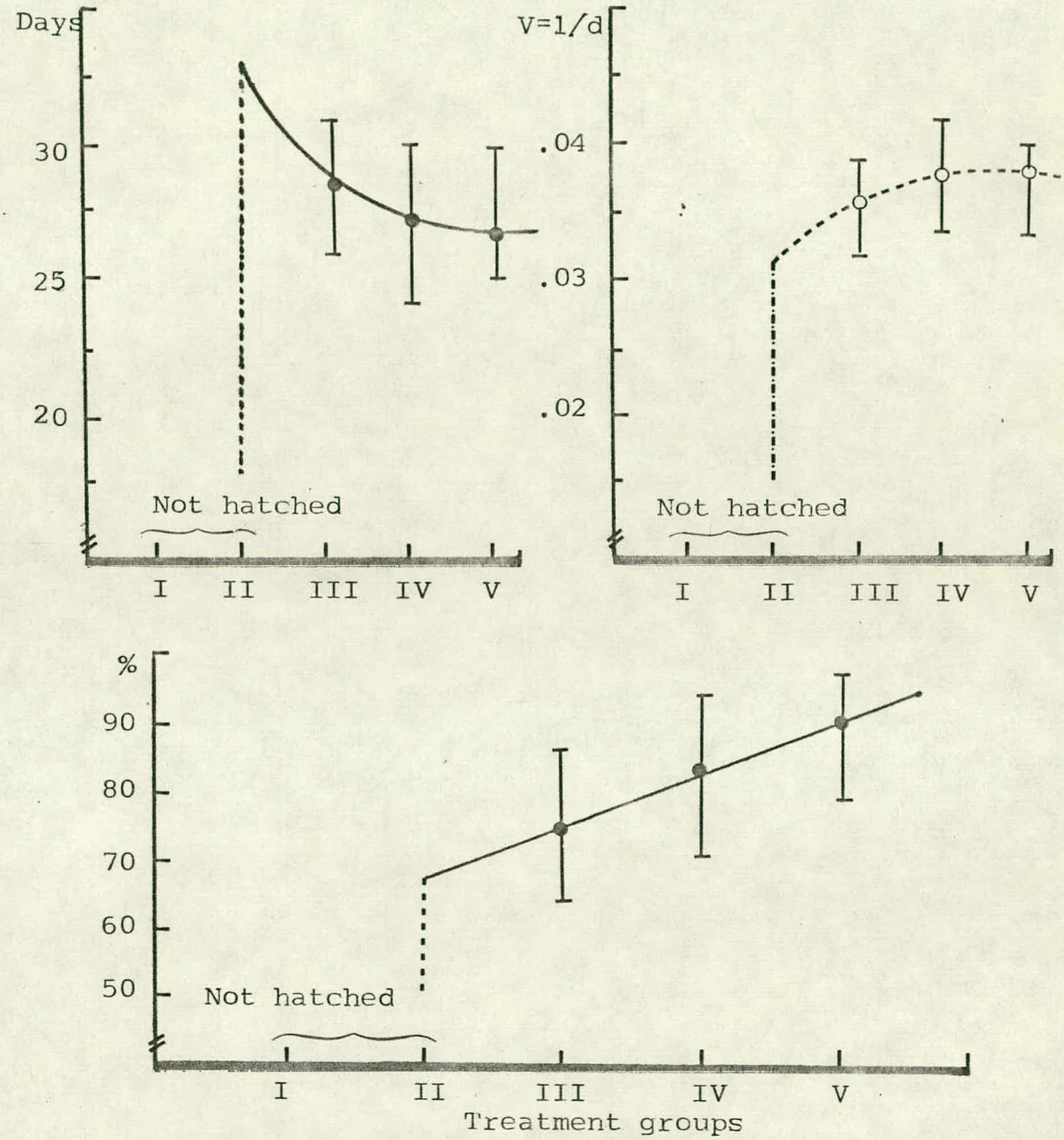

Fig. 25. The incubation periods (Means, Minima & Maxima) in days and velocities, and hatching rates (Means, Minima & Maxima) in % of the eggs maintained at 25°C and various relative humidities (Series A).

(I 12.0% RH, II 32.5% RH, III 55.0% RH, IV 75.5% RH, and V 97.5% RH)

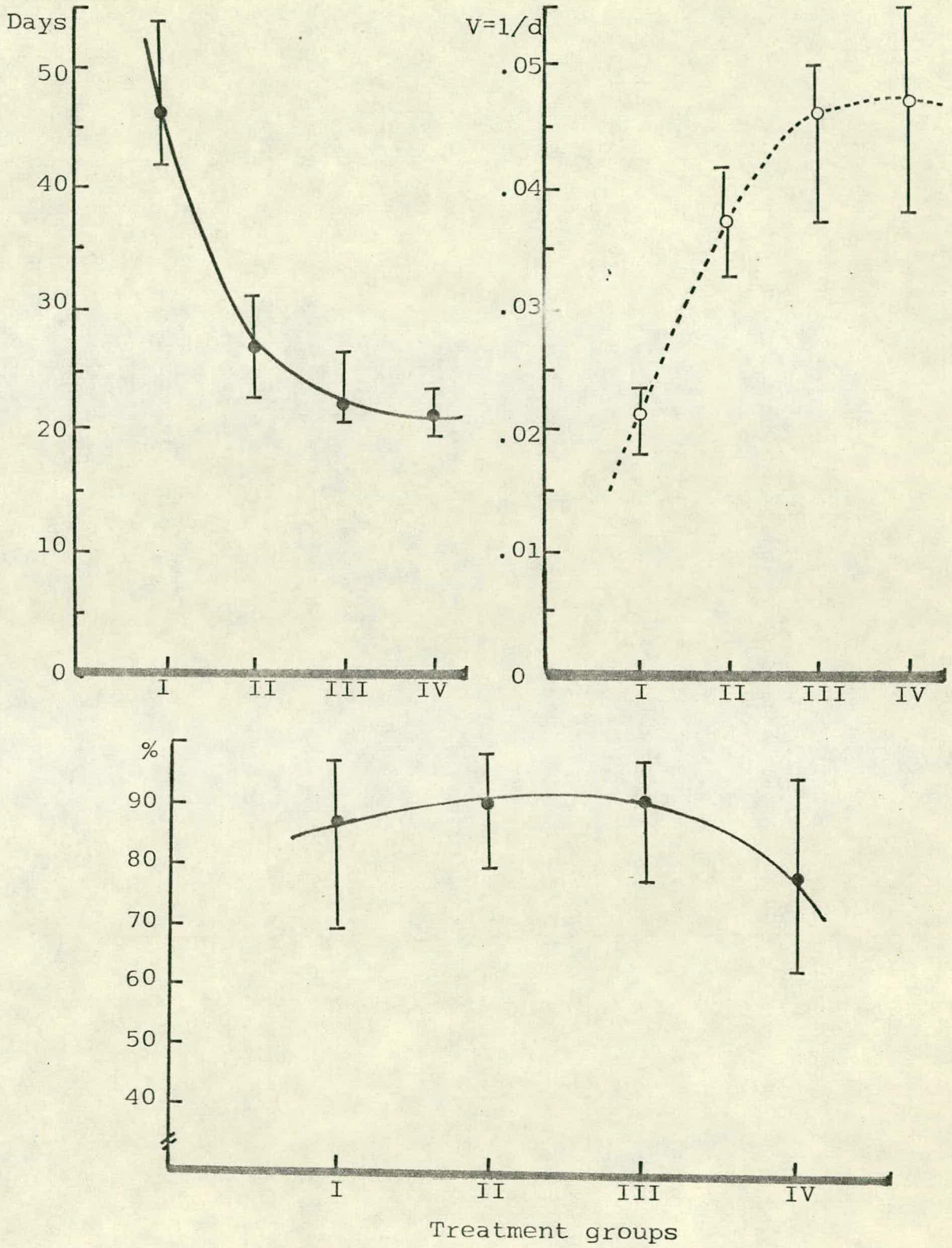

Fig. 26. The incubation periods (Means, Minima & Maxima) in days and velocities, and hatching rates (Means, Minima & Maxima) in % of the eggs maintained at 85.0% relative humidity and various temperatures (Series B).

(I 20°C, II 25°C, III 30°C, and IV 35°C)

Hatching Rate (%);

This term was defined here as the percentage of the number of larvae which emerged to the number of eggs examined, i.e. the hatching rate of the eggs in a cluster. This differed from the hatching rate of the clusters mentioned in the subsection overleaf, as that was defined as the percentage of the number of egg-clusters hatched to the number of egg-clusters examined in the same treatment condition.

With the exception of the egg-clusters not hatched, the mean hatching rate (%) of the eggs at 25°C and various relative humidities, was 85.02%, and it ranged from 63.5% to 97.5% (Table 30). Hatching of the eggs was not possible below 32.5% relative humidities, and it was thus clear that the hatching rate was greatly influenced by humidity and that the critical point was around 55.0% relative humidity, whereas the optimum point was around 85.0% relative humidity to near saturation point (Figure 25). The statistical analysis of variance (Table 31) showed that there was a highly significant difference ($F = 6.038$ with 2/19 degress of freedom, $p < 0.01$) within the 3 relative humidity treatment groups ranging 55.0% relative humidity to 97.5% realtive humidity. However, the non-hatched groups were not included in the analysis. If they were included in the analysis, needless to say the significance level would be increased.

On the other hand, the hatching rate (%) was also influenced by temperatre (Table 32). The maximum mean rate (%) was 91.75 at a combination of 30°C and 84.5% relative humidity, and it was decreased by raising the temperature or lowering it (Figure 26). Therefore, it was clear that the optimum point was 30°C, and the upper critical point was above 35°C. However, the lower critical

		Treatment Groups (% R.H.s)				
		I	II	III	IV	V
		(12.0)	(32.5)	(55.0)	(75.5)	(97.5)
Replicates	1.	-	-	-	85	85.5
	2.	-	-	-	94.5	88.5
	3.	-	-	-	90	97.5
	4.	-	-	71.5	-	94
	5.	-	-	-	77	92
	6.	-	-	-	81.5	-
	7.	-	-	-	-	95.5
	8.	-	-	86	-	79
	9.	-	-	63.5	80.5	90.5
	10.	-	-	-	74	-
	11.	-	-	-	93.5	87
	12.	-	-	-	70.5	93.5
Mean (%)		-	-	73.67	82.94	90.3
N		0	0	3	9	10

Table 30: The Hatching Rates (%) of *H. longicornis* Eggs Maintained at 25°C and Various Relative Humidities (Series A)

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within treatments	704.26	2	352.13	6.038**
Error	1107.99	19	58.315	-
Total	1812.25	21	-	-

Table 31: Analysis of Variance for the Hatching Rates (%) of *H. longicornis* Eggs Maintained at 25°C and Various Relative Humidities (55.0% Relative Humidity, 75.5% Relative Humidity and 97.5% Relative Humidity)(Series A)

		Treatment Groups (Temperatures)			
		I	II	III	IV
		(20°C)	(25°C)	(30°C)	(35°C)
Replicates	1.	88.5	86	92	-
	2.	-	94.5	-	-
	3.	90	-	78	84.5
	4.	76.5	-	94.5	-
	5.	81	79.5	-	95.5
	6.	97.5	96	97.5	-
	7.	93.5	92	84.5	63.5
	8.	85	83.5	-	80
	9.	91	89	-	79.5
	10.	69.5	-	98	-
	11.	96.5	98.5	96.5	72.5
	12.	90.5	97	93	-
Mean (%)		87.23	90.67	91.75	79.25
N		11	10	8	6

Table 32: The Hatching Rates (%) of *H. longicornis* Eggs Maintained at 83-85% Relative Humidity and Various Temperatures (Series B)

Sources of Variation	Sums of Squares	Degrees of Freedom	Mean Squares	F
Within treatments	640.99	3	213.663	3.179*
Error	2016.57	30	67.219	-
Total	2657.56	33	-	-

Table 33: Analysis of Variance for the Hatching Rates (%) of *H. longicornis* Eggs Maintained at 83-85% Relative Humidity and Various Temperatures (Series B)

point was not clarified in this experiment. The analysis of variance (Table 33) showed that there was a significant difference ($F = 3.179$ with 3/30 degrees of freedom, $p < 0.05$) within temperature treatment groups.

Hatchability of the Eggs Laid by Single Females

From the 12 egg-batches produced by the females fed without males and maintained at 25°C and 85.0% relative humidity, no larva emerged. It was concluded that the Weonseong strain of *H. longicornis* used in this project was a normally reproducing bisexual race and that no sporadic parthenogenesis was observed.

CHAPTER THREE : RESULTS

PART III. INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON THE METAMORPHOSES OF THE FED INSTARS

The results were obtained from the two major experiments which were carried out for each fed stage, i.e. the experiment on the metamorphosis of the fed larva to the nymph, and the experiment on the metamorphosis of the fed nymph to the adult (the female or the male tick). Each experiment was designed for observation in two steps: firstly, the preliminary observation of the development of the fed instar at a combination of 25°C and 85.0% relative humidity, and secondly, the observation of the effects of various temperature-humidity combinations on the development of the fed instar. A total of 20 combined conditions in a range of four temperatures (20°C, 25°C, 30°C and 35°C) and a range of five relative humidities (below 12.5% relative humidity, 32.5 - 33.0% relative humidity, 55.0 - 56.0% relative humidity, 75.5 - 76.0% relative humidity and over 96.0% relative humidity) was set up for the experiment. An adequate number of tick groups was used as replicates in every experimental group. Therefore, for the statistical analysis of variance, the two-factor ANOVAR method was applied to the normal variables. Thus, three sources of variation, within temperature treatments, within relative humidity treatments, and within replicates in the same experimental group, were compared to the interactions of each combined factor.

The terms used in these experiments were defined as follows: The term 'premoulting period' was used to describe the time interval which elapsed between the engorgement of the individual tick in a developmental stage and the emergence of the next developmental stage, such as, from an engorged larva to the newly moulted nymph, from an engorged nymph to the newly moulted adult, either female or male. The term 'mean premoulting period' was used for the arithmetic mean of the premoulting periods in the same experimental group. The term 'percentage moulting rate' was used to present the percentage rate of the number of ticks moulted to the total number of ticks examined in the same experimental group as replicate. And the term 'mean percentage moulting rate' was used for the arithmetic mean of the moulting rates (%) in the same treatment group.

1. INFLUENCE ON THE LARVA-TO-NYMPH METAMORPHOSIS

Results from the Preliminary Observation at 25°C and 85.0% Relative Humidity;

A total of 9345 engorged larvae collected from the laboratory animals (rabbits) was maintained at 25°C and 85.0% relative humidity to observe metamorphosis. As the results shown in Appendix VI., the mean percentage moulting rate was 86.80% (8111 nymphs out of 9345 larvae) and it ranged from 73.60% to 96.52%. On the other hand, the overall mean premoulting period was 14.10 days (N = 8111, SD = 1.421, SE = 0.016; and Velocity = 0.071) and it ranged from minimum 12 days (Velocity = 0.083 to maximum 18 days (Velocity = 0.056). The percentage frequency

distribution (Figure 27) showed the slightly skewed histogram and that the day when most observations were obtained was the day 14.

Results from the Experiment at Various Temperature-Humidity Combinations;

A total of 5000 engorged larvae (50 larvae x 5 replicates x 4 temperatures x 5 relative humidities) was used in this experiment upon the larva -to-nymph metamorphosis (Table 34).

	Temperature			
	A ($20^{o}C$)	B ($25^{o}C$)	C ($30^{o}C$)	D ($35^{o}C$)
Relative Humidity				
Group I (Below 12.5%)	A total of 20 experimental groups was used.			
Group II (32.5 - 33.0%)	Each experimental group consisted of 5 groups of replicates containing 50 engorged larvae in each replicate.			
Group III (55.0 - 56.0%)				
Group IV (75.5 - 76.0%)				
Group V (Over 96.0%)				

Table 34: Experimental Design for the Larva-to-nymph Metamorphosis of *H. longicornis* at Various Temperature-humidity Combinations

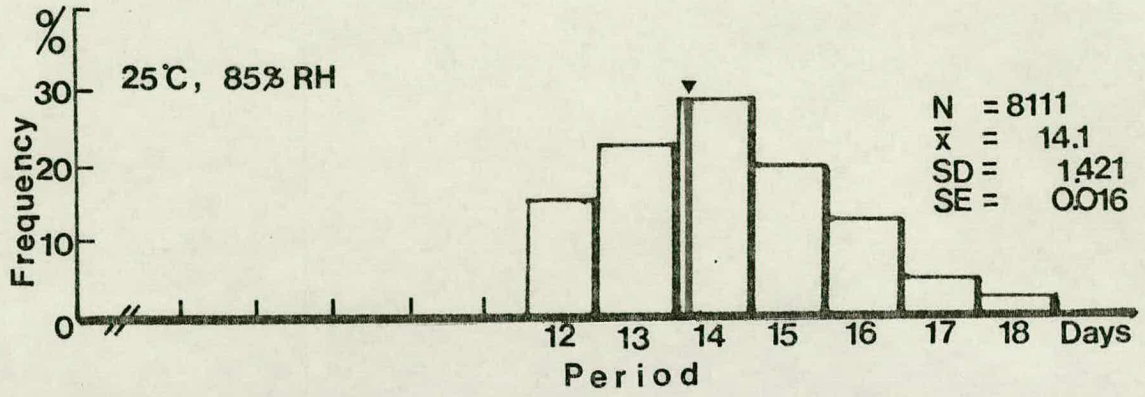

Fig. 27. The frequency(%) distribution of the premoulting periods in days of H. longicornis engorged larvae maintained at 25°C and 85.0% relative humidity.

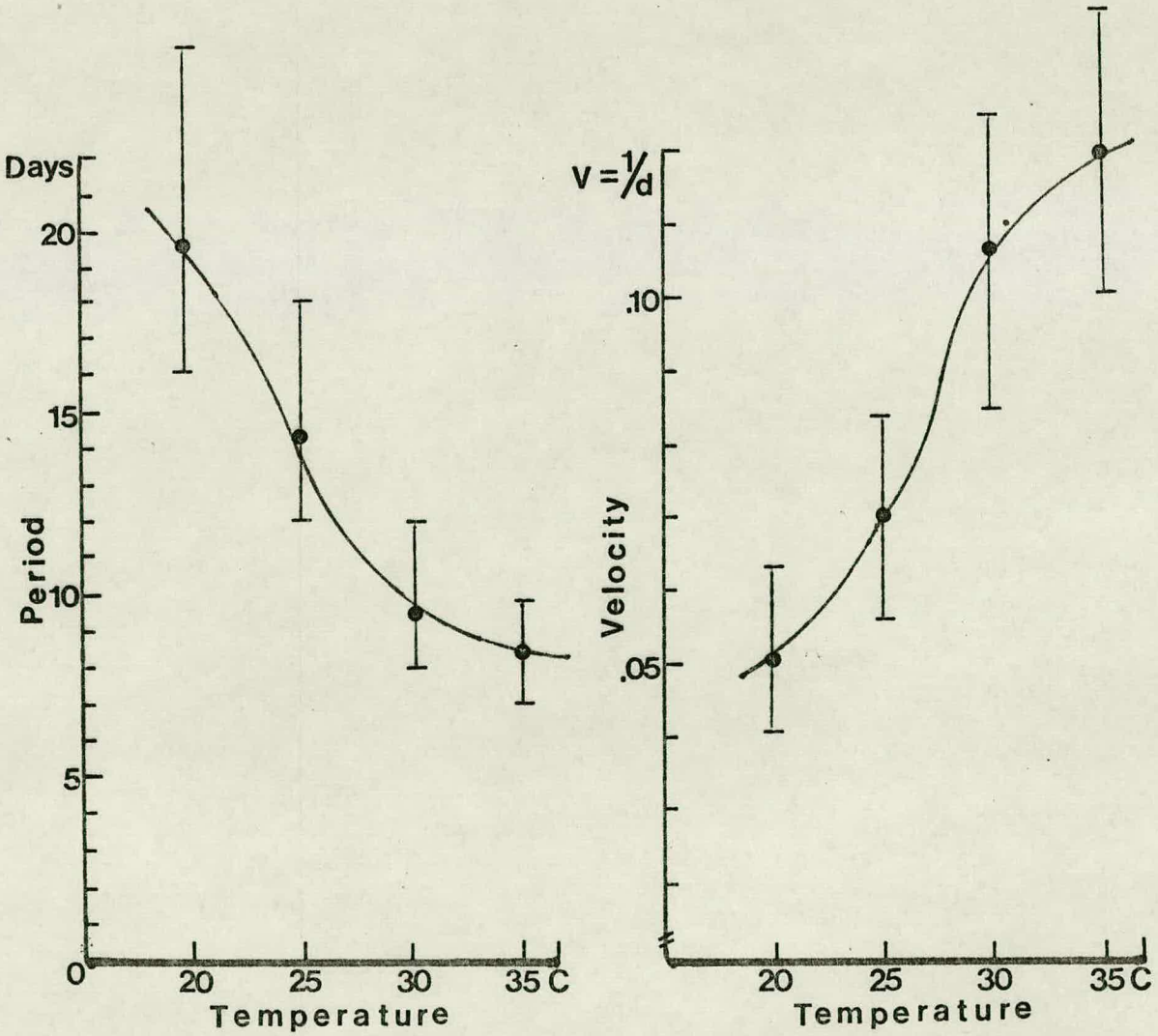

Fig. 28. A comparison of the effect of temperature on the premoulting periods and velocities of engorged larvae.

Regardless of the temperature, no metamorphosis was observed at the lower humidities (below 12.5% relative humidity and 32.5 - 33.0% relative humidity). And, as the relative humidity increased the mean moulting rate (%) also increased. In the experimental Series A (20°C and various relative humidities) the mean moulting rates (%) were 61.2%, 79.6%, and 90.8%; in Series B (25°C and various relative humidities) 80.4%, 86.8% and 93.6%; in Series C (30°C and various relative humidities) 48.0%, 76.4%, and 89.6%; and Series D (35°C and various relative humidities) 34.0%, 66.4%, and 88.4% at relative humidity treatment Group III, Group IV, and Group V, respectively. Details were shown in Appendix VII-1, 2, 3, and 4.

It was thus clear that the moulting rate (%) of engorged larvae was greatly influenced by realtive humidity. The statistical analysis of variance (Table 35) for the actual mean moulting rates excluding the non-moulted groups showed that there was no significant difference within the temperatures ($F = 3.715$ with 3/6 degrees of freedom, $p > 0.05$) and also within the replicates ($F = 0.707$ with 16/32 degrees of freedom, $p > 0.05$), but there was a highly significant difference within the relative humidity treatment groups ($F = 14.021$ with 2/6 degrees of freedom, $p < 0.01$).

Meanwhile, the mean premoulting period of the engorged larvae was affected by temperature (Figure 28). The overall pre-moulting periods ranged from 19.48 days (Velocity = 0.051) at 20°C, to 14.16 days (Velocity = 0.071) at 25°C, 9.46 days (Velocity = 0.106) at 30°C, and 8.35 days (Velocity = 0.120) at 35°C. Consequently, the mean developmental velocities for the larva-to-nymph metamorphosis were progressively accelerated as the treatment temperature increased

(1) Summarised Data for the Moulting Rates (%)

		Temperature				R.H. Totals
		A	B	C	D	
Group Totals						
R.H. Group	III	320	402	240	170	1132
	IV	398	434	382	332	1546
	V	454	468	448	442	1812
Repl. Totals						
Recpliates	1	242	274	204	206	
	2	232	262	230	192	
	3	246	262	212	190	
	4	226	246	210	180	
	5	226	260	214	176	
Temp. Totals		1172	1304	1070	944	4490 (GT)

(2) Two-Factor ANOVAR for the Separate Temperatures

	F-Value	
Temperature	Between R.H. treatments	Between replicates
Series A (20°C)	17.809	0.556
B (25°C)	10.967	6.658
C (30°C)	32.683	0.453
D (35°C)	66.942	0.818

(3) Combined ANOVAR for the Data Analysed Temperature Level by Temperature Level

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Temperatures	4667.40	3	155.8	3.715 (NS)
R.H. Treatments	11742.54	2	5871.27	14.021**
Temperatures x R.H.s interaction	2512.30	6	418.734	-
Replicates	553.61	16	34.6	0.707 (NS)
R.H.s x replicates interaction	1566.39	32	48.95	-
Total	21042.34	59	-	-

Table 35: Statistical Analysis for the Moulting Rates (%) of *H. longicornis* Larvae in the Experiment Carried Out at 4 Temperatures with a Two Factor Layout at Each Temperature for 3 Relative Humidities in 5 Groups of Replicates Containing 50 Larvae in Each Replicate

(1) Summarised Data for the Mean Premoulting Periods

	Temperature				R.H. Totals
	A	B	C	D	
Group Totals					
R.H. Group III	100.03	72.48	48.59	42.39	263.49
IV	97.89	70.07	47.53	41.66	257.15
V	95.09	70.00	46.54	41.57	253.20
Repl. Totals					
Replicates 1	58.60	42.04	28.07	25.06	
2	58.38	42.48	27.97	25.02	
3	58.94	43.22	29.34	25.10	
4	58.23	42.50	28.38	25.37	
5	58.86	42.31	29.80	25.07	
Temp. Totals	293.01	212.55	142.66	125.62	773.84 (GT)

(2) Two-Factor ANOVAR for the Separate Temperatures

	F-Value	
Temperature	Between R.H. Treatments	Between Replicates
Series A (20°C)	27.154	0.676
B (25°C)	10.589	1.695
C (30°C)	5.147	2.736
D (35°C)	1.099	0.179

(3) Combined ANOVAR for the Data Analysed Temperature Level by Temperature Level

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Temperatures	1163.835	3	387.945	2191.78***
R.H. treatments	2.695	2	1.347	7.61*
Temperatures x R.H.s interaction	1.059	6	0.177	-
Replicates	0.851	16	0.053	1.325 (NS)
R.H.s x replicates interaction	1.284	32	0.040	-
Total	1169.724	59	-	-

Table 36: Statistical Analysis for the Mean Premoulting Periods (Days) of *H. longicornis* Larvae in the Experiment Carried Out at 4 Temperatures with a Two-Factor Layout at each Temperature for 3 Relative Humidities in 5 Groups of Replicates Containing 50 Engorged Larvae in each Replicate

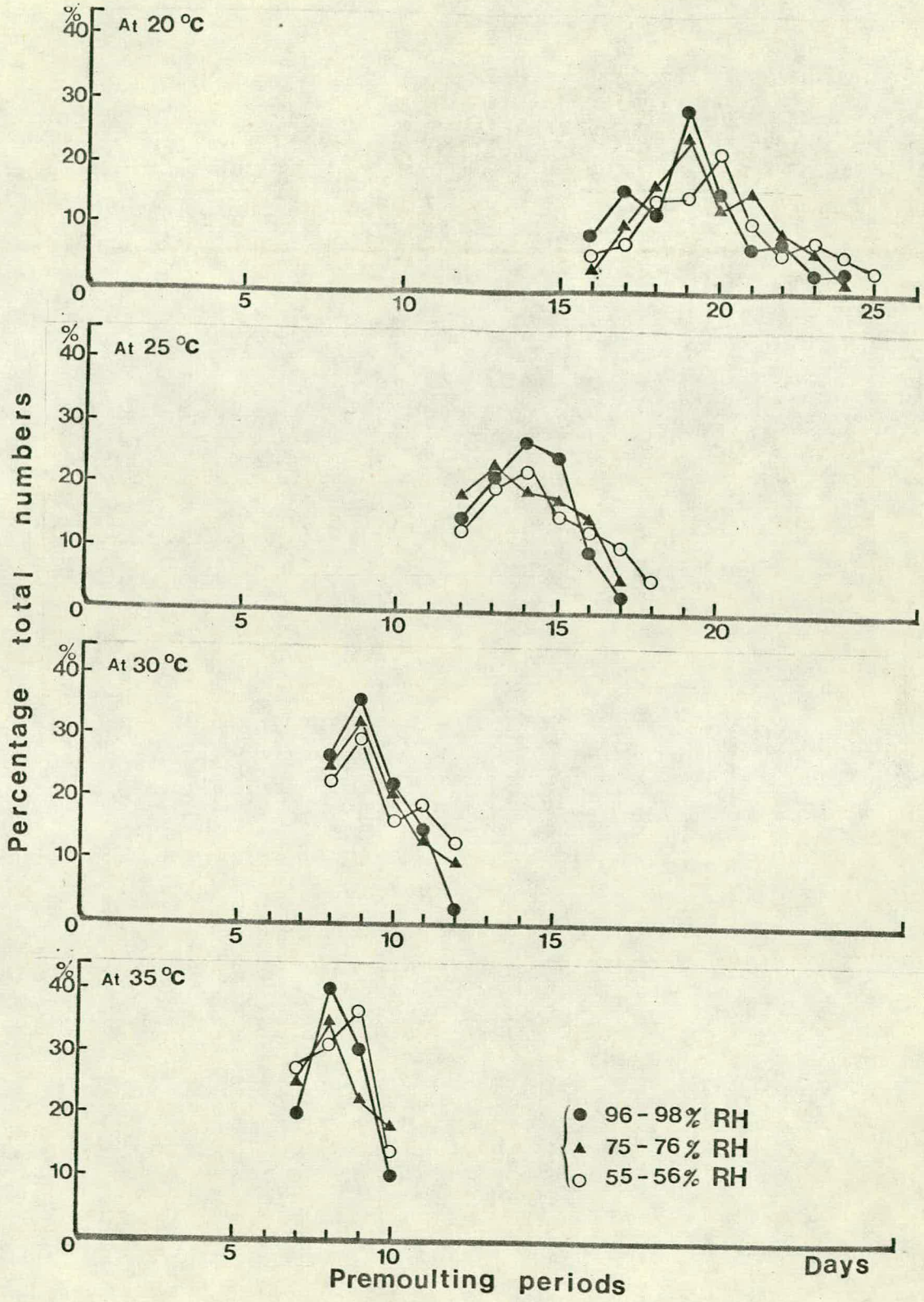

Fig. 29. The frequency(%) distribution of the premoulting periods in days of H. longicornis engorged larvae maintained at various temperature-humidity combinations.

(Figure 28), and, the percentage frequency distribution of the larva-to-nymph premoulting periods showed clearly the influence of temperature on the development of the fed larvae (Figure 29). The statistical analysis of variance (Table 36) for the actual mean premoulting periods excluding the non-moulted groups, showed that there was a very highly significant difference within temperatures ($F = 219.78$) with 3/6 degrees of freedom, $p < 0.001$), and a significant difference within relative humidity treatments ($F = 7.61$ with 2/6 degrees of freedom, $p < 0.05$), but there was no significant difference within replicates ($F = 1.325$ with 16/32 degrees of freedom, $p > 0.05$).

Therefore, it was concluded that the moulting rates (%) was only affected by relative humidity, whereas, on the contrary, the premoulting period was affected by temperature. Also, it was concluded that the critical point of relative humidity was around 32.5 - 56.0% relative humidity regardless of the influence of treatment temperature (20°C to 35°C). It was regretted, that the critical points (upper limit and lower limit) of temperature were not assessed by this experiment on the larva-to-nymph metamorphosis.

2. INFLUENCE ON THE NYMPH-TO-ADULT METAMORPHOSIS

Results from the Preliminary Observation at 25°C and 85.0% Relative Humidity;

A total of 2247 engorged nymphs colonised in the laboratory was maintained at 25°C and 85.0% relative humidity throughout the meta-

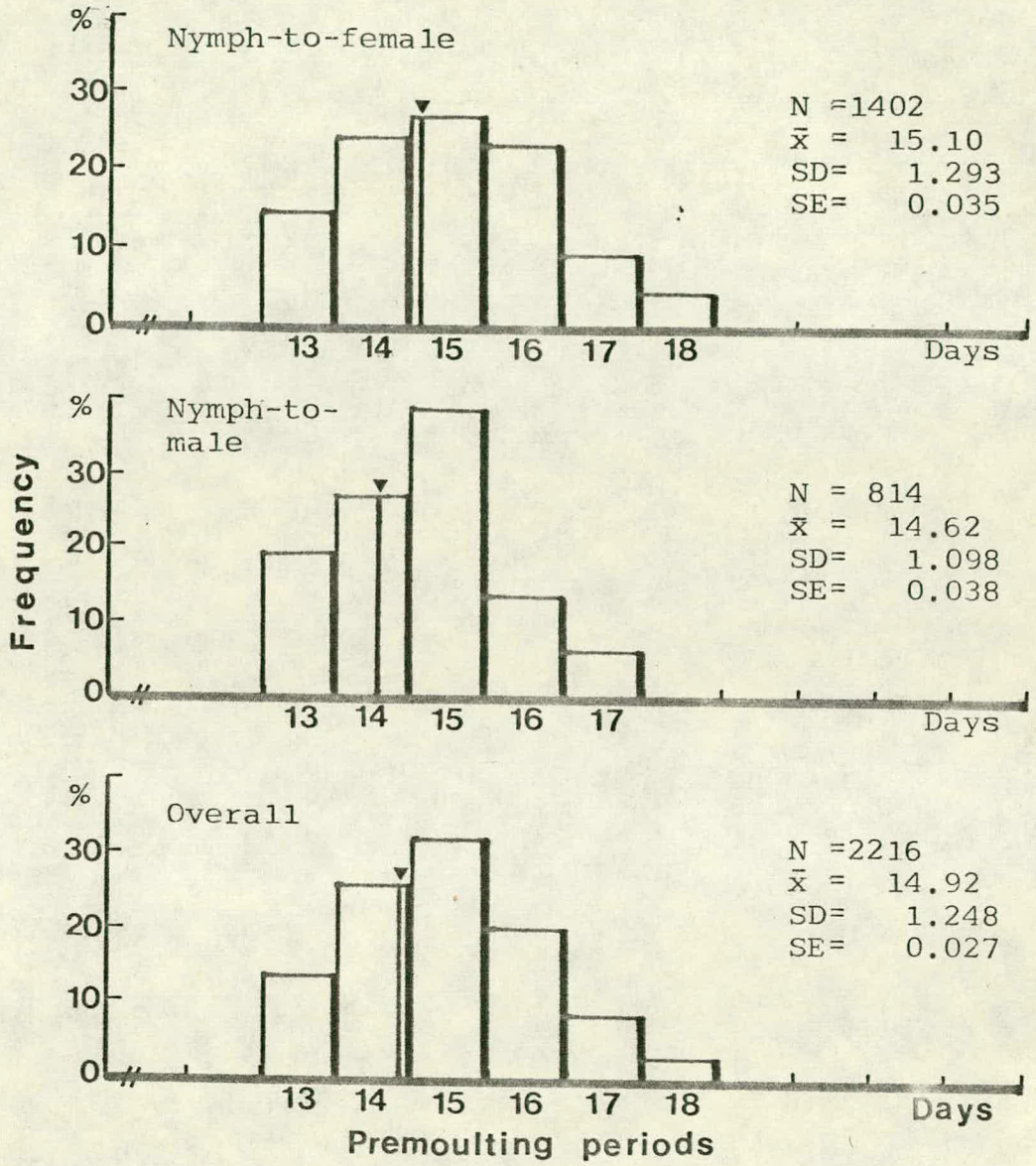

Fig. 30. The frequency(%) distribution of the premoulting periods in days of H. longicornis engorged nymphs maintained at 25°C and 85.0% relative humidity.

morphosis period after engorgement. As the results shown in Appendix VIII, the overall mean moulting rate was 98.62% (2216 adults moulted out of 2247 engorged nymphs) and only 31 nymphs failed to moult (1.38%). The engorged nymphs moulted to either adult females or adult males, and the sex ratio of the newly moulted adults was 1.72:1 (1402 females: 814 males). The overall mean premoulting period in the nymph-to-adult metamorphosis experiment, was 14.94 days (N = 2216, SD = 1.248, SE = 0.027, and Velocity = 0.067), and it ranged from minimum 13 days (Velocity = 0.077) to maximum 18 days (Velocity = 0.056). The percentage frequency distribution of the nymph-to-adult premoulting period showed that the day when most observations were obtained was the Day 15, and it was slightly delayed in comparison to the larva - to-nymph metamorphosis (Figure 30). On the other hand, the mean premoulting periods differed from each other according to the sex of the newly moulted adults. In the nymph-to-female metamorphosis, it was 15.10 (N = 1402, SD = 1.294, SE = 0.035, and Velocity = 0.066), while, in the nymph-to-male metamorphosis it was 14.62 days (N = 814, SD = 1.098, SE = 0.039, and Velocity = 0.068), and the statistical comparison of the two sample means showed that there was a very highly significant difference between the premoulting periods of the nymphs to each sex ($t = 8{,}889^{***}$, $F = 78.95$ with 1/2214 degree of freedom, $p < 0.001$).

Results from the Experiment at Various Temperature-Humidity Combinations;

A total of 720 engorged nymphs (12 nymphs x 3 replicates

x 4 temperatures x 5 relative humidities) was used in this experiment on the nymph-to-adult metamorphosis. The experimental design was as shown in Table 37.

	Temperature			
	A ($20^{o}C$)	B ($25^{o}C$)	C ($30^{o}C$)	D ($35^{o}C$)
Relative Humidity				
Group I (Below 12.5%)	A total of 20 experimental groups was used. Each experimental group consisted of 3 groups of replicates containing 12 engorged nymphs in each replicate.			
Group II (32.5 - 33.0%)				
Group III (55.0 - 56.0%)				
Group IV (75.5 - 76.0%)				
Group V (Over 96.0%)				

Table 37: Experimental Design for the Nymph-to-adult Metamorphosis of *H. longicornis* at Various Temperature-Humidity Combinations.

The results as shown in Appendix IX-1, 2, 3, and 4., were very similar to the results of the experiment on the larva-to-nymph metamorphosis although there was no significant critical point of relative humidity in the experiment on the nymph-to-adult metamorphosis. The overall mean moulting rate (%) throughout the experiment was 95.97% (691 adults out of 720 nymphs), however, there was a general trend that as the relative humidity increased the mean moulting rate (%) also increased. The analysis of variance (Table 38) confirmed its statistical significance ($F = 9.511$ with 4/12 degrees of freedom, $p < 0.01$), and showed that there was also

significant difference within temperatures ($F = 5.571$ with 3/12 degrees of freedom, $p < 0.05$) but there was no significant difference within replicates ($F = 0.823$ with 8/32 degrees of freedom, $p > 0.05$). On the other hand, the mean premoulting periods of the engorged nymphs in each experimental series were greatly affected by temperature (Figure 31). It ranged from 23.68 days (Velocity = 0.042) at 20°C, to 14.98 days (Velocity = 0.067) at 25°C, 10.75 days (Velocity = 0.093) at 30°C, and 9.59 days (Velocity = 0.104) at 35°C. The overall premoulting period was 14.75 days (Velocity = 0.068). The statistical analysis of variance (Table 39) also showed that there was a very highly significant difference within temperatures ($F = 7672.99$ with 3/12 degrees of freedom, $p < 0.001$) and a significant difference within relative humidity treatments ($F = 4.477$ with 4/12 degrees of freedom, $p < 0.05$), but no significant difference within replicates ($F = 1.25$ with 8/32 degrees of freedom, $p > 0.05$). The percentage frequency distribution of the premoulting periods in the nymph-to-adult metamorphosis showed clearly the influence of temperature on the development of the engorged nymphs.

The statistical comparison of the two sample means at each temperature showed that the mean premoulting period in the nymph-to-female metamorphosis was longer than that in the nymph-to-male metamorphosis (Appendix X). The results were summarised as follows:

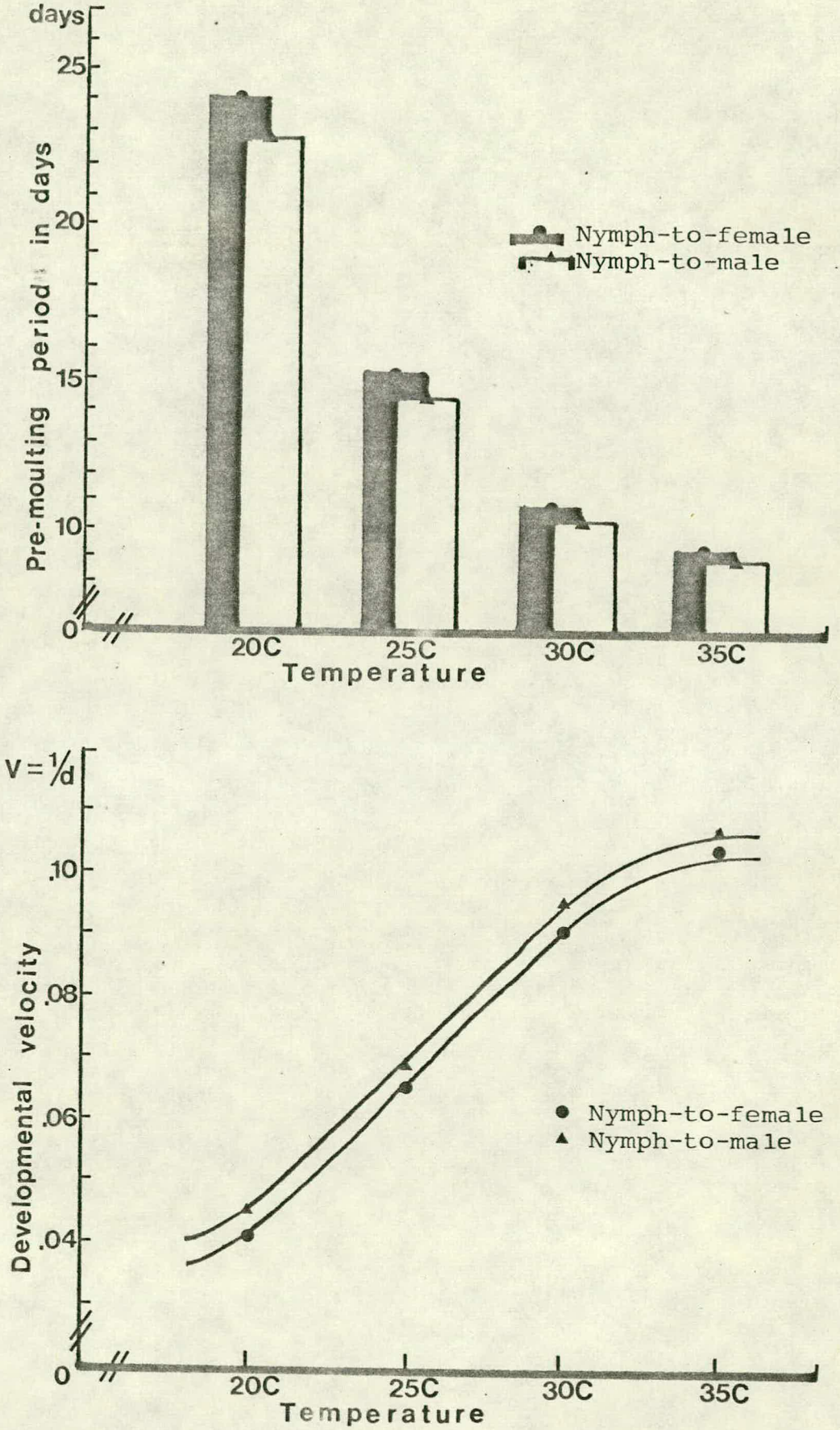

Fig. 31. The mean premoulting periods in days and their developmental velocities of engorged nymphs.

At 20°C, 24.135 days for females and 22.905 days for males, ($t = 4.616$, $F = 21.308^{***}$ with 1/165 d.f., $p < 0.001$),

At 25°C, 15.113 days for females and 14.654 days for males, ($t = 2.617$, $F = 6.847^{**}$ with 1/174 d.f., $p < 0.01$),

At 30°C, 10.920 days for females and 10.609 days for males, ($t = 2.555$, $F = 6.527^{*}$ with 1/173 d.f., $p < 0.05$),

At 35°C, 9.646 days for females and 9.476 days for males, ($t = 2.191$, $F = 4.797^{*}$ with 1/171 d.f., $p < 0.05$).

Figure 32 showed the percentage frequency distribution of the pre-moulting periods in *H. longicornis* nymph-to-adult metamorphosis at various treatment temperatures.

It was thus concluded that the moulting rates (%) of the engorged nymphs was mainly affected by humidity, while the pre-moulting period (days) was greatly affected by temperature; and that there was, however, no significant critical point of relative humidity range; and that the temperature range used in this experiment was within favourable range including the optimum point (25°C to 30°C) for the development of the engorged numphs; and that the developmental velocity in the nymph-to-female metamorphosis was lower than that in the nymph-to-male metamorphosis.

(1) Summarised Data for the Moulting Rates (%)

		Temperature				R.H. Totals
		A	B	C	D	
Group Totals						
R.H. Group	I	258.33	283.34	283.34	266.67	1091.68
	II	266.67	291.67	283.34	291.67	1133.35
	III	291.67	291.67	291.67	291.67	1166.68
	IV	291.67	300	300	291.67	1183.34
	V	283.34	300	300	300	1183.34
Repl. Totals						
Replicate	1	475	491.67	491.67	475	
	2	466.68	491.67	483.34	491.67	
	3	450	483.34	483.34	475.01	
Temp. Totals		1391.68	1466.68	1458.35	1441.68	5758.39 (GT)

(2) Two-factor ANOVAR for the Separate Temperatures

	F-Value	
Temperature	Between R.H. Treatments	Between Replicates
Series A (20°C)	3.667	1.556
B (25°C)	1.000	0.286
C (30°C)	1.429	0.286
D (35°C)	2.839	0.937

(3) Combined ANOVAR for the Data Analysised Temperature Level by Temperature Level

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Temperatures	225.71	3	75.237	5.571*
R.H. Treatments	513.80	4	128.45	9.511**
Temperatures x R.H.s interaction	162.06	12	13.505	-
Replicates	118.46	8	14.808	0.823 (NS)
R.H.s x replicates interaction	575.77	32	17.993	-
Total	1595.80	59	-	-

Table 38: Statistical Analysis for the Moulting Rates (%) of *H. longicornis* Nymphs in the Experiment Carried Out at 4 Temperatures with a Two-Factor Layout at Each Temperature for 5 Relative Humidities in 3 Groups of Replicates Containing 12 nymphs in each Replicate

(1) Summarised Data for the Mean Premoulting Periods

		Temperature				R.H. Totals
		A	B	C	D	
Group Totals						
R.H. Group	I	72.46	45.27	32.81	29.45	179.99
	II	71.18	44.91	32.65	28.53	177.27
	III	70.92	45.17	32.24	28.81	177.14
	IV	71.26	44.74	31.50	28.72	176.22
	V	69.33	44.58	32.01	28.34	174.26
Repl. Totals						
Replicate	1	118.40	75.19	53.74	48.30	
	2	119.36	74.82	53.66	47.56	
	3	117.39	74.66	53.81	47.99	
Temp. Totals		355.15	224.67	161.21	143.85	884.88 (GT)

(2) Two-factor ANOVAR for the Separate Temperatures

	F-Value	
Temperature	Between R.H. Treatments	Between Replicates
Series A (20°C)	2.898	1.344
B (25°C)	1.022	0.544
C (30°C)	9.440	0.114
D (35°C)	6.716	3.142

(3) Combined ANOVAR for the Data Analysed Temperature Level by Temperature Level

Sources of Variation	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Temperatures	1835.763	3	611.921	7672.999***
R.H. Treatments	1.428	4	0.357	4.477*
Temperatures x R.H.s interaction	0.957	12	0.080	-
Replicates	0.475	8	0.059	1.25 (NS)
R.H.s x replicates interaction	1.519	32	0.047	-
Total	1840.142	59	-	-

Table 39: Statistical Analysis for the Mean Premoulting Periods of *H. longicornis* Nymphs in the Experiment Carried Out at 4 Temperatures with a Two-Factor Layout at Each Temperature for 5 Relative Humidities in 3 Groups of Replicates Containing 12 Nymphs in Each Replicate

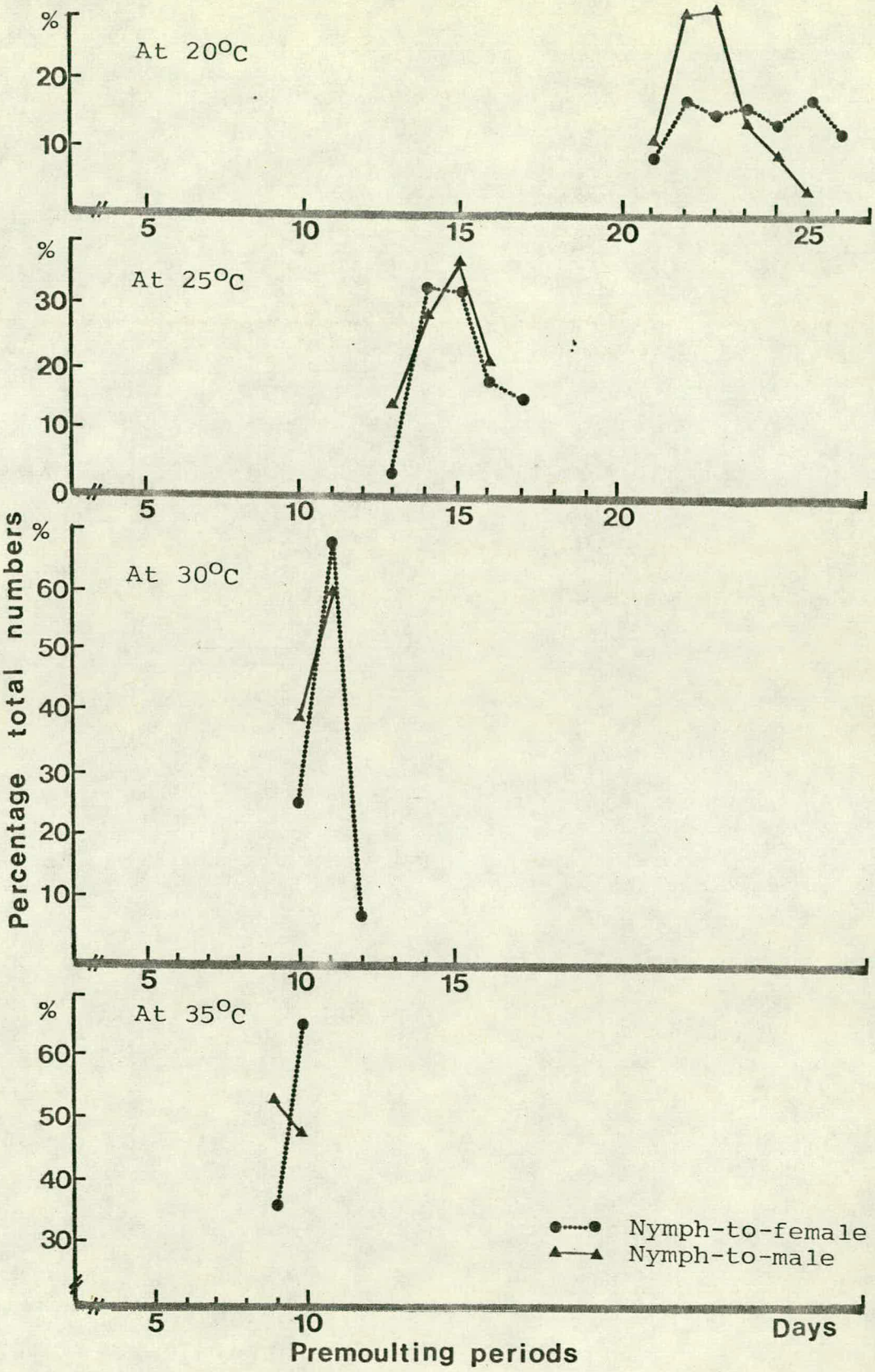

Fig. 32. The frequency(%) distribution of the premoulting periods in days of H. longicornis engorged nymphs.

CHAPTER FOUR : DISCUSSION

In 'The Law of Population', Thomas Robert Malthus (1766-1834) made two postulates: firstly, that food is necessary to the existence of man; secondly, that passion between the sexes is necessary and will remain nearly in its present state. Of course it refers to human population. What about parasitism?

Parasitism is an ecological relationsip between at least two populations, parasites and hosts, and the interactions between them are crucial in determining parasite numbers. When the host population density is very high the percentage of parasitism increases, but when the population density is low the percentage of parasitism becomes low. Bradley (1974) studied the stability in host-parasite systems and attempted to combine mathematical modelling, field data and experimental work. He postulated that these three approaches needed to proceed forwards together: field data alone lead to confusion, laboratory work in isolation may lead to distortion, and mathematical models alone lead to complication. His approaches were largely applicable to protozoa and helminths. However, the parasitic insects would be rather considered as micro-predators, Rogers and Hubbard (1974) studied the parasite-host model in their recent observations and experiments on insect parasites and predators, and concluded that within any particular parasite or predator generation the efficiency of the individual was increased by its tendency to spend more time in regions of high host or prey density; that there would be a dynamic

balance between aggregation of the searching population and the increased interference that is bound to occur; and that selection might determine the quantitative interaction between the two. However, the superficially simple host-parasite relation is often complicated by the presence of free-living stages or by the intervention of extra-ordinary factors.

First of all, the determination of the numbers of the parasite applied is most important for understanding the host-parasite relationship or parasite population ecology in laboratory feeding experiments on the tick. The numbers of ticks applied on a host vary greatly and the determination will depend on the following factors:

a. Purpose of the colonisation
b. Availability of the initial tick material
c. Species, age, and size of the host
d. Site for feeding on the host body
e. Tolerance/resistance of the host to tick infestation, and
f. Species, developmental stage, age, and tenability of the tick.

In this project, the different numbers of ticks at each developmental stage were applied to host animals for assessing the influence of tick-population on the feeding performance of the ticks. Therefore, the mean successful feeding number in a percentage unit, mean feeding period in days (with its reciprocal value, velocity), and mean engorged weight of the tick at each developmental stage, were the main object of the observations. The results showed

that an increase in the number of ticks applied, either larvae, nymphs, or adults, resulted in an overall extension in the duration of the mean feeding periods, and in an overall reduction in the mean rate (%) of the successful engorgements. But the large or small number of ticks applied did not affect the mean engorged weight of the ticks fed in the laboratory. Branagan (1969) observed that an increase in the number of the brown ear tick, *Rhipicephalus appendiculatus* nymphs applied resulted in an overall reduction in the length of the engorgement period. Increasing the number of nymphs applied from 300 to 400 per bovine ear to 500 to 750 per bovine ear reduced the mean engorgement period from 10.03 days to 8.54 days. When very much larger number of ticks were applied (2000 to 3000 per bovine ear), the mean engorgement period was further reduced to 6.29 days. It was the very reverse of the results obtained from the observations and experiments of the host-parasite relation between feeding and population of the tick, *H. longicornis* in present work. Branagan, however, suggested two plausible conjectures to explain the association of shorter nymphal engorgement periods with heavier infestations: the first of those was 'clustering behaviour', the tendency for applied nymphs to congregate to each other before attaching; and the second, 'rejection mechanism', a manifestation of the acquisition by the host of resistance to tick infestation.

It is quite true that ticks possess the clustering behaviour and that hosts get acquired immunity and produce immune responses when they are attacked repeatedly by the tick. However, none of those behavioural characteristics (clustering) of ticks or the immune responses (rejection mechanism) of hosts can explain so easily the

variation of tick-feeding periods in certain circumstances. Therefore, there is some difficulty in accepting Branagan's suggestion. Firstly, as a matter of fact, the clustering habit of ticks is not reckoned as a pre-requisite behaviour for attachment but it could be regarded as an attempt to get out of the tangled tick-pile and to find a host to feed on. If a small number of ticks were maintained in a relatively big container and they were applied to the host one after another, the clustering behaviour would scarcely appear. It has also been noted in the author's experiments that the clustering problem usually disappeared within the few hours before attachment, so that it was really irrelevant. Secondly, with regard to the postulations that the hosts do not carry any innate immunity to the tick infestation or do not have any acquired immunity with the tick infestation at the beginning of the feeding experiment, then Branagan's conjecture of the 'rejection mechanism' is hardly understandable. He tried to insist that the 'rejection mechanism' was involved in reducing the length of nymphal feeding periods and that 'rejection' was accelerated by the application of greater numbers. However, it should be noted that after first meeting the antigen (a kind of protein produced by the tick, for instance) there is an interval of about two weeks before the acquired antibody (which is the agent for the 'rejection mechanism') can be detected. Moreover, the antibody which eventually can be detected in the blood (so called 'primary immune response') does not reach a high level and does not persist unless a second dose of antigen (with succeeding tick infestation, for instance) is given. To obtain a maximum response, the interval between the primary and secondary attack should not be too short and an

interval of less than 10 days is likely to reduce the level of secondary response. As the tick feeding usually ends within two weeks, what sort of immunological rejection mechanism could occur during this initial period? Therefore, it could be regarded as an ecological (or, probably socio-biological) problem relating to the availability of food and the population density of the parasite rather than an immunological one.

Feldman-Muhsam (1964) has alluded to the relation of tick feeding and immune response: during multiple feedings on a host, the first infestation sensitised the host, enabling the second infestation of ticks to feed better, and subsequently the host became immunised. Bailey (1960) has already noticed that it is false economy to use a rabbit more than once for laboratory tick feeding. Later, Gregson (1966) has recommeneded that considering the possibilities of an immunity build-up, it is better to aim at a large and healthy initial infestation than to reinfest and encounter resistance. On the other hand, Gregson (1966) has also indicated that, as another immunological problem, attention must be paid to the formation of immunity in individuals (of the tick stock-line) from repeated infestations on the same species of host, therefore, continued use of the same species of host is not recommended for the feeding of certain species of ticks. Also, there have been several interesting reports on resistance (innate or acquired immunity) in animals to ticks (Trager 1939a and 1939b, Hitchcock 1955, Riek 1962, Francis and Little 1978, Kemp 1978, and Wikel and Allen 1978). Studies on the resistance of ticks to acaricidal chemicals, on the other hand, provides another interesting research field in tick biology (Shaw 1966, Wharton and Roulston 1970, Drummond 1976, and Wharton 1976).

The Weonseong strain of *H. longicornis*, a bisexual race, originally collected from cattle in the Weonseong District of the central part of the Korean Peninsula, underwent a 3-host type of life-cycle in the laboratory as in the field. The whole life-cycle for one generation was divided into 4 stages (Figure 33):

Stage 0 --- From the egg to the newly hatched unfed larva. During this stage the eggs were incubated and developed, and larvae were hatched out from the eggs.

Stage I --- From the unfed larva to the newly moulted unfed nymph. During this stage the larval cuticle hardened, the larval feeding on the host (I) occurred, and the larva-to-nymph metamorphosis occurred.

Stage II --- From the unfed nymph to the newly moulted unfed adult. During this stage the nymphal feeding on the host (II) occurred, and the nymph-to-adult metamorphosis occurred (the nymph moulted to either an adult female or an adult male).

Stage III --- From the unfed adult to the end of the generation. During this stage the adult cuticle hardened, the adult (both female and male) feeding and mating on the host (III) occurred, the ovaries developed, and the oviposition occurred. Then the generation finished.

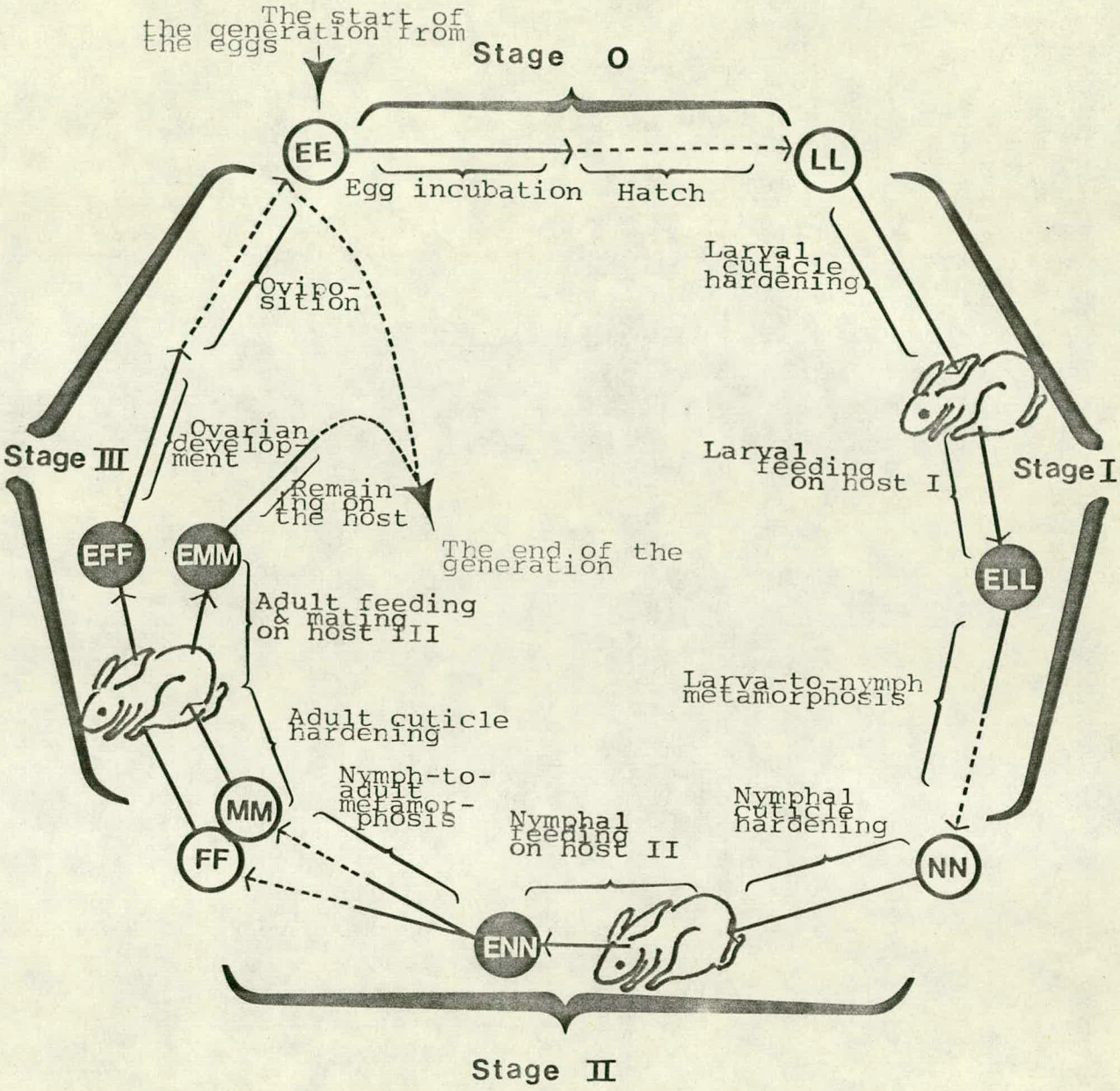

Fig. 33. A model for the life-cycle pattern and developmental stages of the tick H. longicornis based on the laboratory colonisation using the rabbit host.

(EE=Eggs, LL=Larvae, NN=Nymphs, FF=Adult Females, MM=Adult Males, ELL=Engorged Larvae, ENN=Engorged Nymphs, EFF= Engorged Adult Females, EMM=Engorged Adult Males)

Although the ticks require 1 or 2 years to complete their life-cycle in the field, it was possible to reduce it to a minimum of 2 months including approximately 10 days of cuticle hardening (3 to 4 days of post-moulting developmental periods at each instar) in the laboratory. This reduction of generation interval was recognised as the major benefit of laboratory colonisation. Also the laboratory colonisation provided a great quantity of specimens of known history and homogeneous quality at any developmental stage of the tick. Loomis (1961) described the laboratory life histories of *Amblyomma americanum*, *Dermacentor andersoni* and some argasid species, and recorded the possible minimum periods of 71 days for *A. americanum* and 96 days for *D. andersoni*.

The results obtained from the laboratory observation on the life-cycle of the Weongseong strain of *H. longicornis* were summarised and compared with those of the Shimane strain of *H. longicornis* (a bisexual race, Japanese origin) and the Gumma strain of *H. longicornis* (a parthenogenetic race, Japanese origin) both reported by Hoogstraal *et al.* (1968) (Table 40). It had been noted that the Weonseong strain showed apparently shorter generation interval than the Shimane strain of same bisexual type. The explanation of the difference of the generation intervals of the two strains was not determined, but it was assumed to be due to the different behaviour of oreintation resulting from the different climatic geographical origin of the ticks. On the other hand, the life-cycle of the Weonseong strain was very similar to that of the Australian parthenogenetic strain reported by Bremner (1959, as *H. bispinosa*). It was of most interest that the female-feeding periods of the parthenogenetic Gumma strain (mean 15.0 days) and

Observed by	Hoogstraal et al. (1968)		Author (Kang 1980)
Strains & Origins	Gumma Japan	Shimane Japan	Weonseong Korea
Reprodutive Type	Partheno-genetic	Bisexual	Bisexual
Host	Rabbit	Rabbit	Rabbit
Lab Condition	26°C - 28°C and 20% - 30% R.H.		*Various controlled Conditions
Events	Mean Periods in Days		
Egg Incubation	27.5	38.6	46.82 (45-57) at 20°C* 26.89 (24-31) at 25°C* 21.75 (20-27) at 30°C* 21.17 (18-26) at 35°C*
Larval Feeding	4.5	6.0	5.24 (3-9)**
Larva-to-nymph Metamorphosis	15.5	21.0	14.10 (12-18) at 25°C, 85% R.H. 19.48 (16-25) at 20°C* 14.16 (12-18) at 25°C* 9.46 (8-12) at 30°C* 8.35 (7-10) at 35°C*
Nymphal Feeding	6.0	4.5	4.75 (3-8) **
Nymph-to-adult Metamorphosis	14.0	15.0	14.94 (13-18)*** at 25°C 85% R.H. 23.68 (21-27) at 20°C* 14.98 (13-17) at 25°C* 10.75 (10-12) at 30°C* 9.59 (9-10) at 35°C*
Adult Feeding	15.0	12.0	9.73 (7-14)**** 15.32 (10-20)*****
Ovarian Development	5.0	4.5	15.2 (12-21) at 20°C* 7.6 (6-10) at 25°C* 4.8 (4-6) at 30°C* 4.5 (4-5) at 35°C* 10.83 (7-14)******

*	With variation by R.H. treatments at each temperature level.
**	With variation by tick-population density.
***	It is an overall mean for females (15.10) and males (14.62
****	For females fed with same number of males. With variation by tick-population density.
***** & ******	For females fed without males, and for their resultant egg

Table 40: Comparison of the Laboratory Observations on the Life-cycles of the Different Populations of *H. longicornis*

the Weonseong strain females fed without males (mean 15.32 days) were identical.

The results obtained from the laboratory observations on the weight records of each developmental stage and the oviposition records of the Weonseong strain of *H. longicornis* were summarised as shown in Table 41, and they were compared with the different geographical origin of the ticks reported by Kitaoka (1961d). It had already been reported that the parthenogenetic population was slightly larger in size than the bisexual population (Kitaoka (1961d), and Hoogstraal *et al.* (1968)). However, as shown in Table 41., it was of interest that each developmental stage of the Weonseong strain had an apparently heavier body weight both of unfed or engorged, than the Japanese bisexual population (the experimental host of the Weonseong strain was rabbit while the Japanese bisexual strain fed on cattle), although it was lighter in body weight than the Japanese parthenogenetic population.

On the other heand, the feeding efficiency (%) defined as the rate of body weight increase was reckoned as an appropriate parameter to estimate the net body-weight increase by blood-sucking of the tick. The feeding efficiencies at the each developmental stage of the Weonseong strain were approximately 1389% for the larva, 1343% for the nymph, 13660% for the adult female, and 63% for the adult male. The feeding efficiencies for the Japanese bisexual population calculated from the Kitaoka's data (1961d) showed 1145% and 1045% for the larva and for the nymph, respectively. At the mean time, the feeding efficiencies of the Japanese parthenogenetic population from the same source (Kitaoka 1961d) were calculated as 1155% and 1023% for the larva and for the nymph, respectively. It

Observed by	Kitaoka (1961)		Author (Kang, 1980)
Strains & Origins	Honshu Japan	Kyushu Japan	Weonseong Korea
Reprodutive Type	Partheno-genetic	Bisexual	Bisexual
Weight Records of Each Developmental Stage (mg unit)			
Host for Feeding	Cattle	Cattle	Rabbit
Egg	0.0686	0.0529	0.0500
Larva			
Unfed	0.047	0.040	0.040 (0.036*)
Engorged	0.590	0.498	0.536
Nymph			
Unfed	0.390	0.235	0.260 (0.240*)
Engorged	4.380	2.690	3.463
Adult			
Unfed			
Female	2.780	2.030	2.643 (1.831*)
Male	-	1.600	1.519 (1.140*)
Engorged			
Female	-	-	251.950 (71.840**)
Male	-	-	1.854 -
Oviposition Records			
Lab Conditions	30°C and 100% R.H.		Various Temp x R.H.
Number of eggs per mg body weight	8.26	11.51	9.49 (7.80**)
Percentage of total egg weight to body weight	58.2	61.4	47.45 (38.91**)
Oviposition potential (eggs/day/female)			139.94 (66.28**)
Total weight of eggs laid per female (mg)			123.18 (37.87**)

* Approximately 4 weeks after moulting (at the time of feeding) with variation of the weight according to the maintaining conditions.

** Data for the females fed without males.

Table 41: Comparison of the Laboratory Observations on the Weight Records of Each Developmental Stage and the Oviposition Records of the Different Populations of *H. longicornis* (as *H. bispinosa* in Kitaoka (1961))

was thus clear that the Weonseong strain showed higher feeding efficiencies at each developmental stage than both of the Japanese strains, bisexual or parthenogenetic.

The females of the Weonseong strain produced the mean of 123.18 mg of eggs (i.e. equivalent to approximately 2464 eggs). They also showed other parameters for their oviposition potential: 47.45% of engorged weight, 140 eggs per day per female, and 9.5 eggs per mg of engorged body weight. The Japanese bisexual population in the Kitaoka's date (1961d) showed their oviposition ability of 61.4% of engorged weight, 11.51 eggs per mg of engorged body weight, while the parthenogenetic population showed 58.2% and 8.26 eggs. On the other hand, Hoogstraal *et al.* (1968) have observed the life-cycle of 3 females of bisexual population (the Shimane strain) 33 females of parthenogenetic population (the Gumma strain) all collected from Japan, and reported that the females of the Shimane strain produced the mean of 2740 eggs while the females of the Gumma strain produced the mean of 2024 eggs. It was thus concluded that the Weonseong strain showed lower oviposition ability than the Japanese bisexual population, although they showed lower rate (%) of oviposition ability to body weight but a higher number of eggs per mg of body weight as compared with the Japanese parthenogenetic population. In general, under the favourable constant conditions, the oviposition ability of the tick was not influenced by humidity or temperature treatments. However, besides the physical factors, there was a very highly significant correlation between the fecundity and the initial engorged weight of the female tick. Similar results

have been shown in any other ixodid ticks, for example, in *Amblyomma maculatum* (Wright 1971), in *Boophilus microplus* (Hitchcock 1955), in *Haemaphysalis pospelovashtromae* (as *H. warburtoni*, Sartbaev 1959), in *Hyalomma anatolicum anatolicum* (Snow and Arthur 1966), in *Hy. asiaticum* (Balashov 1972), in *Rhipicephalus sanguineus* (Sweatman 1967 , and Sweatman and Koussa 1968) and in *R. turanicus* (Balashov 1972). In the present experiment on hatchability, no larva hatched out from the eggs laid by the Weonseong strain of *H. longicornis* females maintained at and below 32.5% relative humidites. The eggs maintained at 25°C showed the overall mean hatching rate of 85.02% at 55.0% relative humidity to near saturation, and those maintained at 20°C to 35°C showed the overall mean 87.79% hatchability at around 83-85% relative humidities. It was thus clear that the successful hatching rate (%) was influenced by humidity. In other words, the eggs were very sensitive to dryness, so that the eggs maintained at low relative humidities (high saturation deficit conditions) failed to hatch. However, Hoogstraal *et al.* (1968) have reported the extremely high percentages (96% and 94%) of hatching from the eggs laid by the females of the Shimane strain, a bisexual race of *H. longicornis*, and of the Gumma strain, a parthenogenetic race of *H. longicornis*, at the conditions of 26°C to 28°C and 20% to 30% relative humidity. Most of all successful experiments and observations on the hatchability of tick-eggs were done at the conditions of reasonably high relative humidities (%) or near saturation. It therefore had been generally accepted that ixodid ticks are highly susceptible to desiccation at their immature stages especially in the egg stage. Hitchcock (1955) showed that the maximum hatch of *Boophilus microplus* eggs occurred at 70%

relative humidity or lower. The adverse effect of dryness on the eggs of ticks had already been reported (MacLeod 1935a, and Arthur 1951). Although the low relative humidities (%) of completely desiccating conditions did not affect the egg production of *Rhipicephalus sanguineus*, the eggs deposited at low relative humidity conditions failed to hatch (Sweatman 1967). This indicated that tick eggs must be deposited in high relative humidities in their natural habitat to insure survival and successful development. Howe (1967), however, indicated that humidity usually interacts with temperature but many insect eggs are not influenced by it.

Gregson (1966) mentioned that the feeding period of females of some tick species is regularly influenced by their mating habits, and that in the absence of mating, *Dermacentor andersoni* females remained attached for another week or more. The females needed to feed for 1 day and the males for 5 days, before mating took place. Absence of copulation in the Weonseong strain of *H. longicornis* led to markedly prolonged feeding periods or even caused the death of the females fed without males. A mean period of 15.32 days has been shown in 25 females (52.08%) out of 48 females fed initially in the absence of males. It has also been assumed that the maximum feeding period of 20 days could not be the limit and a much prolonged feeding period could be possible.

Deposition of viable parthenogenetic eggs by unfertilised females have been described for some ixodid species (Stone 1963, and Balashov 1972), and sporadic parthenogenesis may happen occasionally in many species of insects (Wigglesworth 1972). Bremner (1959) observed the mode of reporduction by parthenogenesis in the Australian pop-

ulation of *H. longicornis* (as *H. bispinosa*), and reported that males appeared in a ratio of 1 to every 400 females in Australia. Since then the Australian strain of *H. longicornis* has been regarded as a parthenogenetic population. Later, Oliver & Bremner (1968) indicated that the rare parthenogenetic males in Australia failed to produce sperm or to show an interest in mating. However, Khalil (1970 & 1972) reported that the female genital system of the Australian parthenogenetic *H. longicornis* was structually similar to that of bisexual amblyommina species, for example *Hyalomma anatolicum excavatum*. On the other hand, Kitaoka (1961d) in Japan, investigated the reprodutive pattern of the Japanese *H. longicornis* (as *H. bispinosa*), and reported a significantly different geographical distribution of the two races, partheno-genetic and bisexual, of the Japanese *H. longicornis* species. Also, he (Kitaoka, 1961d) observed a second generation of hybrids from a hybridisation experiment between a bisexual race (the Kumamoto strain from Kyushu, Japan) and a parthenogenetic race (the Aomori strain from Honshu, Japan). Later, however, Oliver, Tanaka and Sawada (1973) verified that it was incorrect information from personal communication with Kitaoka, and added that additional attempts to hybridise bisexual and parthenogenetic races should be made concurrently with karyotype studies. They (Oliver *et al.* 1973) failed to achieve hybridisation between diploid and triploid races (both Japanese origin), but succeeded between bisexual diploid males (Japanese origin) and parthenogenetic aneuploid females (the Jeju strain, Korean origin). They reported that parthenogenetic ability was almost completely lost in F_1 and F_2 females produced from the hybridisation, and they confirmed that only the females of the Jeju strain retained the ability to reproduce bisexually and

thus have two modes of reproductive pattern (parthenognetic/ bisexual). The trial to induce a sporadic parthenogenesis in this project showed that the females of the Weonseong strain (a bisexual *H. longicornis*, Korean origin) fed without males produced very poor oviposition records, and no larva hatched out from the eggs laid by the single females. Therefore, the trial to induce a sporadic parthenogenesis from the bisexual race ended in a failure. Wigglesworth (1972) indicated that parthenogenesis may be induced artificially in certain insect eggs by various stimuli, such as high temperature, acids, alcohol, and various histological fixatives, etc., which serve to activate the eggs. No more attempts have been made in this project, but it would be worthy of more detailed research in this field.

For the laboratory colonisation of the tick, *H. longicornis*, the temperature of experimental micro-environments was the key factor controlling the developmental processes including the developmental velocities. It was clear that the velocities for the developmental processes at each developmental stage of the ticks were associated by high treatment temperatures and retarded by low. It could be explained by certain biochemical phenomena that the kinetic movements of most enzyme actions are accelerated by a rise of temperature, because developmental processes depend upon the metabolism which consists of a reticulum of enzyme actions. Ryabova (1971) compared the effects of temperature on the developmental duration in immature *Haemaphysalis japonica* and *H. concinna* and reported that the larval and nymphal *H. japonica* needed higher temperatures for development than the immature *H. concinna*. The lowest threshold of development for larva-to-nymph metamorphosis in

H. japonica was 10.5°C and for nymph-to-adult 13.6°C while for larva-to-nymph metamorphosis in *H. concinna* it was 9.1°C and nymph-to-adult 12.7°C. Branagan (1973a) reported that the development of *Rhipicephalus appendiculatus* engorged instars became very prolonged as temperatures fell below 18°C, being negligible at 15°C and having apparently ceased altogether at 9 °C. Continuous exposure for more than two or three days to temperatures of 4°C and below were lethal to all engorged instars.

It was of interest that *Hyalomma anatolicum anatolicum* may undergo either a 2-host or 3-host type of life-cycle. Feldman-Muhsam (1948, as *Hy. excavatum*) observed the life-cycle in the laboratory and confirmed that it is normally a 3-host species. Hoogstraal (1956, as *Hy. excavatum*) made a theoretical conclusion saying that under primitive conditions this tick spends part or all of its life-cycle on small animals and in these situations it undergoes a 1- or 2-host type of life-cycle; however, on larger domestic animals the tick undergoes a 3-host type of life-cycle. He added a comment that the matter was obviously in need of further investigation. After that, he and his colleague (Hoogstraal and Kaiser 1959) described again that the tick undergoes an ambiguous type of life-cycle. On the other hand, Balashov (1972) reported that temperature greatly influences the developmental life-cycle of ticks. As an example in his experiments, most larval *Hy. anatolicum* on rabbits showed a 2-host feeding type but when the air temperature was lower the percentage of 2-host development was greater: at 20° to 22°C, up to 80% of the ticks had a 2-host cycle and only 20% a 3-host cycle, but at 30°C, 60% had 2-host and 40% had 3-host development. Based on the present author's own exper-

ience of *Hy. a. anatolicum* feeding, the ticks underwent two types of feeding, 2-host and 3-host type, and all of the above three theories (Feldman-Muhsam 1948, Hoogstraal 1956, and Balashov 1972) were not satisfying. Although no satisfying answer is available up to date, an approach of genetical or evolutional research would be of interest.

Anyhow, temperature has been regarded as an important factor in laboratory tick feeding although most of the tick-feeding experiments have been accomplished at controlled room-temperature (around 18°C) or at various natural temperatures. Saito (1960) indicated that *Haemaphysalis flava* refuses to attach if the ambient temperature is over 30°C, but behaves well if the host is subjected to around 15°C. It would be worthy of more detailed study to find the optimum temperature for each tick feeding in the laboratory. In addition, the seasonal fluctuation of the tick prevalence in nature with particular relevance to the physiological phenomena of diapauses in the eggs, larvae, and adult ticks, would be extremely worthy to assess the ecological significance of the ticks. MacCulloch & Hall (1970) reported that *H. longicornis* in Korea hibernates mainly as a nymph or unfed adult, while *B. microplus* survives as an egg with larva appearing in spring in the same area.

Although certain information (Campbell 1948, Yeoman 1966, and Branagan 1973a, and 1973b) has shown that relative humidity had no or little significance upon the rate of development of certain species of ixodid ticks, there are some contrasting reports on the effects of relative humidity (MacLeod 1934, Wilson 1946 and 1950, Snow and Arthur 1966, and Sweatman 1967).

In fact, humidity is not an independent factor. Humidity itself is closely related to temperature, since the saturation vapour pressure of air increases greatly with an increase in temperature. It was assumed in this project that humidity with temperature interaction remarkably influenced the development of the ticks especially in immature stages, i.e., hatchability of the eggs, rates (%) of successful metamorphosis in both larva-to-nymph and nymph-to-adult. Humidity also influenced the oviposition period of the ticks. And this result corresponded to the references which emphasised the effect of humidity on the eggs and on the survival and activity of ticks (MacLeod 1935a, Lees 1946, Arthur 1948, Sweatman & Koussa 1968, Hafez, El-Ziady and Hefnawy 1970b, Sauer and Hair 1971, and Hefnawy, Bishara and Bassal 1975), like other terrestrial arthropods (Birch 1942, Birch and Andrewartha 1942, Davidson 1942, Sharif 1948, Willis and Roth 1950, Smith 1951, Murray 1960, 1963a, 1963b and 1963c, Shaw and Stobbart 1963, Bursell 1970, Howell and George 1973, and Okasha 1973). It, therefore, appeared that humidity is also a very important factor controlling the ecological and physiological significance of ticks. If temperature be regarded as the major factor, humidity should be in second place.

CHAPTER FIVE : CONCLUSIONS

To present the principal biological data on the laboratory colonisation of the tick, *Haemaphysalis longicornis* (the Weonseong strain, bisexual race, originally collected from cattle in Korea), which is a very important vector and curious parasite; from the epidemiological/epizootological point of view, as the vectors of bovine theileriosis, Q fever, Russian spring-summer encephalitis and other infectious disease, and from the biological/genetical point of view, as a curious creature possessing three distinctive reproductive and chromosome types, i.e., diploid bisexual, triploid parthenogenetic, and aneuploid parthenogenetic/bisexual; and to provide the detailed data on the development of each stage of the ticks relating to the influence of temperature and humidity under various controlled laboratory conditions, several experiments and observations were carried out, and the results obtained were summarised as follows:

(1) The overall successful feeding rates (%) of each developmental stage were 89.89, 92.57, and 86.11 for larvae, nymphs, and adult females, respectively. The overall mean feeding periods were 5.24 days for larvae, 4.75 days for nymphs, and 9.73 days for adult females. Adult males usually tended to remain on the host for a long period, and no significant time limit was observed for them. The overall mean fed weights were 0.536 mg, 3.463 mg, 251.950 mg, and 1.854 mg for larvae, nymphs, adult females, and adult males, respectively, and their feeding efficiencies (%) were in the around of 1389, 1343, 13660, and 63, respectively.

(2) The population density of ticks applied influenced the mean feeding periods and the mean successful feeding rates (%) of each instar. An increase in the number of ticks applied resulted in an overall retardation in the velocity of engorgement and an overall reduction in the successful feeding rates (%). But, there was no significant difference of the mean engorged weights of each instar within the treatment groups fed with different numbers of ticks.

(3) The overall mean pre-oviposition period was 8.16 days, and it was not significantly influenced by humidity at 25°C (Series A), but greatly influenced by temperature at 83-85% relative humidity (Series B). The velocity for the ovarian development was accelerated by raising the treatment temperature and retarded by lowering it.

(4) The overall mean oviposition periods was 17.75 days, and it was not significantly influenced by humidity at 25°C (Series A), but influenced by temperature at 83-85% relative humidity (Series B). The result coincided with that of the pre-oviposition period experiment.

(5) The engorged females produced a mean of 123.18 mg of eggs (i.e. equivalent to approximately 2464 eggs). The overall mean of the total weight of eggs laid per female was 47.45% of engorged weight and it ranged individually from 41.63% to 52.59%. And 1 mg increase of body weight showed 9.5 egg increase. On the other hand, the overall mean egg output potential was recorded as 139.94 eggs per day per tick and it ranged individually from 93.55 to 217.47. There was a very highly significant correlation between the total

weight of eggs laid and the engorged weight of the female regardless of the effects of humidity or temperature (20°C to 35°C).

(6) The observed daily oviposition pattern greatly varied in individuals, however, there was a common trend that the peak of the oviposition curve was seen within 2 to 5 days after the onset of egg-laying, with a successive gradual decline thereafter. The entire oviposition period was possibly divided into 3 phases, i.e. inductive phase, prosperous phase, and debilitated phase. No significantly different oviposition pattern influenced by humidity or temperature was observed.

(7) At 25°C, the overall mean incubation period of the eggs was 27.09 days, and there was no significant difference within humidity treatment groups. Relative humidity influenced the successful hatching rates (%) of the eggs maintained at 25°C and various relative humidities. No larva emerged from the eggs maintained at or below 32.5% relative humidities and the eggs maintained at 55.0% relative humidity or near saturation showed the overall mean hatching rate of 85.02%. On the other hand, those maintained at 83-85% relative humidity and various temperatures (20°C to 35°C) showed 87.79% hatchability. Temperature greatly influenced the incubation periods of the eggs maintained at 83-85% relative humidity and various temperature combinations. The mean incubation period at 20°C and 85.0% relative humidity was 46.82 days, and it was shortened as temperature increased. Also, temperature influenced the hatching rates. The maximum hatching rate of the eggs was 91.75% at a combination of 30°C and 84.5% relative humidity, and it was decreased by raising the temperature or lowering it.

(8) At a combination of 25°C and 85.0% relative humidity, the mean premoulting period was 14.10 days and the mean moulting rate was 86.8% in the preliminary experiment on the larva-to-nymph metamorphosis. On the other hand, at the various combinations of temperatures and humidities, the moulting rates were greatly influenced by humidity, while the premoulting periods were mainly affected by temperature.

(9) In the preliminary experiment on the nymph-to-adult metamorphosis at 25°C and 85.0% relative humidity the overal mean moulting rate was very high at 98.62% and the mean premoulting period was 14.94 days. The duration of the nymph-to-female premoulting period (15.10 days) was significantly longer than that of the nymph-to-male premoulting period (14.62 days). The sex ratio of the newly moulted adults was recorded as 1.72:1 (1402 females: 814 males). At the various combinations of temperatures and humidities, the moulting rates were mainly influenced by humidity, whereas, the premoulting periods were greatly influenced by temperature. As shown in the preliminary experiment, the developmental velocity in the nymph-to-female metamorphosis was lower than that in the nymph-to-male metamorphosis,and it was more apparent at low temperatures.

(10) When the females were applied to the host in absence of males, the feeding rate was relatively low (52.08%), the mean feeding periods was prolonged (15.32 days), and the mean fed weight did not reach the average of the females fed normally with males. The mean pre-oviposition period was delayed, but the mean oviposition period was relatively short, and they showed poor oviposition records; 38.91% of body weight, 66.28 eggs per day per tick, and 7.80 eggs

per mg body weight. There was also a very highly significant correlation between the total weight of eggs laid and the fed weight of the female. However, no larva emerged from the eggs laid by the single females, and it meant that no sporadic parthenogenesis was observed in this experiment.

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APPENDIX 1-1 Feeding Records for *H. longicornis* Larvae of the Experimental Group 1. (500 Larvae per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K12-L	K15-R		
Days of collection				
3.	128	119	247	25.68
4.	113	141	254	26.40
5.	84	95	179	18.61
6.	95	64	159	16.53
7.	69	54	123	12.78
Total No. of larvae collected	489	473	962	
%	97.80	94.60	96.20	
Feeding periods (days) ΣfX	2309	2158	4467	
$\bar{x}$	4.72	4.56	4.64	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 100 larvae for each trial

	Trials					
	K12-L			K15-R		
	54.6	57.2	52.9	53.9	54.0	56.8
	46.8	53.5	54.0	57.2	58.6	55.3
	55.3	56.7	58.1	49.2	51.7	52.5
	49.7	50.6	48.3	54.4	55.8	50.4
	57.7	59.2	58.5	57.3	56.1	52.0
Total		813.1			815.2	
Mean		54.21			54.35	
Overall Total			1628.3			
Mean			54.28			

APPENDIX I-2 Feeding Records for *H. longicornis* Larvae of the Experimental Group II. (1500 Larvae per Rabbit Ear).

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K13-L	K14-R		
Days of collection				
3.	262	211	473	17.36
4.	393	377	770	28.25
5.	497	299	796	29.21
6.	159	208	367	13.47
7.	112	207	319	11.71
Total No. of larvae collected	1423	1302	2725	
%	94.87	86.80	90.83	
Feeding periods (days) ΣfX	6581	6333	12914	
$\bar{x}$	4.62	4.86	4.74	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 100 larvae for each trial

Trials					
K13-L			K14-R		
50.6	52.3	47.9	52.7	51.8	57.5
54.8	56.1	54.2	50.0	54.1	55.6
55.7	46.3	57.4	53.9	54.2	56.3
56.2	58.5	59.2	49.4	47.0	51.8
57.0	48.2	51.6	53.4	52.2	58.3
Total	806.0			798.2	
Mean	53.73			53.21	
Overall Total	1604.2				
Mean	53.47				

APPENDIX I-3 Feeding Records for *H. longicornis* Larvae of the Experimental Group III. (2500 Larvae per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials				Overall	Mean Period
	K4-L	K4-R	K3-L	K3-R		
Days of Collection						
3.	109	318	133	85	645	6.90
4.	625	615	802	676	2718	29.09
5.	805	481	709	782	2777	29.72
6.	324	511	447	408	1690	18.08
7.	217	394	308	191	1110	11.88
8.	122	100	-	183	405	4.33
Total No. of larvae collected	2202	2419	2399	2325	9345	
%	88.08	96.76	95.96	93.00	93.45	
Feeding Periods (days) ΣfX	11291	12443	11990	12118	47842	
$\bar{x}$	5.13	5.14	4.98	5.21	5.12	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 100 larvae for each trial

Trials			
K4-L	K4-R	K3-L	K3-R
54.9 53.8	52.4 53.7	55.2 52.5	56.1 51.4
56.0 54.9	54.6 58.7	50.6 48.5	59.2 46.1
58.6 52.6	51.8 51.0	54.2 45.6	54.7 58.3
55.6 55.8	55.3 52.4	57.4 51.2	54.3 53.6
53.7 54.7	53.2 46.0	50.3 56.1	54.9 56.0
45.8 55.7	59.7 55.3	54.3 53.4	52.6 50.1
56.5 55.4	56.4 49.8	49.7 55.8	54.5 55.3
42.8	52.4	55.3	53.8
Total 806.8	802.7	790.1	810.9
Mean 53.79	53.51	52.67	54.06
Overall Total	3210.5		
Mean	53.51		

APPENDIX I-4 Feeding Records for *H. longicornis* Larvae of the Experimental Group IV. (3500 Larvae per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	Mean Period
	K13-R	K14-L		
Days of collection				
3.	272	233	505	8.17
4.	703	692	1395	22.58
5.	821	875	1696	27.45
6.	761	580	1341	21.70
7.	296	421	717	11.60
8.	189	234	423	6.85
9.	-	102	102	1.65
Total No. of larvae collected	3042	3137	6179	
%	86.91	89.63	88.27	
Feeding periods (days) ΣfX	15883	17059	32942	
$\bar{x}$	5.22	5.44	5.33	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 100 larvae for each trial

	Trials					
	K13-R			K14-L		
	49.6	52.9	54.0	53.2	50.6	51.1
	55.5	58.2	59.1	55.0	54.2	50.7
	57.3	55.4	53.6	48.8	52.7	52.2
	44.2	49.0	54.7	56.3	50.1	50.8
	52.4	58.6	57.7	54.5	55.9	56.0
Total		812.2			792.1	
Mean		54.15			52.81	
Overall Total			1604.3			
Mean			53.48			

APPENDIX I-5 Feeding Records for *H. longicornis* Larvae of the Experimental Group V. (4500 Larvae per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	Mean Period
	K12-R	K15-L		
Days of collection				
3.	128	314	442	5.70
4.	877	492	1369	17.65
5.	1214	858	2072	26.71
6.	1008	956	1964	25.32
7.	536	719	1255	16.18
8.	88	267	355	4.58
9.	110	190	300	3.87
Total No. of larvae collected	3961	3796	7757	
%	88.02	82.13	85.08	
Feeding periods (days) ΣfX	21456	21815	43271	
$\bar{x}$	5.42	5.75	5.58	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 100 larvae for each trial

Trials					
K12-R			K15-L		
55.3	44.9	48.6	50.6	52.3	57.4
59.2	57.3	55.8	54.5	56.2	47.4
54.9	58.1	57.3	52.3	50.0	49.8
55.0	49.6	50.9	57.8	53.6	54.4
51.8	56.6	53.3	48.8	58.1	51.2
Total	808.6			794.4	
Mean	53.91			52.96	
Overall Total	1603.0				
Mean	53.43				

APPENDIX II-1 Feeding Records for *H. longicornis* Nymphs of the Experimental Treatment Group I. (100 Nymphs per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K5-L	K6-R		
Days of collection				
3.	25	19	44	22.45
4.	35	42	77	39.29
5.	21	27	48	24.49
6.	12	8	20	10.20
7.	4	3	7	3.57
Total No.of nymphs collected	97	99	196	
%	97	99	98	
Feeding Periods (days) ΣfX	420	429	849	
$\bar{x}$	4.33	4.33	4.33	

(2) Record for engorged weights (mg)
Sample size: 8 groups of 10 nymphs for each trial

	Trials	
	K5-L	K6-R
	32.9 30.7	33.4 35.5
	36.5 34.8	36.2 35.2
	33.6 35.1	36.9 34.7
	37.2 36.0	33.5 36.3
Total	276.8	281.7
Mean	34.60	35.21
Overall Total	558.5	
Mean	34.91	

APPENDIX II-2 Feeding Records for *H. longicornis* Nymphs of the Experimental Treatment Group II. (300 Nymphs per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K10-L	K11-R		
Days of collection				
3.	37	54	91	15.56
4.	148	123	271	46.32
5.	69	99	168	28.72
6.	30	5	35	5.98
7.	12	8	20	3.42
Total No. of nymphs collected	296	289	585	
%	98.67	96.33	97.50	
Feeding periods (days) ΣfX	1312	1235	2547	
$\bar{x}$	4.43	4.27	4.35	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 10 nymphs for each trial

Trials					
K10-L			K11-R		
38.2	34.6	36.4	32.4	35.6	33.7
37.8	33.7	34.5	37.2	32.9	31.4
35.5	32.4	35.0	35.3	38.6	38.8
33.9	36.8	33.6	35.7	36.0	34.3
31.3	32.3	34.9	37.2	36.2	34.5
Total	520.9			529.8	
Mean	34.73			35.32	
Overall Total			1050.7		
Mean			35.02		

APPENDIX II-3 Feeding Records for *H. longicornis* Nymphs of the Experimental Treatment Group III. (500 Nymphs per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials				Overall	%
	K1-L	K2-R	K5-R	K6-L		
Days of collection						
3.	105	86	97	103	391	20.34
4.	193	180	206	179	758	39.44
5.	76	111	93	105	385	20.03
6.	88	66	54	63	271	14.10
7.	23	31	26	37	117	6.09
Total No. of nymphs collected	485	474	476	487	1922	
%	97.0	94.8	95.2	97.4	96.1	
Feeding Periods (days) ΣfX	2156	2146	2086	2187	8575	
$\bar{X}$	4.45	4.53	4.38	4.49	4.46	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 10 nymphs for each trial

Trials			
K1-L	K2-R	K5-R	K6-L
35.9 38.5	33.7 36.8	36.3 32.9	35.7 34.2
36.3 34.1	32.8 34.6	33.4 37.0	32.5 34.9
33.3 31.5	35.4 38.1	34.1 36.3	37.8 30.3
34.4 35.1	34.6 36.7	36.2 35.6	36.2 33.3
34.2 37.9	32.9 34.5	37.7 34.9	34.8 37.3
32.2 35.0	33.0 34.2	34.2 34.8	32.0 35.3
35.9 34.3	35.9 33.6	33.8 33.5	34.5 36.3
36.6	33.7	35.1	32.9
Total 525.2	520.5	525.8	518.0
Mean 35.01	34.70	35.05	34.53
Overall Total	2089.5		
Mean	34.83		

APPENDIX II-4 Feeding Records for *H. longicornis* Nymphs of the Experimental Treatment Group IV. (700 Nymphs per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K1-R	K2-L		
Days of collection				
3.	119	81	200	15.53
4.	222	188	410	31.83
5.	132	167	299	23.21
6.	124	96	220	17.08
7.	47	68	115	8.93
8.	15	29	44	3.42
Total No. of nymphs collected	659	629	1288	
%	94.14	89.86	92.00	
Feeding periods (days) ΣfX	3098	3114	6212	
$\bar{x}$	4.70	4.95	4.82	

(2) Record for engorged weights(mg)
Sample size: 15 groups of 10 nymphs for each trial

	Trials					
	K1-R			K2-L		
	34.7	36.2	36.0	32.5	34.7	33.6
	34.6	35.1	35.9	35.8	34.3	36.1
	34.5	34.6	33.2	35.4	32.9	37.5
	33.6	31.0	34.9	30.5	30.8	34.6
	37.2	36.2	35.4	33.6	33.8	33.5
Total		523.1			509.6	
Mean		34.87			33.97	
Overall Total			1032.7			
Mean			34.42			

APPENDIX II-5 Feeding Records for *H. longicornis* Nymphs of the Experimental Treatment Group V. (900 Nymphs per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K10-R	K11-L		
Days of collection				
3.	76	34	110	7.04
4.	164	157	321	20.54
5.	222	253	475	30.39
6.	191	242	433	27.70
7.	85	79	164	10.49
8.	32	28	60	3.84
Total No. of nymphs collected	770	793	1563	
%	85.56	88.11	86.83	
Feeding periods (days) ΣfX	3991	4224	8215	
$\bar{x}$	5.18	5.33	5.26	

(2) Record for engorged weights (mg)
Sample size: 15 groups of 10 nymphs for each trial

	Trials					
	K10-R			K11-L		
	33.9	33.3	34.2	34.5	31.1	35.5
	36.3	31.8	35.4	38.2	31.9	34.1
	32.4	31.5	33.6	36.2	33.0	37.7
	34.6	34.9	35.7	33.3	32.0	30.6
	30.2	35.2	36.3	35.8	31.2	32.5
Total		509.3			507.6	
Mean		33.95			33.84	
Overall Total			1016.9			
Mean			33.90			

APPENDIX III-1 Feeding records for *H. longicornis* Adult Females of the Experimental Group I. (4 Pairs of Ticks per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K46-L	K48-R		
Days of collection				
7.	-	2	2	25.0
8.	1	2	3	37.5
9.	2	-	2	25.0
10.	1	-	1	12.5
Total No. of females collected	4	4	8	
%	100	100	100	
Feeding periods (days) ΣfX	36	30	66	
$\bar{x}$	9.0	7.5	8.25	

(2) Record for engorged weights (mg)

	Trials	
	K46-L	K48-R
	246.2	242.8
	254.6	263.7
	261.7	250.8
	238.5	287.3
Total	1001.0	1044.6
Mean	250.25	261.15
Overall Total	2045.6	
Mean	255.7	

APPENDIX III-2 Feeding Records for *H. longicornis* Adult Females of the Experimental Group II. (8 Pairs of Ticks per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K47-L	K49-R		
Days of collection				
7.	2	-	2	13.33
8.	2	2	4	26.67
9.	2	3	5	33.33
10.	1	1	2	13.33
11.	-	1	1	6.67
12.	-	1	1	6.67
Total No. of females collected	7	8	15	
%	87.5	100	93.75	
Feeding periods (days) ΣfX	58	76	134	
$\bar{x}$	8.286	9.500	8.93	

(2) Record for engorged weights (mg)

Trials			
K47-L		K49-R	
219.5	275.2	274.3	205.8
258.4	243.6	284.2	223.4
273.6	232.3	254.8	244.6
294.8		234.9	268.5
Total	1797.4	1990.5	
Mean	256.77	248.81	
Overall Total	3787.9		
Mean	252.53		

APPENDIX III-3 Feeding Records for *H. longicornis* Adult Females of the Experimental Group III. (12 Pairs of Ticks per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials				Overall	Mean Period
	R40-L	R40-R	R41-L	R41-R		
Days of collection						
7.	1	1	2	1	5	11.36
8.	4	3	3	2	12	27.27
9.	2	3	-	3	8	18.18
10.	1	1	2	2	6	13.64
11.	2	-	2	2	6	13.64
12.	2	1	1	1	5	11.36
13.	-	1	1	-	2	4.55
Total No. of females collected	12	10	11	11	44	
%	100	83.33	91.67	91.67	91.67	
Feeding periods (days) ΣfX	113	93	105	104	415	
% $\bar{x}$	9.417	9.300	9.545	9.455	9.432	

(2) Record for engorged weights (mg)

	Trials			
	R40-L	R40-R	R41-L	R41-R
	232.8 280.5	338.1 260.4	298.2 232.7	176.2 301.4
	255.0 288.3	160.1 297.6	296.5 245.0	266.5 213.7
	315.5 253.7	215.4 278.1	214.1 275.4	288.6 206.5
	227.6 292.8	208.3 210.1	246.8 213.9	329.5 185.6
	358.7 226.2	316.2 279.4	275.5 310.6	236.0 270.0
	219.4 214.0		279.4	265.3
Total	3165	2663.7	2888.1	2739.3
Mean	263.75	256.37	262.55	249.03
Overall Total		11356.1		
Mean		258.09		

APPENDIX III-4 Feeding Records for *H. longicornis* Adult Females of the Experimental Group IV. (16 Pairs of Ticks Per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K46-R	K48-L		
Days of collection				
7.	1	1	2	7.41
8.	2	3	5	18.52
9.	1	4	5	18.52
10.	3	4	7	25.93
11.	2	2	4	14.81
12.	3	-	3	11.11
13.	1	-	1	3.70
Total No. of females collected	13	14	27	
%	81.25	87.50	84.38	
Feeding Periods (days) ΣfX	133	129	262	
$\bar{x}$	10.23	9.21	9.70	

(2) Record for engorged weights (mg)

Trials			
K46-R		K48-L	
269.1	248.6	293.0	304.2
223.4	248.0	252.7	216.7
279.7	224.3	189.6	245.4
163.6	215.7	223.9	265.7
304.2	182.6	211.3	232.0
263.7	284.2	270.1	305.4
245.4		284.2	149.4
Total	3152.5	3443.6	
Mean	242.50	245.97	
Overall Total	6596.1		
Mean	244.30		

APPENDIX III-5 Feeding Records for *H. longicornis* Adult Females of the Experimental Group V. (20 Pairs of Ticks per Rabbit Ear)

(1) Record for feeding rates (%) and feeding periods (days)

	Trials		Overall	%
	K47-R	K49-L		
Days of collection				
7.	1	1	2	6.67
8.	3	-	3	10.00
9.	1	1	2	6.67
10.	2	1	3	10.00
11.	2	4	6	20.00
12.	3	4	7	23.33
13.	2	3	5	16.67
15.	-	2	2	6.67
Total No of females collected	14	16	30	
%	70.0	80.0	75.0	
Feeding Periods (days) ΣfX	144	185	329	
$\bar{x}$	10.29	11.56	10.97	

(2) Record for engorged weights (mg)

Trials					
K47-R			K49-L		
241.3	287.2	212.3	311.5	276.8	263.2
184.2	235.7	285.4	222.5	236.6	241.4
205.7	252.3	298.2	250.8	293.3	312.7
269.8	274.3	164.5	243.4	263.8	248.9
211.4	254.9		232.9		
Total	3377.2		4078.9		
Mean	241.23		254.93		
Overall Total	7456.1				
Mean	248.54				

APPENDIX IV-1 Records for the Oviposition of *H. longicornis* Females Maintained at 25°C and Various Relative Humidities (Series A)

(1) Pre-oviposition and oviposition periods

Experiment No.	Tick	Engorged Wt. (mg)	Pre-Oviposition Period (Days)	Oviposition Period (Days)
I-1	R40L-1	232.8	8	16
2	R40R-1	338.1	6	17
3	R41L-1	298.2	8	16
4	R41L-2	232.7	8	14
5.	R41R-1	176.2	8	15
Total		1278.0	38	78
Mean		255.60	7.6	15.6
S.D.			0.894	1.140
S.E.			0.400	0.510
II-1	R40L-2	280.5	7	20
2	R40L-3	255.5	10	17
3	R40L-4	288.3	9	19
4	R40L-5	315.5	8	16
5	R40R-2	260.4	7	18
Total		1400.2	41	90
Mean		280.04	8.2	18.0
S.D.			1.304	1.581
S.E.			0.583	0.707
III-1	R40R-3	1601	8	18
2	R40R-4	297.6	9	22
3	R41L-3	296.5	8	18
4	R41L-4	245.0	7	20
5	R41L-5	214.1	7	17
Total		1213.3	39	95
Mean		242.66	7.8	19.0
S.D.			0.738	2.000
S.E.			0.334	0.894
IV-1	R41R-2	301.4	9	18
2	R41R-3	266.5	8	19
3	R40L-6	253.7	10	21
4	R40L-7	227.6	8	14
5	R40R-£	215.4	8	20
Total		1264.6	43	92
Mean		252.92	8.6	18.4
S.D.			0.894	2.702
S.E.			0.400	1.208
V-1	R40R-6	278.1	8	19
2	R40R-7	208.3	9	16
3	R41R-4	323.7	7	17
4	R41R-5	288.6	10	14
5	R41R-6	206.5	8	22
Total		1195.2	42	88
Mean		239.04	8.4	17.6
S.D.			1.140	3.050
S.E.			0.510	1.364

(2) Oviposition ability

Experiment No.	Total Wt. of Eggs (mg)	Total Wt. of Eggs/Engd.Wt. (%)	Daily Mean Oviposition (Eggs)	Egg Output/ mg Body Wt. (Eggs)
I-1	101.4	43.56	125.75	8.71
-2	153.1	45.28	180.12	9.06
-3	138.7	46.51	173.37	9.30
-4	98.4	42.29	140.57	8.46
-5	90.3	51.25	120.40	10.25
Total	581.9	228.89	741.21	45.78
Mean	116.38	45.78	148.24	9.16
S.D.	27.724	3.457	27.127	0.691
S.E.	12.399	1.546	12.132	0.309
II-1	129.5	46.17	129.50	9.23
-2	121.3	47.48	142.71	9.49
-3	143.0	49.60	150.53	9.92
-4	134.7	42.69	168.37	8.54
-5	118.6	45.55	131.78	9.11
Total	647.1	231.49	722.89	46.29
Mean	129.42	46.30	144.58	9.26
S.D.	9.941	2.544	15.780	0.507
S.E.	4.446	1.138	7.057	0.227
III-1	84.2	52.59	93.55	10.52
-2	131.6	44.22	119.64	8.84
-3	144.2	48.63	160.22	9.73
-4	129.8	52.98	129.80	10.60
-5	106.3	49.65	125.06	9.93
Total	596.1	248.07	628.27	49.62
Mean	119.22	49.61	125.65	9.92
S.D.	23.888	3.544	23.870	0.711
S.E.	10.683	1.585	10.675	0.318
IV-1	140.9	46.75	156.55	9.35
2	127.4	47.80	134.00	9.56
3	126.5	49.86	120.48	9.97
4	99.7	43.80	142.43	8.76
5	108.8	50.51	108.80	10.10
Total	603.3	238.72	662.26	47.74
Mean	120.66	47.74	132.45	9.55
S.D.	16.349	2.677	18.611	0.535
S.E.	7.312	1.197	8.323	0.239
V-1	139.6	50.20	146.95	10.04
2	89.3	42.87	111.63	8.57
3	150.5	46.49	177.06	9.30
4	127.4	44.14	142.00	8.83
5	103.8	50.27	94.36	10.05
Total	610.6	233.97	672.00	46.79
Mean	122.12	46.79	134.40	9.36
S.D.	25.257	3.399	32.237	0.679
S.E.	11.296	1.520	14.417	0.304

APPENDIX IV-2 Records for the Oviposition of *H. longicornis* Females Maintained at 83-85% Relative Humidity and Various Temperatures (Series B).

(1) Pre-oviposition and oviposition periods

Experiment No.	Tick No.	Engorged Wt. (mg)	Pre-oviposition Period (Days)	Oviposition Period (Days)
I-1	R40L-8	292.8	21	24
2	R40R-8	210.1	13	17
3	R41L-6	275.4	12	21
4	R41L-7	246.8	16	20
5	R41R-7	329.5	14	25
Total		1354.6	76	107
Mean		270.92	15.2	21.4
S.D.			3.564	3.209
S.E.			1.594	1.435
II-1	R41R-8	185.6	8	14
2	R40L-9	358.7	6	22
3	R40L-10	226.2	7	17
4	R41L-8	213.9	10	18
5	R41L-9	275.5	7	18
Total		1259.9	38	89
Mean		251.98	7.6	17.8
S.D.			1.517	2.864
S.E.			0.678	1.281
III-1	R41R-9	236.0	4	16
2	R41R-10	270.0	6	18
3	R40L-11	219.4	4	16
4	R40L-12	214.0	6	15
5	R40R-9	316.2	4	15
IV-1	R41L-10	310.6	4	14
2	R41R-11	265.3	5	19
3	R40R-10	279.4	5	15
4	R41L-11	279.4	4	14
Total		1134.7	18	62
Mean		283.67	4.5	15.5
S.D.			0.577	2.380
S.E.			0.289	1.190

(2) Oviposition ability.

Experiment No.	Total Wt. of Eggs (mg)	Total Wt. of Eggs/Engd. (%)	Daily Mean Oviposition (Eggs)	Egg Output/ mg Body Wt. (Eggs)
I-1	140.7	48.05	117.25	9.61
2	110.5	52.59	130.00	10.52
3	137.0	49.75	130.48	9.95
4	124.6	50.49	124.60	10.09
5	147.6	44.80	118.08	8.96
Total	660.4	245.68	620.41	49.13
Mean	132.08	49.14	124.08	9.83
S.D.	14.672	2.921	6.303	0.769
S.E.	6.562	1.306	2.819	0.344
II-1	94.9	51.13	135.29	10.20
2	166.5	46.42	151.36	9.28
3	109.2	48.28	128.47	9.65
4	100.7	47.08	111.89	9.41
5	114.7	41.63	127.44	8.33
Total	596.0	234.54	654.45	46.87
Mean	117.2	46.91	130.89	9.37
S.D.	28.593	3.458	14.293	0.682
S.E.	12.788	1.546	6.392	0.305
III-1	107.2	45.42	134.00	9.08
2	119.5	44.26	132.78	8.85
3	99.6	45.40	124.50	9.08
4	104.0	48.60	138.67	9.72
5	163.1	51.58	217.47	10.32
Total	593.4	235.26	747.42	47.05
Mean	118.68	47.05	149.48	9.41
S.D.	25.909	3.004	38.347	0.603
S.E.	11.587	1.343	17.150	0.270
IV-1	149.6	48.16	213.71	9.63
2	139.4	52.54	146.74	10.51
3	130.5	46.71	174.00	9.34
4	121.7	43.56	173.86	8.71
Total	541.2	190.97	708.31	38.19
Mean	135.30	47.74	177.08	9.55
S.D.	11.962	3.730	27.581	0.748
S.E.	5.981	1.865	13.790	0.374

APPENDIX V Records for the Oviposition of *H. longicornis* Females Fed Without Male Ticks and Maintained at 25°C and 85.0% Relative Humidity.

(1) Pre-oviposition periods and oviposition periods

Experiment No.	Tick No.	Engorged Wt. (mg)	Pre-oviposition Period (Days)	Oviposition Period (Days)
S-I	K5L-1	82.9	7	11
	2	98.7	12	10
	3	56.5	-	-
	4	122.4	14	12
	5	84.9	-	-
	6	27.2	-	-
	7	20.5	-	-
S-II	K9R-1	53.6	9	8
	2	75.7	-	-
	3	60.3	-	-
	4	131.2	12	17
	5	69.8	-	-
S-III	K49L-1	27.9	-	-
	2	58.4	13	12
	3	64.3	10	11
	4	115.7	8	14
	5	49.7	-	-
S-IV	K49R-1	74.0	-	-
	2	68.3	10	7
	3	43.5	-	-
	4	94.0	13	-
	5	89.5	14	10
	6	142.6	8	13
	7	24.2	-	-
	8	50.3	-	-
Overall total			130	134
N			12	12
Mean			10.83	11.16
S.D.			2.480	2.725
S.E.			0.716	0.787

(2) Oviposition ability

Tick No.	Total Wt. of Eggs (mg)	Total Wt. of Eggs/Engd. Wt. (%)	Daily Mean Oviposition (Eggs)	Egg-Output/ mg Body Wt. (Eggs)
K5L-1	34.6	41.74	62.91	8.35
2	27.8	28.17	55.6	5.63
3	Not oviposted			
4	54.3	44.36	90.5	8.87
5	Not oviposted			
6	Not oviposted			
7	Not oviposted			
K9R-1	12.7	23.69	31.75	4.74
2	Not oviposted			
3	Not oviposted			
4	59.8	45.58	70.35	9.12
5	Not oviposted			
K49L-1	Not oviposted			
2	18.5	31.68	30.83	6.34
3	30.0	46.66	54.55	9.33
4	42.8	36.99	61.14	7.40
5	Not oviposted			
K49R-1	Not oviposted			
2	17.4	25.48	49.71	5.09
3	Not oviposted			
4	34.2	36.38	76	7.28
5	51.6	57.65	103.2	11.53
6	70.7	49.58	108.77	9.91
7	Not oviposted			
8	Not oviposted			
Total	454.4	466.96	795.31	93.59
N	12	12	12	12
Mean	37.87	38.91	66.28	7.80
S.D.	18.233	10.765	25.008	2.086
S.E.	5.263	3.108	7.219	0.602

APPENDIX VI Moulting Record for *H. longicornis* Engorged Larvae Maintained at 25°C and 85.0% Relative Humidity (Preliminary Observation)

Experiment No.	No. of tested	Premoulting Period (Days)							N	Mean Period
		12	13	14	15	16	17	18		
K4L-1	109	15	30	23	16	8	3	-	95	13.80
R-1	318	47	49	69	57	36	14	-	272	14.10
L-2	625	69	108	182	85	72	37	9	562	14.23
R-2	615	72	124	145	73	48	26	12	500	14.05
L-3	805	97	173	204	185	91	16	11	777	14.12
R-3	481	79	104	99	77	49	12	-	420	13.88
L-4	324	67	53	80	47	48	4	-	299	13.89
R-4	511	88	99	97	66	68	47	3	468	14.17
L-5	217	23	21	74	33	17	-	8	176	14.18
R-5	394	39	58	83	79	24	7	-	290	14.04
L-6	122	9	32	30	18	12	-	-	101	13.92
R-6	100	14	15	22	24	4	7	-	86	14.12
K3L-1	133	11	29	29	13	19	7	6	114	14.39
R-1	85	19	12	18	19	-	6	-	74	13.82
L-2	802	59	153	154	148	93	18	7	632	14.23
R-2	676	67	126	166	94	72	26	4	555	14.13
L-3	709	94	139	184	105	88	22	15	647	14.12
R-3	782	107	132	229	113	62	33	11	687	14.05
L-4	447	64	103	86	99	42	18	3	415	14.04
R-4	408	45	77	85	83	43	9	8	350	14.17
L-5	308	33	43	89	34	42	19	-	260	14.25
R-5	191	18	32	63	27	19	7	-	166	14.11
L-6	-	-	-	-	-	-	-	-	-	-
R-6	183	29	26	46	45	12	7	-	165	14.04
Total	9345	1165	1738	2257	1540	969	345	97	8111	14.10
Daily Moulting (%)		14.4	21.4	27.8	19.0	12.0	4.3	1.2		

Overall successful moulting rate (%) = 86.8% (73.60% - 96.52%)
Overall Mean Pre-moulting Period (days) = 14.10 days (N = 1 8111, S.D. = 1.421, S.E. = 0.016)

APPENDIX VII-1 Moulting Record for *H. longicornis* Engorged Larvae Maintained at 20°C and Various Relative Humidities (Series A)

Experiment No.	Premoulting Period (Days)										N	Mean Period
	16	17	18	19	20	21	22	23	24	25		
I-1	Not moulted										-	-
2	-										-	-
3	-										-	-
4	-										-	-
5	-										-	-
Total	-										-	-
II-1	Not moulted										-	-
2	-										-	-
3	-										-	-
4	-										-	-
5	-										-	-
Total	-										-	-
III-1	1	3	7	4	6	3	4	3	2	2	35	20.20
2	-	4	3	10	9	1	3	2	1	-	33	19.67
3	2	-	7	2	7	5	3	-	-	4	30	20.23
4	3	2	6	3	10	4	-	2	4	-	34	19.79
5	2	4	-	5	4	6	-	5	2	-	28	20.14
Total	8	13	23	24	36	19	10	12	9	6	160	20.00
IV-1	-	4	7	11	11	4	5	-	1	-	43	19.56
2	2	3	6	5	3	8	6	2	-	-	35	19.77
3	-	4	8	15	5	5	2	4	1	-	44	19.59
4	5	2	10	5	2	5	6	1	1	-	37	19.27
5	-	6	2	13	6	8	-	5	-	-	40	19.70
Total	7	19	33	49	27	30	19	12	3	-	199	19.58
V-1	6	7	4	12	7	2	3	1	1	-	43	18.84
2	8	6	4	15	4	7	-	1	3	-	48	18.94
3	4	5	6	17	9	1	5	2	-	-	49	19.12
4	-	9	7	13	5	2	3	1	2	-	42	19.17
5	1	9	8	10	9	3	5	-	-	-	45	19.02
Total	19	36	29	67	34	15	16	5	6	-	227	19.02

APPENDIX VII-2 Moulting Record for *H. longicornis* Engorged Larvae Maintained at 25°C and Various Relative Humidities (Series B)

Experiment No.	Pre-moulting Period (Days)							N	Mean Period
	12	13	14	15	16	17	18		
I-1	Not moulted							-	-
2	-							-	-
3	-							-	-
4	-							-	-
5	-							-	-
Total	-							-	-
II-1	Not moulted							-	-
2	-							-	-
3	-							-	-
4	-							-	-
5	-							-	-
Total	-							-	-
III-1	6	12	10	5	3	3	2	41	14.10
2	3	9	8	10	7	4	2	43	14.67
3	3	7	9	8	5	6	4	42	14.93
4	5	4	11	5	6	5	-	36	14.50
5	9	8	6	4	6	3	3	39	14.28
Total	26	40	44	32	27	21	11	201	14.50
IV-1	11	7	9	11	7	2	-	47	14.04
2	6	10	13	5	3	4	-	41	14.02
3	8	10	5	12	10	-	-	45	14.13
4	9	8	7	8	9	-	-	41	14.00
5	7	16	8	4	3	5	-	43	13.88
Total	41	51	42	40	32	11	-	217	14.02
V-1	6	13	14	12	4	-	-	49	13.90
2	14	8	9	9	4	3	-	47	13.79
3	5	7	13	14	5	-	-	44	14.16
4	4	11	19	7	3	2	-	46	14.00
5	6	11	9	15	6	1	-	48	14.15
Total	35	50	64	57	22	6	-	234	13.99

APPENDIX VII-3 Moulting Record for *H. longicornis* Engorged Larvae Maintained at 30°C and Various Relative Humidities (Series C)

Experiment No.	Pre-moulting Period (Days)					N	Mean Period
	8	9	10	11	12		
I-1	Not moulted					-	-
2	-					-	-
3	-					-	-
4	-					-	-
5	-					-	-
Total	-						
II-1	Not moulted					-	-
2	-					-	-
3	-					-	-
4	-					-	-
5	-					-	-
Total	-					-	-
III-1	11	7	2	3	4	27	9.33
2	11	5	9	4	-	29	9.21
3	1	9	3	7	5	25	10.24
4	3	7	2	5	1	18	9.67
5	1	8	4	3	5	21	10.14
Total	27	36	20	22	15	120	9.68
IV-1	9	11	6	7	2	35	9.49
2	15	9	3	3	8	38	9.47
3	3	13	16	8	-	40	9.73
4	9	16	9	1	6	41	9.49
5	11	12	6	6	2	37	9.35
Total	47	61	40	25	18	191	9.51
V-1	11	13	11	5	-	40	9.25
2	14	16	8	10	-	48	9.29
3	9	22	-	6	4	41	9.37
4	13	19	5	9	-	46	9.22
5	11	10	25	3	-	49	9.41
Total	58	80	49	33	4	224	9.31

APPENDIX VII-4 Moulting Record for *H. longicornis* Engorged Larvae Maintained at 35°C and Various Relative Humidities (Series D)

Experiment No	Pre-moulting Period (Days) 7	8	9	10	N	Mean Period
I-1	Not moulted				-	-
2	-				-	-
3	-				-	-
4	-				-	-
5	-				-	-
Total	-				-	-
II-1	Not moulted				-	-
2	-				-	-
3	-				-	-
4	-				-	-
5	-				-	-
Total	-				-	-
III-1	4	7	6	2	19	8.32
2	2	9	8	3	22	8.55
3	3	6	8	1	18	8.39
4	2	2	8	2	14	8.71
5	4	3	1	4	12	8.42
Total	15	27	31	12	85	8.47
IV-1	9	8	10	9	36	8.53
2	11	7	9	3	30	8.13
3	5	11	8	6	30	8.50
4	10	13	4	10	37	8.38
5	6	19	6	2	33	8.12
Total	41	58	37	30	166	8.34
V-1	9	25	9	5	48	8.21
2	10	13	17	4	44	8.34
3	5	27	15	-	47	8.21
4	8	15	13	3	39	8.28
5	12	7	13	11	43	8.53
Total	44	87	67	23	221	8.31

APPENDIX VIII Moulting Record for *H. longicornis* Engorged Nymphs Maintained at 25°C and 85.0% Relative Humidity (Preliminary Observation)

(1) Successful moulting rate (%) = 98.62% (2216 adults moulted out of 2247 engorged nymphs observed)

(2) Sex ratio of the newly moulted adults;

Female:Male = 1.72:1 (1402 females: 814 males)
(63.27%:36.73%)

(3) Course of moulting and pre-moulting periods

Sex	Pre-moulting Period (Days)						N	Mean
	13	14	15	16	17	18		
Females	146	345	389	325	133	64	1402	15.10
%	10.4	24.6	27.7	23.2	9.5	4.6		
Males	149	212	304	101	48		814	14.62
(%)	18.3	26.0	37.4	12.4	5.9			
Overall	295	557	693	426	181	64	2216	14.92
(%)	13.3	25.1	31.3	19.2	8.2	2.9		

(4) Comparison of two sample means (Nymph-to-female and nymph-to-male pre-moulting periods).

$\bar{x}_1$ = 15.10 days (n_1 = 1402), $\bar{x}_2$ = 14.62 days (n_2 = 814)

s^2 = 1.5029, $S_{\bar{d}}$ = 0.054, t = 8.889*** (df = 2214)

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Sources of Variance	Sums of Squares	Degrees of Freedom	Mean Squares	F
Between sexes	118.6548	1	118.6548	78.95***
Interaction (Among females & among males)	3327.46	2214	1.5029	-
Total	3446.1148	2215	-	-

APPENDIX IX-1 Moulting Record for *H. longicornis* Engorged Nymphs Maintained at 20°C and Various Relative Humidities (Series A)

Experiment. No.	Pre-moulting Period (Days)							N	Mean Period
	21	22	23	24	25	26	27		
I-1	-	-	3	3	1	1	2	10	24.60
2	-	3	-	3	1	3	1	11	24.36
3	2	-	3	2	2	1	-	10	23.50
Total	2	3	6	8	4	5	3	31	24.16
II-1	1	2	2	3	2	1	-	11	23.55
2	-	4	2	1	2	1	1	11	23.73
3	-	2	3	2	1	1	1	10	23.90
Total	1	8	7	6	5	3	2	32	23.72
III-1	-	4	3	1	2	1	1	12	23.67
2	-	3	3	1	2	2	1	12	24.00
3	-	3	5	1	1	1		11	23.25
Total	-	10	11	3	5	4	2	35	23.66
IV-1	2	4	2	-	2	1	1	12	23.25
2	-	2	2	2	2	3	-	11	24.18
3	2	1	3	2	1	1	2	12	23.83
Total	4	7	7	4	5	5	3	35	23.74
V-1	2	3	2	2	1	1	1	12	23.33
2	4	2	1	1	-	2	1	11	23.09
3	2	4	2	1	1	-	1	11	22.91
Total	8	9	5	4	2	3	3	34	23.12
Overall %	15	37	36	25	21	20	13	167 92.78	23.68

APPENDIX IX-2 Moulting Record for *H. longicornis* Engorged Nymphs Maintained at 25°C and Various Relative Humidities (Series B)

Experiment No.	Premoulting Period (Days) 13	14	15	16	17	N	Mean Period
I-1	-	3	4	2	2	11	15.27
2	-	4	5	2	1	12	15.00
3	1	2	5	2	1	11	15.00
Total	1	9	14	6	4	34	15.09
II-1	-	4	4	2	2	12	15.17
2	1	3	5	-	2	11	14.91
3	-	6	2	4	-	12	14.83
Total	1	13	11	6	4	35	14.97
III-1	1	4	3	3	1	12	14.92
2	-	2	7	1	2	12	15.25
3	1	3	3	3	1	11	15.00
Total	2	9	13	7	4	35	15.06
IV-1	1	2	5	3	1	12	15.08
2	2	3	3	3	1	12	14.83
3	-	5	5	1	1	12	14.83
Total	3	10	13	7	3	36	14.92
V-1	1	5	3	2	1	12	14.75
2	2	4	2	2	2	12	14.83
3	-	5	3	3	1	12	15.00
Total	3	14	8	7	4	36	14.86
Overall (%)	10	55	59	33	19	176 97.78	14.98

APPENDIX IX-3 Moulting Record for *H. longicornis* Engorged Nymphs Maintained at 30°C and Various Relative Humidities (Series C)

Experiment No.	Premoulting Period (Days) 10	11	12	N	Mean Period
I-1	1	9	2	12	11.08
2	3	7	1	11	10.82
3	1	10	-	11	10.91
Total	5	26	3	34	10.94
II-1	3	7	1	11	10.82
2	1	9	1	11	11.00
3	2	10	-	12	10.83
Total	6	26	2	34	10.88
III-1	3	9	-	12	10.75
2	4	8	-	12	10.67
3	3	7	1	11	10.82
Total	10	24	1	35	10.74
IV-1	7	5	-	12	10.42
2	6	6	-	12	10.50
3	6	5	1	12	10.58
Total	19	16	1	36	10.50
V-1	4	8	1	12	10.67
2	4	8	-	12	10.67
3	5	6	1	12	10.67
Total	13	22	1	36	10.67
Overall	53	114	8	175	10.75
(%)				97.22	

APPENDIX IX-4 Moulting Record for *H. longicornis* Engorged Nymphs Maintained at 35°C and Various Relative Humidities (Series D)

Experiment No.	Premoulting Period (Days) 9	10	N	Mean Period
I-1	1	9	10	9.90
2	4	7	11	9.64
3	1	10	11	9.91
Total	6	26	32	9.81
II-1	4	8	12	9.67
2	6	6	12	9.50
3	7	4	11	9.36
Total	17	18	35	9.51
III-1	4	8	12	9.67
2	6	6	12	9.50
3	4	7	11	9.64
Total	14	21	35	9.60
IV-1	4	7	11	9.64
2	6	6	12	9.50
3	5	7	12	9.58
Total	15	20	35	9.57
V-1	7	5	12	9.42
2	7	5	12	9.42
3	6	6	12	9.50
Total	20	16	36	9.44
Overall	72	101	173	9.59
(%)			96.11	

APPENDIX X Comparisons for the Two Sample Means (Nymph-to-Female and Nymph-to-Male) of *H. longicornis* Nymphal Premoulting Periods.

(1) Comparison for the two sample means at 20°C (Series A)

Sex	Premoulting Period (Days)							N	Mean
	21	22	23	24	25	26	27		
Females	8	18	16	16	15	18	13	104	24.135
Males	7	19	20	9	6	2		63	22.905
Overall	15	37	36	25	21	20	13	167	23.671

$s^2 = 2.785$ $S_{\bar{d}} = 0.2664$ $t = 4.616^{***}$

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S.V.	S.S.	dF	M.S.	F
Between sexes	59.3419	1	59.3419	21.308***
Interaction	459.5451	165	2.785	-
Total	518.887	166	-	-

(2) Comparison for the two sample means at 25°C (Series B)

Sex	Premoulting Period (Days)					N	Mean
	13	14	15	16	17		
Females	3	40	40	22	19	124	15.113
Males	7	15	19	11		52	14.654
Overall	10	55	59	33	19	176	14.977

$s^2 = 1.1275$ $S_{\bar{d}} = 0.1754$ $t = 2.617^{**}$

ANOVAR

S.V.	S.S.	df	M.S.	F
Between sexes	7.72	1	7.72	6.847**
Interaction	196.19	174	1.1275	-
Total	203.91	175	-	-

(3) Comparison for the two sample means at 30°C (Series C)

Sex	Premoulting Period (Days)			N	Mean
	10	11	12		
Females	28	75	8	111	10.9198
Males	25	39		64	10.6094
Overall	53	114	8	175	10.7429

$s^2 = 0.2753$ $S_{\bar{d}} = 0.0824$ $t = 2.555^*$

ANOVAR

S.V.	S.S.	df	M.S.	F
Between sexes	1.797	1	1.797	6.527*
Interaction	47.632	173	0.2753	-
Total	49.429	174	-	-

(4) Comparison for the two sample means at 35°C (Series D)

Sex	Premoulting Period (Days)		N	Mean
	9	10		
Females	39	71	110	9.6455
Males	33	30	63	9.4762
Overall	72	101	173	9.5838

$s^2 = 0.2391$ $S_{\bar{d}} = 0.0773$ $t = 2.191^*$

ANOVAR

S.V.	S.S.	df	M.S.	F
Between sexes	1.147	1	1.147	4.797*
Interaction	40.888	171	0.2391	-
Total	42.035	172	-	-