Effect of temperature and photoperiod on growth, development and reproduction of *Nysius huttoni* White (Heteroptera: Lygaeidae)

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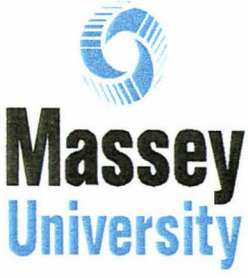

Abstract

The influence of temperature, host plant and photoperiod on *Nysius huttoni* White growth, development and reproduction was investigated under laboratory conditions.

The growth and development rate increased in linear fashion with temperature over the range of 15-30°C. The estimated lower temperature thresholds for all life stages were above 10°C except for the third instar on shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.). *Nysius huttoni* completed its life cycle at 20, 25 and 30°C on twin cress (*Coronopus didymus* (L.) Sm.) and shepherd's purse, but could not develop through to the adult stage at 15°C on any of the test host plants or on chickweed (*Stellaria media* (L.) Vill.) at any of the test temperatures. Thermal requirement for completing the life cycle in *N. huttoni* was 625.00 degree-days on twin cress and 714.29 degree-days on shepherd's purse. The time needed for a life cycle of both sexes was similar but the adult longevity decreased as temperature increased. The estimated lower temperature thresholds for mating and oviposition on twin cress were 12.3°C 16.8°C, respectively. Females failed to lay eggs when both nymphs and adults were fed with shepherd's purse. Sunflower seed was conducive to sexual maturity and fecundity.

The growth and development of *N. huttoni* slowed down as photoperiod decreased. Adults and fifth instar nymphs were sensitive to diapause-inducing photoperiod. When fifth instar nymphs and sequential adults were held at 12:12 and 10:14 h (L:D), 100% of females entered diapause. Females that had oviposition breaks over 50 days at 10:14 and 12:12 h (L:D) apparently entered diapause. However, exposure of the entire life cycle to 10:14 and 12:12 h (L:D) gave a significantly lower diapause incidence. The critical photoperiod for diapause was estimated between 13:11 to 13.5:10.5 h (L:D). Fecundity appeared to decrease with the decrease in photoperiod. The time needed for a life cycle and the longevity of both sexes were similar at a given photoperiod but increased as photoperiod decreased.

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General introduction

1.1 Introduction

Bergroth (1923) recognised 44 families of the Heteroptera of the world, of which 19 are represented in New Zealand. The Lygaeidae is one of the most speciose heteropteran families in New Zealand (Myers 1926). Myers (1926) listed three species of *Nysius* in Lygaeidae: *N. huttoni* White, *N. anceps* White and *N. clavicornis* (Fabr.). However, Eyles (1960a) considered *N. huttoni* as the only species of *Nysius* recorded from New Zealand.

The New Zealand wheat bug (*N. huttoni*) was described by White (1878). It is endemic to New Zealand and occurs throughout the North and South Islands (Myers 1926, Usinger 1942, Gurr 1957, Cumber 1959, Eyles and Ashlock 1969). It is also found in some adjacent islands such as Stephens Island (Myers 1926), Chatham Islands (Kirkaldy 1909) and Three Kings Islands (Woodward 1954).

1.2 Host plants and habitats

Nysius huttoni is a very adaptable feeder and when the necessity arises can live on many cultivated plants as well as a variety of weeds (Gurr 1957). Some of the largest populations have been found in association with shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), twin cress (*Coronopus didymus* (L.) Sm.) and Curnow's curse (*Calandrinia caulescens* H.B.K) (Gurr 1952, 1957). The known host plants and habitats are shown in Table 1.1.

Table 1.1: The known host plants and habitats in New Zealand of *N. huttoni*

Common name	Botanical name	Importance of host	Author
Broom	*Cytisus scoparius* L.		Gurr 1957
Brown top	*Agrostis tenuis* Sibth.	Major autumn shelter host	Eyles 1965b
Catchfly	*Silene gallica* L.	Medium spring and summer host	Eyles 1965b
Catsear	*Hypochaeris redicata* L.	Shelter host	Eyles 1965b
Chickweed	*Stellaria media* (L.) Vill.	Spring host	Farrell and Stufkens 1993
Curnow's curse	*Calandrinia caulescens* H.B.K.		Gurr 1957
Linen flax	*Linum usitatissimum* L.		Gurr 1957
Lucerne	*Medicago sativa* L.	Minor summer host	Myers 1921, 1926
Moss	*Triquetrella papillata* Broth.	Medium host	Eyles 1965b
Nasella tussock	*Nasella trichotoma* (Ness) Hack.		Gurr 1957
Onehunga weed	*Soliva sessilis* Ruiz & Pav.	Medium summer, autumn and winter shelter host	Eyles 1965b
Paspalum	*Paspalum dilatatum* Poil.	Minor autumn shelter host	Eyles 1965b
Perennial ryegrass	*Lolium perenne* L.	Major winter shelter host	Eyles 1965b
Red clover	*Trifolium pratense* L.	Minor summer host	Myers 1921, 1926
Sand spurrey	*Spergularia rubra* L.	Major summer host	Eyles 1965b
Scarlet pimpernel	*Anagallis arvensis* L.	Minor summer host	Eyles 1965b
Sheep's sorrel	*Rumex acetosella* L.	Major spring and summer host	Eyles 1965b
Shepherd's purse	*Capsella bursa-pastoris* (L.) Medik.	Major spring and summer host	Gurr 1957
Subterranean clover	*Trifolium subterraneum* L.		Gurr 1957
Suckling clover	*Trifolium dubium* Sibth.	Major spring host	Eyles 1965b
Toad rush	*Juncus bufonius* L.	Shelter host	Eyles 1965b
Twin cress	*Coronopus didymus* (L.) Sm.	Major spring and summer host	Gurr 1957
White clover	*Trifolium repens* L.	Major summer host	Eyles 1965b
Wireweed	*Polygonum aviculare* agg.	Major spring and summer host	Eyles 1965b
Yorkshire fog	*Holcus lanatus* L.	Major winter shelter host	Eyles 1965b

Nysius huttoni is a migratory insect. When the annual host plants begin to die off, or before the winter, the bug migrates seeking food (Eyles 1965b) or overwintering sites (Farrell and Stufkens 1993).

The summer is the major feeding and fast developing season for *N. huttoni*. Eyles (1965b) stated that *N. huttoni* lived on or very close to the ground and had often been observed under 3 mm of leaf litter and under small stones. During the warmest part of the day, both nymphs and adults seek the shade of host plants, and sometimes move into the edge of thicker vegetation; at night they shelter under leaf debris (Eyles 1965b).

In the autumn, the bugs become sluggish and tend to shelter more under grass straw, leaf litter, and at the bases of tufts of brown top (Eyles 1965b). In the winter, the adults shelter under vegetable debris, and at the bases of weeds and grasses (Gurr 1957) where much dead leaf litter is around the bases, giving good protection to the insects. In the early spring, overwintered adults migrate to patches of annual weeds, and feed and reproduce (Farrell and Stufkens 1993).

1.3 General ecology

In all Heteroptera, there are usually five nymphal instars (Myers 1926). The life cycle of *N. huttoni* includes eggs, five nymphal instars, and adults. The variation in adult and immature stages of *N. huttoni* was described by Eyles (1960a). He indicated that the population of this species consists of three size groups, the large, medium and small individuals (Table 1.2); and moreover, in both the large and medium groups, there are three forms, the macropterous, sub-brachypterous and brachypterous. Thus, there are seven different "kinds" of individuals depending on size and wing-form (Eyles 1960a). Fig. 1.1 shows the mating adults of *N. huttoni*.

Table 1.2: The three different sizes (mm) of individuals in *N. huttoni* population

	Female		Male	
Group	length	width	length	width
Larger individual	3.74-4.34	1.61-1.75	3.55-3.86	1.32-1.39
Medium individual	3.36-3.74	1.44-1.53	3.00-3.48	1.15-1.32
Small individual	2.47-3.19	1.20-1.32	2.38-3.00	0.94-1.15

Fig. 1.1: Mating adults of *N. huttoni*.

1.3.1 Preimaginal development

The colour of freshly laid eggs is creamy white (Gurr 1957). Egg laying occurs from 8 a.m. until 7.30 p.m. with a peak early in the afternoon (Eyles 1963a), and 95% of the hatching occurs in the morning with a peak between 8 a.m. and 9 a.m. and nymphal moulting occurs at any time during daylight hours (Eyles 1963b).

Eyles (1963a,b) observed the egg incubation period, nymph evelopment, fecundity and oviposition rhythms in an unheated greenhouse. He reported that when eggs were placed in refrigerators at 3°C, none of them hatched for two months; however, transferred to the greenhouse (in March) after 32 d, some of them hatched in 16 d. Ninety percent of eggs hatched at 45.2°C, indicating that this temperature was not the upper limit of temperature tolerance for eggs. In the first generation, the length of the stadium for each

instar decreased generally from first instar to adult: 15, 14, 13, 12 and 10 d; in second generation, the average of stadium was 6 d per instar; and in the third, it usually needed about 16 d per instar. Therefore, the total nymph development duration of each generation was about 64, 30 and 90 d, respectively. However, Those parameters have not been reported under controlled temperature and photoperiod conditions, making correct forecast of pest outbreaks difficult.

1.3.2 Adult reproduction

Some details of courtship and copulation were described by Eyles (1965b). For previously unmated adults there is a courtship period of from five minutes to three days; and for experienced males, courtship is brief or non-existent (Eyles 1965b). In all generations, maturity in the males is reached a little earlier than in females, and copulation may occur at any time (excluding winter) (Eyles 1963a) and is repeated many times (Eyles 1965b). Copulation normally continues for several hours but may be for only several minutes (Eyles 1965b).

Eggs of *N. huttoni* were found in soil (Gurr 1957, Eyles 1965b) but not in or on plants where the bugs congregate (Gurr 1957). However, Farrell and Stufkens (1993) reported that eggs were attached to flowers of chickweed and shepherd's purse in the field. Eggs were also found on axillae and growing points of twin cress in the laboratory (He and Wang 2000).

Female has 14 ovarioles but the maximum number of eggs laid per day ranged from 17 (Eyles 1963a) to 30 eggs (Gurr 1957). Food affects female fecundity. Under laboratory conditions, each female deposits an average of 75.6 ± 20.3 eggs when fed on the shepherd's purse (Gurr 1957) and 98.6 ± 10.6 eggs on grains of milky-ripe wheat (Farrell and Stufkens 1993). The influence of history that females have experienced on the fecundity (total egg number per female), daily egg production (daily output per female) and longevity was observed by Eyles (1963a) with very small samples (Table 1.3). The mean number of eggs per female laid after winter was greater than that

laid before winter, while daily number of eggs per female laid after winter was fewer than that laid before winter.

Table 1.3: Reproductive parameters and longevity of *N. huttoni* females (data from Eyles 1963a)

		Reproductive phase (d)			Daily egg		
Female source	No.	Preovi.	Ovi.	Postovi.	Fecundity	production	Longevity (d)
Before winter:							
from 5th instar nymphs	3	14	18	13	48	2.4	55
from field	4	----	20	2	41	2.2	----
After winter:							
from 5th instar nymphs	5	155	64	1	63	1.2	203
from field	2	----	56	1	69	1.2	----

*Preovi. = preoviposition period, Ovi. = oviposition period, Postovi. = postoviposition period

The longevity of both sexes was similar. Overwintered females lived more than twice as long as those of first generation (Eyles 1963a).

Eyles (1963a) reported the presence of a male was not necessary for the formation of eggs, virgin and mated once females producing the normal total number of eggs but virgin females having longer oviposition periods (Table 1.4). One mating per week increased the number of eggs laid per day on the days of mating or immediately following days (Table 1.4). One mating is sufficient to fertilise a female for life; the female may carry viable sperm over winter and remain fertile for a complete oviposition period (Eyles 1963a).

Table 1.4: Effect of mating on reproductive parameters and longevity of *N. huttoni* females (data from Eyles 1963a)

Female	No.	Reproductive phase (d)			Fecundity	Daily egg preduction	Longevity (d)
		Preovi.	Ovi.	Postovi.			
Permanently paired	6	6	81	13	204	2.7	97
Virgin	1	5	104	13	178	1.7	122
Mated once	2	10	76	6	203	2.8	94
Mated once/week	1	----	107	-----	323	3.0	115

*Preovi. = preoviposition period, Ovi. = oviposition period, Postovi. = postoviposition period,

1.3.3 Overwintering and diapause

There are three to four generations a year in the field. It is likely that late emerged adults of the second and third generations overwinter with the fourth generation (Eyles 1965b). The adults start to overwinter at about the end of April and become active again at about the beginning of August in the Nelson district (Gurr 1952). Farrell and Stufkens (1993) noted that the flight activity recorded in sticky traps in the vicinity of overwintering sites was slight in winter, increased in late August and reached a peak in mid September in Canterbury. Therefore, the coincidence of population decline in overwintering sites and peak flights in their vicinity suggests that overwintered adults migrate to new habitats in mid September (Farrell and Stufkens 1993). Only adults have been seen at the beginning of the spring, it is thus likely that in Nelson only adults survive the winter (Gurr 1952). However, the overwintering fifth instar was also recorded in Palmerston North (Eyles 1963b). Farrell and Stufkens (1993) found that adults of the second generation migrated to overwintering sites in the autumn and aggregated under dead leaves of woolly mullein (*Verbascum thapsus* L.), under bark of dead pinus radiata (*Pinus radiata* D. Don), in gorse hedges (*Ulex europaeus* L.), and swards of browntop grass (*Agrostis capillaris* L.) in Canterbury. However, for the females, only those emerging late in the season and not laying eggs before winter are able to survive (Eyles 1963a). Whether this relates to energy requirement of reproduction and oviposition before winter is unknown. Moreover, Farrell and Stufkens (1993) reported that

no gravid females were found on overwintering sites in 1990, and only 5-10% of females in bark sites were gravid in later August-early September in 1991.

The overwintering *N. huttoni* undergo quiescence and diapause. Eyles (1965b) stated that when female fifth instar nymphs were taken in the field in early May and placed in a constant temperature of 24°C, they moulted to adults in 5 d and oviposited after further 15 d; and adult females taken in the field at the same time completed ovarian development at the warmer temperature and oviposited in 20 d. Overwintering *N. huttoni* did not remain in one position throughout the winter. During the less intense quiescent period, i.e., before and after June and July, *N. huttoni* often moved to the tops of the grasses to bask in the sunshine, whereas during the coldest four weeks from min-June to min-July the majority of the time was passed motionless in the refuge (Eyles 1965b).

Reproductive diapause is induced by the shortening photoperiod of late summer; diapause is defined as the absence of oviposition for 30 d after adult emergence (Farrell and Stufkens 1993). Farrell and Stufkens (1993) reported that a high proportion of second generation females did not develop eggs during February and March. When the third and fourth instar nymphs in the second generation collected on 17 January (14.4:9.6 L:D) were transferred to a long photoperiod (16:8 L:D) in the laboratory, eighty-two percent of adults commenced oviposition on average 15.9 d after emergence; whereas, when exposing to a short photoperiod (12:12 L:D) condition, no oviposition occurred during the first 30 d. Sixty-four percent of females commenced oviposition an averaged of 27.1 d after being transferred from short photoperiod to long one following 30 days' diapause; whereas, only one female (7%) laid one egg 75 d after adult emergence under the continuous short photoperiod regime. Ovipositing females laid an average of 98.6 ± 10.6 eggs under long photoperiod and 43.0 ± 11.9 eggs under short/long photoperiods.

1.4 Economic importance

Nysius huttoni is well known as a pest that attacks wheat grains in the milky-ripe stage and causes sticky dough in wheat-flour (Morrison 1938, Blair and Morrison 1949, Gurr 1952 and 1957). Similarly, Every *et al.* (1990) stated that wheat is most vulnerable to damage at the flowering and grain-filling stages of growth in late November and early December. Some mature wheat crops in Mid Canterbury were found to be infested at a rate of 500 grains/m^2 in early January 1989 (J. Farrell unpubl. data) and yielded severely bug-damaged grain (D. Every pers. comm., cited by Farrell and Stufkens 1993). There have been five major outbreaks of damage since the problem was first reported in 1936; in the worst outbreak in 1970 about 10,000 tonnes of wheat was damaged (Swallow and Cressey 1987). In 1950, 98 (7%) of 1400 samples were defined as 'bug wheat' at the Wheat Research Institute in Christchurch (Gurr 1957). Gurr (1957) stated that as little as 1% of bugged wheat used in the production of a flour has made it unusable for baking, whereas Meredith (1970) stated 0.3% and Blair and Morrison (1949) stated 5% of damaged wheat is sufficient to ruin good wheat. When feeding, *N. huttoni* injects into plants saliva, contain powerful enzymes that digest the grain carbohydrate and/or protein so that solubilised nutrient can be drawn through the styli. The *N. huttoni*-damaged grains contain a *N. huttoni* salivary proteinase (Cressey 1987, Cressey and McStay 1987, Every 1993) or a high level active protease (Every *et al.* 1992). The salivary enzymes can cause serious problems in bread baking, such as runny, sticky dough, and poor quality loaves (Morrison 1938). In the past 20 years, no major outbreaks of bug damage have occurred on wheat in New Zealand. Swallow and Cressey (1987) stated that the diminished damage might be the result of the development of more resistant cultivars.

In contrast with wheat, which is not the preferred food of *N. huttoni* and is attacked at the edge of the maturing crop only after weeds around the crop have died off in summer, a crucifer crop is a suitable habitat for *N. huttoni*, and the plants are readily attacked from the seedling stage (Gurr 1957). Gurr (1957) reported that *N. huttoni* thrived best under hot and dry conditions and preferred situations where the direct sunlight struck through to the ground. The

spacing of plants makes a young crucifer crop suitable for *N. huttoni*, which is a pest particularly in areas of the South Island that have very dry summers, such as Marlborough, Mid Canterbury, and Central Otago (Gurr 1957).

The damage to rape (*Brassica napus* L.), chou moellier (marrow-stem kale) (*Brassica oleracea* L.) and some species of turnips has been reported in Marlborough, Mid Canterbury, and Central Otago (Gurr 1957). The feeding punctures are made around the stems of the seedlings at ground level and cause a cankerous growth of the tissue (Gurr 1957). In recent years, *N. huttoni* has caused serious damage to brassica crops that are used as winter fodder for stock by many farmers in Maniototo and Central Otago (Ferguson 1994). The bugs preferred the stems of the swede (*Brassica napus rapifera* Metzger) seedlings (He and Wang 1999, 2000), and up to 70% of immature plants could be lost through wind breakage (Ferguson 1994). In addition, some adults may migrate to strawberries and kiwifruit, where their presence on harvested fruit cause a contamination problem (W. Thomas pers. comm., cited by Farrell and Stufkens 1993). Moreover, although *N. huttoni* does not feed on apples, it has been observed to occur in apple packages (Lay-Yee *et al.* 1997). It is thus also a pest causing quarantine problems for the apple export industry of New Zealand.

1.5 Pest management

Eyles (1965a) stated that in 1957 and 1958, there were none or very low numbers of *N. huttoni* on the field crucifers at Palmerston North. He suggested that the thick pasture swards in that district, and the vigorous growth of grass and weeds around the edges of the crops did not favour this pest; or probably as the areas were in pasture previously and were surrounded by pasture, the chance of infestation was small. In a sweep-sampling survey of North Island pasture insects, Cumber (1959) only collected 24 *N. huttoni* out of 221 samples, and in a survey of North Island fodder crops, Eyles (1960b) only took one specimen of *N. huttoni*. The habitat requirements of *N. huttoni* may prevent it from becoming a pest of crucifers in Palmerston North. High populations of *N. huttoni* in shepherd's purse, twin cress and Curnow's curse

were reported by Gurr (1952, 1957). When cultivated crucifers were grown adjacent to suitable habitat for *N. huttoni* in areas of the North Island that are dry in summer, damage by this pest may result; in the wetter areas, unsuitable environment probably prevents *N. huttoni* from becoming a pest (Eyles 1965a).

Ferguson (1994) reported that application of insecticide (isazophos) could successfully control *N. huttoni* in direct drilled swede seedlings. In his field observation, 87% of plants were damaged in the untreated areas, and only up to 1% damage was observed in the areas with single or double applications of isazophos. However, in a nearby area of swede sown after cultivation at the same time as the direct drilled crop, no damage was observed. This suggests that the damaging population of *N. huttoni* was associated with the drilled crop.

1.6 Aims of the project

The ecology of this pest has been studied in natural and unheated greenhouse conditions; however, under controlled conditions, the effect of temperature, photoperiod and host plant on the development and reproduction of *N. huttoni* has not been reported. This knowledge is essential to modelling the population dynamics of this insect and to developing management strategies for producers because most growers can be easily trained to use the heat–unit accumulations in timing control in crop systems. Such data will also help manage risk from this serious quarantine pest.

On the basis of these observations, I initiated the project aiming at:

(1) determining the effect of temperature and food on growth, development and reproduction;

(2) investigating the influence of photoperiod on growth, development, reproduction and diapause.

Chapter two
Literature review

The growth and development of insects depend upon a number of factors, including food, temperature, and photoperiod. Two or three factors may interact to complicate interpretation of growth characteristics and patterns. In this chapter, I will review the knowledge of insect growth and development, and the main factors affecting these as the bases to build on.

2.1 Insect growth and development

2.1.1 Introduction

Insect growth is usually characterised as being discontinuous and allometric. Allometric growth involves the unsclerotized parts of insect cuticle in which it can stretch and grow during the entire inter-moult period (Nijhout 1994). Discontinuous growth involves sclerotized cuticular parts of the body in which its expansion is limited by the rigid cuticle (Gullan and Cranston 1994); it can increase in size only during the brief time around moulting (Nijhout 1994).

The growth rate of insect parts at moulting varies widely among insects and is a species-specific character. However, within a species, the cuticle of any given part of the body expands by a nearly constant factor over its size during the previous stage (Nijhout 1994). Based on measurements of the change in width of larval head capsule of Lepidoptera at each moult, Dyar's law was established that growth follows a geometric progression; the proportionate increase in size for a given structure is constant from one instar to the next (Gillott 1980, Wigglesworth 1980). The constant value (x/y) is usually 1.2-1.4, where x is the size in a given instar, and y is the size in previous instar (Gillott 1980). Dyar's law holds not only for the linear measures of the sclerotized structures but also for many other features of size and growth. For example, the proportionate increase in body weight of the lepidepteran *Manduca* is also constant from one instar to the next (Nijhout 1994) (Fig. 2.1). When the

logarithm of the size of a structure is plotted against instar number, a straight line is obtained (Gillott 1980, Nijhout 1994).

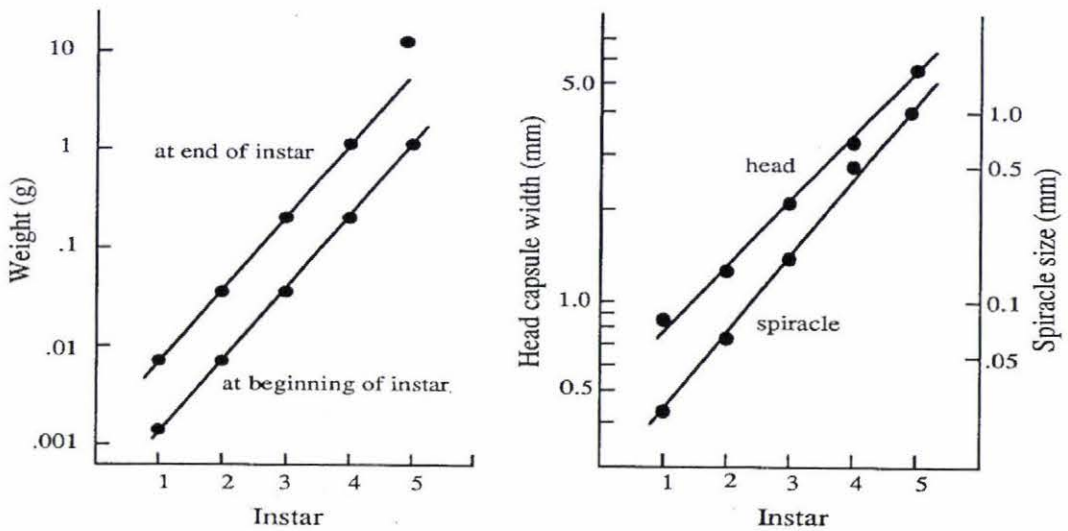

Fig. 2.1: Exponential growth in larvae of the moth *Manduca sexta* (L.) under laboratory conditions. The weight and dimensions of body parts increase by a constant factor during each instar (from Nijhout 1994).

Insect development is characterised by cyclic growth and a series of moults in successive instars. At the moulting (or ecdysis) of the old cuticle, the insect advances to the next development stage – larva or nymph, pupa and adult. However, the moult and the development stage attained at moulting are usually governed by three insect hormones: prothoracicotropic (PTTH), moulting (MH) and juvenile hormones (JH).

The PTTH is produced by the lateral cells of the insect brain (Agui *et al.* 1979, Riddiford 1980b, Muszyńska-Pytel 1987). Cymborowski (1992) suggested that the activity of the lateral cells of the brain may be controlled by nerve connections with the visual organs and the involvement of the optic lobes, and it is assumed that the control of PTTH release is mainly by environmental factors such as photoperiod, temperature and food. Truman

(1972) has shown that the timing of PTTH secretion during the larval stage of the moth *Manduca sexta* (L.) is controlled by photoperiod. Thus, the information from the external environment is transformed in the neurosecretory cells in the insect brain to chemical information in the form of the neurohormone PTTH.

The function of PTTH is to regulate the secretion of MH by the prothoracic glands. The prothoracic glands secret MH prior to each moult, and the direct action (of 20-hydroxyecdysone) on the epidermal cells causes them to undergo apolysis, cell division, digestion of the old cuticle, and secretion of the new cuticle (Nijhout 1994).

The level of MH in insect haemolymph directly corresponds to the developmental stage. Shaaya and Kaelson (1965) demonstrated that the concentration peaks before each larval moult, whereas this hormone is completely absent during the remainder of the period between moults.

JH is probably the most versatile hormone in the entire animal kingdom, and plays a role in almost every aspect of insect development and reproduction (Nijhout 1994). JH is secreted by the *corpora allata*. The *corpora allata* are switched off towards the end of the larval life, except in species having a larval diapause (Cymborowski 1992). Thus, the level of JH is variable in individual developmental stages of insects. When JH is absent, or at least in extremely low titre, adult characteristics are manifested in the newly moulted insect (Beck 1968).

It is evident that MH and JH control the development and metamorphosis of insects, and the PTTH regulates the synchronisation of these processes by being released into the haemolymph and initiates a number of physiological changes in the insect body. The interaction of these two principal hormones during development and metamorphosis has been demonstrated in the moth *Manduca sexta* (Gilbert *et al.* 1980, Riddiford 1980a) (Fig. 2.2). An

increase in the concentration of MH in the last larval instar just before larval moulting is due to the disappearance of JH at this time.

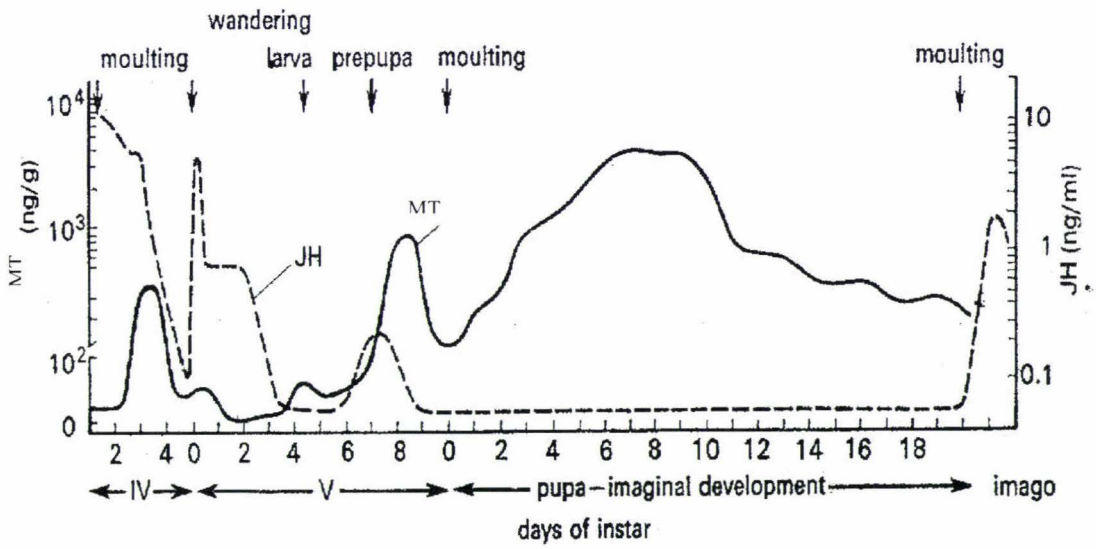

Fig. 2.2: Changes in JH and MH levels during larval development and metamorphosis in *Manduca sexta* (from Riddiford 1980a).

2.1.2 Effect of environmental factors on the growth and development of insects

2.1.2.1 Temperature

Insects are cold-blooded animals whose body temperature varies with the environmental temperature. Therefore, heat is the force driving the rates of growth and development when food is not limiting (Gullan and Cranston 1994). In insects, the developmental rate increases almost linearly with the temperature over the normal range of operating temperatures (Gilbert and Raworth 1996). A good example is the damsel bugs, *Nabis americoferus* Carayon and *N. roseipennis* Reuter over the range of 18 to 33°C (Braman *et al.* 1984). The effect of temperature on the nymphal development of the Australian stinkbug, *Biprorulus bibax* Breddin, was studied by James (1990). The complete nymphal duration decreased by 47.7 d (from 68.6 to 20.9 d) as the temperature increased 12.5°C (from 20 to 32.5°C). Romoser (1973) stated that exposure to temperatures above or below the extremes of this range resulted in

death; the definition of this optimal range of temperature, of course, varies from species to species and also with the physiological stage of an individual. For example, in the morrill lace bug, *Corythucha morrilli* Osborn and Drake, no nymphs developed at temperatures exceeding 34.4°C, and there was only little nymphal development at 17.8°C (Stone and Watterson 1985).

Optimal temperatures of egg laying vary greatly among species, possibly reflecting the temperatures the species normally encounter during reproductive periods (Engelmann 1970). Some species may oviposit over a broad temperature range, such as the bean weevil, *Bruchus obtectus* Say, which has a optimal egg laying temperature range of 21 to 30°C and possible egg laying temperature range of 8 to 40°C (Menusan 1935), or relatively narrow, as in the fire brat, *Thermobia domestica* (Packard), which has an optimal temperature of 37°C and a range of 32 to 41°C (Sweetman 1938). An interesting observation on fecundity was reported by Oldiges (1959) for the greater wax moth, *Galleria mellonella* (L.). Females obtained from larvae reared at 26°C laid more eggs than those reared at either lower or higher temperatures. At 26°C the larvae consumed more food and consequently become heavier than those reared at other temperatures, the resulting heavier adults laid more eggs than did the lighter ones. On the other hand, the low temperatures at which a given species will inhibit oviposition vary among species. The tropical or subtropical cockroach, *Nauphoeta cinerea* (Olivier) matured no eggs below temperatures of 15-17°C (Springhetti 1962), and similarly the levant housefly, *Musca domestica vicina* Macquarr laid no eggs below 14°C (Feldman-Muhsam 1944). However, the stored product beetle, *Ptinus tectus* Boieldieu, still laid a few eggs at 5°C (Howe 1951); in this case, since temperatures fluctuated during the day-night rhythm, for short periods insects could probably sustain lower temperatures and still lay eggs as soon as the temperature rose (Engelmann 1970).

Within certain ranges, both the longevity and fecundity of the adult are usually greater at low than at high temperatures. For example, adults of black carpet beetle, *Attagenus alfierii* Pic., live 10 to 13 d at 25°C and lay an average

of 41 to 47 eggs, whereas at 38°C they live only 6 to 7 d and lay 11 to 20 eggs (Ayyappa *et al.* 1964). However, in the reverse case, the angoumois grain moth, *Sitotroga cerealella* (Olivier), reared at 20°C lives an average of 11.3 d but lays more eggs at 25°C, at which temperature its life is shorter (Grewal and Atwal 1969).

Moreover, Huffaker and Rabb (1984) stated that more eggs might be laid under a fluctuating temperature regime of a given arithmetic mean value than at a constant temperature at this value, or at either of the respective constant low, or high, temperatures. For example, the cricket *Gryllus bimaculatus* DeGeer laid 276 eggs at 20°C and 900 eggs at 30°C, however, 1433 eggs were laid under a fluctuating temperature regime of 19-31°C in a daily rhythm of 8:16 (L:D), respectively (Engelmann 1984). Similarly, the parasite *Bracon mellitor* Say laid 310 eggs when temperatures fluctuated between 21-32°C in a daily rhythm of 10:14 (L:D), but just 213 eggs at constant 32°C and only 171 at constant 21°C (Barfield *et al.* 1977).

The relationships between temperature and development may be described by degree-days and development rate. Degree-days (DD) is a measure of amount of heat required over time for an insect to complete development or a stage of development, and development rate is the reciprocal of development time for an insect to complete development or a stage of development (Gullan and Cranston 1994). Because each species and each stage in the life history may develop at its own rate in relation to temperature, DD is a meaningful measure of development time. Moreover, DD models based on simple linear regressions are widely used and provide adequate predictions of phenological events within the range of temperatures encountered in the field (Campbell *et al.* 1974). On the other hand, based on the linear regression of development rate of each life stage at different temperature ranges, the development threshold for each life stage is obtained by extending the line to the x-axis (which represents the development rate). Thus, the knowledge of temperature-development relationships may provide adequate prediction of preimaginal feeding periods, generation length, population size and time of

adult emergence under variable temperature conditions that exist in the field. Such predictions are especially important for pest control timing.

2.1.2.2 Food

Growth, development and reproduction of insects are directly dependent on the quantity and quality of food ingested (Huffaker and Rabb 1984). A major fraction of the food intake may provide exogenous protein or amino acids that are required for tissue construction; another major fraction of intake is required for metabolism that provides energy, and this fraction may be carbohydrate, fat, some portion of the amino acids, or various combinations of these classes of nutrient (Huffaker and Rabb 1984).

It is clear that different food (or different nutrients) will affect the developmental rate of nymphs of most species. For example, the nymph developmental time of the rice stink bug, *Oebalus pugnax* (F.), is influenced by host plant type (Naresh and Smith 1983). At 27°C, development time is significantly shorter when on grain sorghum (*Sorghum bicolor* L.) (25.82 d), than on rice (*Oryza sativa* L.) (28.12 d), or on vasey grass (*Paspalum urvillei* Stuedel) (28.89 d). For some phytophagous insects, a combination of different kinds of plants appears necessary for survival and/or normal rate of juvenile development (Gillott 1980). An example was reported by Pickford (1962) for the migratory grasshopper, *Melanoplus sanguinipes* (Walker). He stated that when the grasshoppers fed on wheat (*Triticum aestivum* L.), a smaller percentage of insects survived from hatching to adulthood, and the development was slower than that fed on wheat plus flixweed (*Descurainia sophia* L.) or dandelion (*Taraxacum officinale* Weber).

Both the quantity and quality of food influence total egg production and egg laying by a given species. In many cases the normal food is superior to other foods in promoting egg production. The tuber flea beetle, *Epitrix tuberis* Gentner, laid more eggs if fed on potato, its normal food plant, than if fed on tomato, bean, or March elder (Hill 1946). This may be due to superior nutritional

value or it may be caused by the animals having eaten greater quantities of the normal food than of other foods tested (Engelmann 1970). In the flour beetle *Tribolium confusum* Jacquelin du val, females laid 521 eggs when fed on whole wheat flour, 333 on bran, and only 187 on white flour (Good 1933). Obviously, whole wheat flour was the most nutritious material for *T. confusum*. The effects of quantity and quality of food eaten by insects frequently cannot be separated, those species which require specific nutrients for egg maturation will often mature more eggs if given greater amount of the essential foodstuffs (Engelmann 1970). For example, the bed bug, *Cimex lectularius* L., laid 541 eggs if allowed to take 335 mg of blood but only 216 eggs on 195 mg (Titschak 1930).

The quality of available food may influence certain reproductive processes. Bonjour *et al.* (1993) reported that the squash bug, *Anasa tristis* (DeGeer), fed with pumpkin (*Cucurbita pepo* L. var. *pepo* 'Jack O' Lantern'), had shorter premating and preoviposition phases, but had longer oviposition and longevity phases than with watermelon (*Citrullus lanatus* (Thunberg) Matsumura and Nakai 'Crimson Sweet'). In addition, females had higher fecundity when fed with pumpkin seedlings.

Obviously, different types of plants may provide different nutrients or different nutritional levels affecting insect development and reproduction.

2.1.2.3 Photoperiod

The photoperiod influences insects in two ways: it may either induce short-term (daily) behavioural responses which occur at specified times in the 24-hour cycle, or bring about long-term (seasonal) physiological responses which keep insects in tune with changing environmental conditions (Gillott 1980). In short-term responses the time interval between the onset of light or darkness and commencement of the activity is important. For seasonal responses, the absolute daylength (number of hours of light in a 24-hour period) is usually

critical, though in some species it is the day-to-day increase or decrease in the light period.

The photoperiod affects a variety of long-term physiological processes in insects and allows a species to (1) exploit suitable environmental conditions, and (2) survive periods when climatic conditions are adverse (Gillott 1980). Among the processes known to be affected by the photoperiod are the rate of development, reproduction, synchronised adult emergence, and induction of diapause. For some species growth is accelerated under long photoperiod conditions and inhibited in short photoperiods. For example, the tambo cricket, *Velarifictorus parvus* Chopard, shows a long-day response: nymphs grow rapidly in long photoperiods (15:11, 16:8 (L:D)) and slowly in short photoperiods (12:12, 14:10 (L:D)) (Tanaka *et al.* 1999). However, opposite responses to daylength have also been reported. The growth of the larvae of tussock moth, *Dasychira pudibunda* L. (Geyspitz and Zarankina 1963) and the nymphs of predatory bug, *Podisus maculiventris* (Say) (Chloridis *et. al.* 1997), are accelerated by short photoperiods and slowed by long photoperiods.

The effect of photoperiod on growth rate is often correlated with the nature of diapause induction. Some species that grow more slowly under short photoperiod conditions tend also to enter diapause as a result of short photoperiods (Beck 1968, Saunders 1982). For example, in the species of the flesh fly genus *Sarcophaga* Meigen, short photoperiods induce slow development and a subsequent pupal diapause whereas long photoperiods induce rapid development and non-diapausing pupae (Denlinger 1972, Saunders 1972). Denlinger (1972) suggested that short-day larvae developed more slowly because they were 'diapause-committed'. However, the tendency for prediapause growth to be slower than nondiapause growth cannot be taken as an infallible rule, because in some species, larval growth rates are not affected by photoperiod. For example, in the European corn borer, *Ostrinia nubilalis* (Hübner), although all larvae reared under the short photoperiods were destined to enter the diapause stage, the larvae grow at similar rates in short

photoperiod (12:12, L:D) as in long photoperiod (16:8, L:D) (Beck and Hance 1960, Beck 1962).

2.2 Insect diapause

2.2.1 Introduction

Diapause is a period of arrested development that has evolved as an adaptation to survive seasonally recurring adverse conditions. In temperate zones, diapause is a strategy for overwintering. In tropical regions, it provides a way of surviving seasonal periods of drought and scarcity of food. During diapause insects become quiescent, their metabolic rate becomes greatly reduced, and they develop various physiological and biochemical adaptations to desiccation and cold-tolerance (Tauber *et al.* 1986). Diapause may occur at any growth stage – egg, larva (or nymph), pupa, or adult. However, diapause usually occurs in only one stage although insects with life cycle of a year or longer may enter diapause in one or more stages (Tauber *et al.* 1986).

Depending on the species, diapause is either genetically controlled and obligatory, or it is facultative and induced by specific environmental cues. Insects with an obligatory diapause always enter diapause when they reach a specific point in their life cycle and therefore have only one generation per year. Examples of these are the silk moth family (Saturniidae) and the species of tent caterpillars (*Malacosoma* Hübner), with a diapause in the pupal and egg stage, respectively (Nijhout 1994). Insects that have a facultative diapause generally (but not necessarily) have more than one generation a year. Such insects enter diapause only when they are exposed to a very specific set of environmental conditions that herald the approach of an unfavourable season.

When insects encounter diapause-inducing environmental conditions during their sensitive period, their physiology and metabolism undergo subtle changes. Often their feeding and growth are altered and an increasing portion of their food is channelled away from growth or reproduction and toward storage and accumulation of reserves in the fat body (Tauber *et al.* 1986).

Finally, when the stage in the life cycle is reached at which the species normally goes into diapause, metabolism drops precipitously and development comes to a virtual standstill.

Whether obligatory or facultative, and irrespective of the stage at which it occurs, diapause seems to be universally controlled by the endocrine system (Nijhout 1994). For example, hormonal regulation of adult diapause is best known in the Colorado potato beetle, *Leptinotarsa decemlineata* Say (de Wilde 1963, 1965). The removal of the corpora allata from nondiapausing beetles leads to a state characterisic of normal diapause; and implantation of active corpora allata into these insects interrupts diapause. Thus, under conditions of short photoperiod the production and release of JH are inhibited, probably owing to the inhibitory action of the brain on the corpora allata, leading to adult diapause (de Wilde and de Bore 1969, Schooneveld 1970, Hodková 1977). Adult diapause is generally characterised by the cessation of egg development, re-sorption of any eggs that are in the process of development, and increases in the fat body, and changes in metabolism and behaviour (Cymborowski 1992).

Diapause always begins well before the actual onset of unfavourable conditions and usually persists for a period of time after favourable conditions return (Nijhout 1994). Because environmental cues that signal future environmental changes are perceived by the insect, often long in advance of the diapausing stage itself, this information is stored and later translated into neuroendcrine functions in the form of diapause induction (Tauber *et al.* 1986).

Insect diapause may be evoked by major seasonal factors such as photoperiod, temperature and food supply. Of these factors, temperature and food supply show wide variations within a given environment and are difficult to forecast, so they are not good indicators of seasonal changes. However, except at the equator, the photoperiod is the most regular and therefore provides the most reliable long-term cue to future conditions for insects (Tauber *et al.* 1986).

Photoperiod has an especially important influence on the seasonal cycles of species in the temperate zone, where seasonal changes in photoperiod are large and highly correlated with seasonal changes in temperature and food supply that affect development (Tauber *et al.* 1986). For temperate species, temperature and food may modify the effect of photoperiod. This situation is often different among equatorial and tropical insects, in which the role of photoperiod may be superseded by the effect of temperature and moisture (Tauber *et al.* 1986).

2.2.2 Photoperiodic regulation of diapause

2.2.2.1 Induction of diapause

Numerous experiments have demonstrated that insects are highly sensitive to a critical photoperiod, below or above which they enter diapause. The curve relating insect responses to photoperiod is usually plotted on the basis of experimental data on insects kept under a constant photoperiod, longer or shorter than the critical value (Cymborowski 1992). Such conditions of course never prevail in the natural environment. Because the natural photoperiod undergoes continuous variation, it increases or decreases continuously. The question thus raises as to the sensitivity of insect to increase or decrease in photoperiod. However, such a problem does not exist for insects, since they are never sensitive to photoperiod throughout their entire period of development (Saunders 1982). In some species, for instance, the sensitive period is in autumn, and in others it is in spring. In silkworm *Bombyx mori* (L.), the most sensitive stage is the egg, although the first larval stage is also somewhat sensitive (Kogure 1933). In the saturniid *Philosamia cynthia* Drury, only the 4th and 5th larval stages are sensitive to the short photoperiod that induces pupal diapause (Pammer 1965). There are no records in which the characteristic of diapause has been altered either by artificial selection in laboratory or by natural selection acting on recently introduced and colonising species (Tauber *et al.* 1986).

When the percentage incidence of diapause is plotted against daylength, four types of diapause induction curves shown in Fig. 2.3 maybe obtained (Beck

1968). Type I induction curves: long-day response curves are typical of a great many species, in which relatively long photoperiods tend to favour continuous (nondiapause) growth and development. The examples displaying this type of diapause induction curve include the Colorado potato beetle, *Leptinotarsa decemlineata* (de Wilde 1958), a noctuid *Acronycta rumicis* L. (Danilevskii 1961), pink bollworm, *Pectinophora gossypiella* (Saunders) (Adkisson *et al.* 1963) and spider mite, *Panonychus ulmi* Koch (Lees 1953a, b).

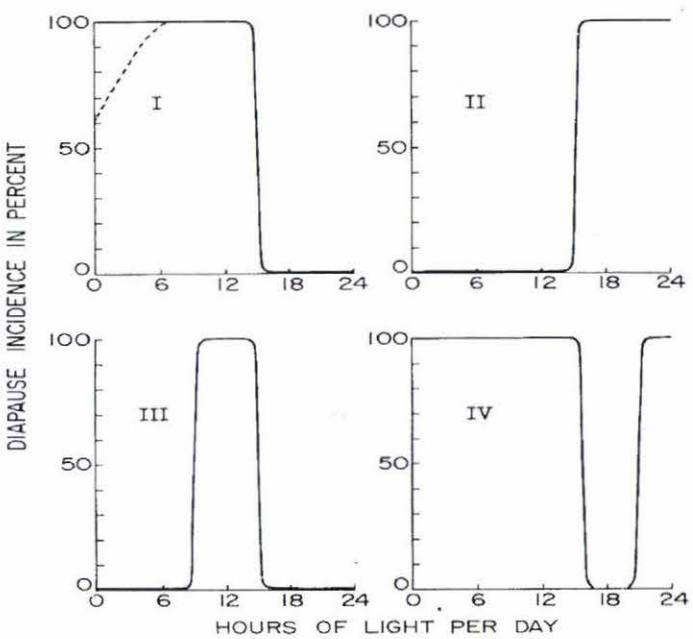

Fig. 2.3: Different types of diapause incidence-daylength relations observed among insects. I = long-day response type, II = short- day response type, III = short-day -- long-day response type, and IV = long-day -- short-day response type (from Back 1968).

Type II induction curves: short-day response curves are where nondiapause development is promoted by the short photoperiods. Some of the examples are the commercial silkworm, *Bombyx mori* (Kogure 1933), the geometrid *Abraxas miranda* (Butler) (Masaki 1956, 1959) and the noctuid *Mamestra brassicae* Linné (Masaki and Sakai 1965).

Type III induction curves: short-day -- long-day response curves are where diapause is observed at daylengths of from 10 to 16 hours, but daylengths of less than 8 or more than 16 hours are not diapause inducing. Examples of insects displaying this type of diapause induction curves include the European corn borer, *Ostrinia nubilalis* (Beck 1962), oriental fruit moth, *Grapholita molesta* (Busck) (Dickson 1949) cabbage worm, *Pieris brassicae* (L.) (Danilevskii 1961) and the mirid *Lygus hesperus* Knight (Bearda and Strong 1966).

Type IV induction curves: long-day -- short-day response curves are where there is an absence of diapause incidence over a very restricted range of relatively long photoperiods, and all other photoperiodic conditions result in a high incidence of diapause. This type of response to photoperiod has been demonstrated in only a few insect species, all of which are lepidopterans: *Leucoma salicis* (L.), *Euproctis chrysorrhea* (L.), *Euproctis similis* (Fuessly) (Geyspitz 1953) and the peach fruit moth, *Carposina niponensis* Walsingham (Toshima *et al.* 1961).

2.2.2.2 Termination of diapause

As mentioned above, diapause may occur in response to photoperiod, photoperiod may also hasten the termination of diapause. In some species such as the damselfly *Lestes disjunctus* Selys (Sawchyn and Church 1973), photoperiodic sensitivity may persist throughout diapause; without the appropriate photoperiodic cue, diapause would persist long beyond the time when development should begin (Tauber *et al.* 1986). That short photoperiod induces and long photoperiod terminates the diapause is common in some species. Those examples include the pupae of the parasitoid *Trogus mactator* Tosquinet (Omata 1989), prepupal stage of parasitoid *Phytodietus vulgaris* Cresson (Coop and Croft 1990), pupae of oriental tobacco budworm, *Heliothis assulta* Guenée (Boo *et al.* 1990), and adults of the bruchid beetle, *Bruchus rufimanus* Boheman (Darquenne *et al.* 1993).

However, in the saturniid *Antheraea yamamai* Guerin, aestival diapause in the pupae is induced by long photoperiod (16:8, L:D) during the larval stage but it is terminated in a few days after transferring diapausing pupae to short photoperiods (Kato and Sakate 1981). However, if diapausing pupae are maintained under long photoperiods, diapause persists more than three months (Tauber *et al.* 1986). In this case, diapause is induced by long photoperiods and terminated in respond to short photoperiods. Other species include adults of the mirid *Lygus hesperus* (Bearda and Strong 1966, Steenwyk and Stern 1976), the larvae and pupae of the lasiocampid *Dendrolimus pini* L. (Danilevskii 1961, Geyspitz *et al.* 1972) and the adults of the Emperor dragonfly, *Anax imperator* (Hansemann) (Corbet 1956).

2.2.3 Modification of photoperiodic response by temperature and food

Temperature and food influence growth rates of insects and therefore exert a strong effect on the proportion of individuals completing their photosensitive stages before or after the occurrence of diapause-inducing photoperiods. In some species, various temperatures have been observed to modify insects' reaction to photoperiod. For example, the critical photoperiods for diapause induction of the European corn borer, *Ostrinia nubilalis* (Beck and Hance 1960), and cabbage worm, *Pieris brassicae* (Bünning and Joerrens 1962), decreased approximately 15 minutes for every 5°C rise in ambient temperature. However, much greater effects have been reported in other species. A 5°C change in the constant ambient temperature induced a 1 hour change in the critical photoperiod for diapause induction in the Asian bollworm, *Chloridae obsoleta* F. (Goryshin 1958), and 1.5 hours change for the noctuid *Acronycta rumicis* (Danilevskii 1961).

More commonly, especially among temperate-zone insects, temperature acts as a modifier of photoperiodic response. In such cases, temperature may considerably modify, or even abolish the insect's reaction to photoperiod. For example, in the cabbage worm, *Pieris brassicae* (Danilevskii 1961), short photoperiod (12:12, L:D) caused 100% of diapause incidence at

all temperatures below 25°C, but at temperatures above 28°C completely averted diapause induction, even in the presence of the short photoperiod.

That food modifies the insect primary response to photoperiod is found in the Colorado potato beetle, *Leptinotarsa decemlineata*. Photoperiodic induction of diapause is enhanced when the insects feed on physiologically aged rather than young of mature potato leaves (Hare 1983).

Chapter three

Effect of temperature and host plant on *N. huttoni* development and reproduction

3.1 Introduction

Some attempts had been made (Eyles 1963a, b) to determine the development times for the preimaginal stages and fecundity and oviposition rhythms of *N. huttoni*. These have generally been based on observations in an unheated greenhouse with a small number of individuals.

Prediction of the seasonal occurrence of insect life stages is vital to accurate census sampling and management timing for *N. huttoni* in the field and is also helpful in risk management of this quarantine pest. This requires an understanding of the growth and development of the insect under known environmental conditions. The degree-days (DD) requirement for insect development is often used as a basis for prediction (Taylor 1981). Development rate increases almost linearly with temperature over the normal range of operating temperatures for an insect species, and the widely adopted measure of the DD requirements for their development relies on that linearity (Gilbert and Raworth 1996).

Many studies have investigated the effect of temperature and host plant on insect development. Information about the effect of different host plants and temperatures on development time and survival of nymphs, and fecundity is crucial in determining potential insect populations. The relationship between *N. huttoni* growth or development and temperature, and host plant, has not been reported. Furthermore, there are no previous attempts to determine the low temperature threshold and degree-days requirements for preimaginal development, mating and oviposition of *N. huttoni*.

In this study, the influences of temperature and host plant on preimaginal growth and development, and female reproduction were

determined under laboratory conditions. The effects of food supply and mating on reproduction were also tested.

3.2 Effect of temperature and host plant on preimaginal development, growth and survival

3.2.1 Materials and methods

3.2.1.1 Breeding colony

A breeding colony was maintained in the Entomology and IPM Laboratory of Massey University at 20 ± 1°C, 60 ± 10% RH, with a photoperiod of 16:8 (L:D). Food for *N. huttoni* consisted of sunflower seeds and stems of twin cress (TC) (*Coronopus didymus* (L.) Sm.). The breeding colony was housed in a clear plastic container (8.5 × 10 cm), with a 4.5 cm gauze-covered hole in the lid for ventilation (Fig. 3.1). A 6.5 cm long stick of cottonwool was provided as an oviposition site. Eggs were collected every 24 hours from the cottonwool stick and allowed to hatch in plastic Petri dishes (9.0 × 1.3 cm). All experiments were carried out in glass vials (2.5 × 8.0 cm) with a 1.5 cm mesh covered hole in the lids (Fig. 3.2).

Fig. 3.1: Containers used for breeding colonies of *N. huttoni*.

Fig. 3.2: An experiment setup in glass vials.

3.2.1.2 Eggs

Newly laid eggs (< 24 h old) were immediately transferred to one of the five temperatures for incubation: 10, 15, 20, 25 and 30 ± 1°C. Sixty replicates were set up for each temperature with 10 eggs per replicate. The egg development time and hatch rate were recorded. Because eggs failed to hatch at 10 ± 1°C, only four treatments were analysed. Ten newly hatched nymphs (as one replicate) from the same temperature treatment were weighed together using a Dual Range Balance (Mettler AE163, Switzerland) with a readability of 10 µg (Fig. 3.3). The number of repeated weighings for each temperature ranged from 28 to 40 depending on the various hatch rates at different temperatures (Table 3.1).

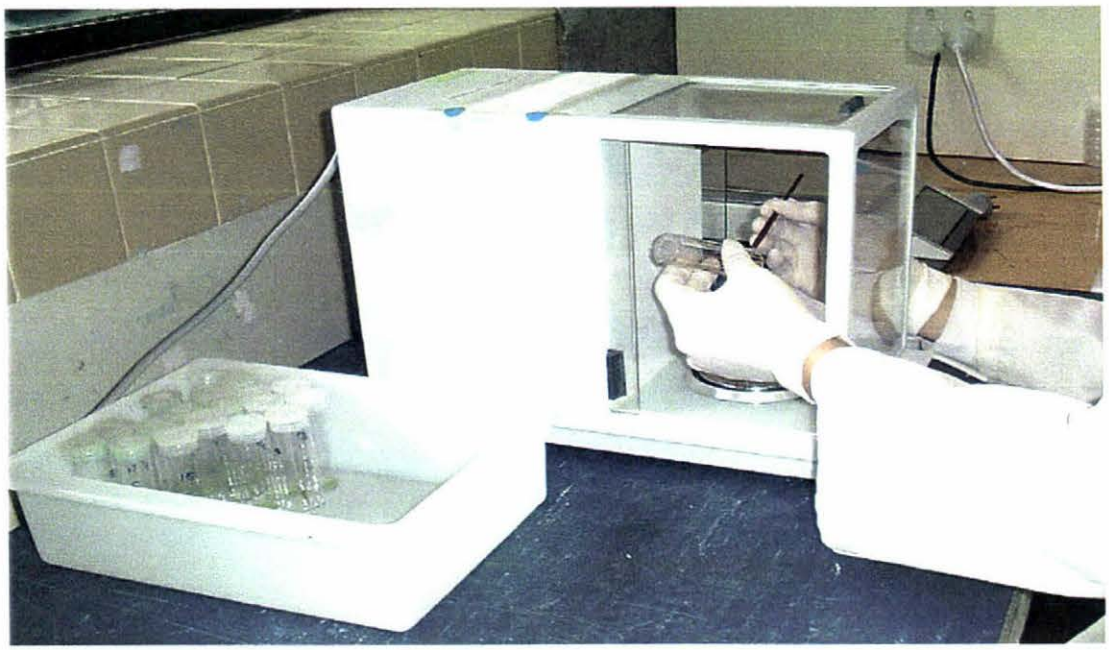

Fig. 3.3: Weighting bugs using a Dual Range Balance.

3.2.1.3 Nymphs

Newly hatched nymphs under four different temperatures (15, 20, 25 and 30 ± 1°C) were transferred to glass vials (10 individuals each) and fed with one of the three host plants under the same temperatures at which the eggs were incubated. The three host plants were: twin cress (TC), shepherds' purse (*Capsella bursa-pastoris* (L.) Medik) (SP) and chickweed (*Stellaria media* (L.) Vill.) (CW). A total of 12 treatments (4 temperature x 3 host plants) were performed with each vial as a replicate. In addition, 150 newly hatched nymphs of each instar were obtained from the breeding colony, transferred to 15 glass vials, 10 individual each, and put at 10 ± 1°C and fed with TC.

Nymphs were checked every 24 hours and weighed together when 50% or more individuals in each vial moulted. Newly emerged females and males were sexed according to (Eyles 1963b) and weighed individually. The dates were recorded as the moulting dates; the numbers of live nymphs were also recorded. Nymph survival of each instar was calculated by the following formula:

Survival = No. of surviving nymphs or adults/No. of previous surviving nymphs.

Development rate was the reciprocal of time (1/day) required for eggs and nymphs to complete development (Campbell *et al.* 1974), and growth rate was defined as the daily increase in body weight, i.e.,

Growth rate = (Body weight of each instar or adults - body weight of previous instar)/Development time.

3.2.1.4 Statistics

The data of this experiment were analysed using ANOVA (General Linear Models procedure) on (SAS Institute 1990). Where significant differences in variables occurred, means were separated using Tukey's Studentized Range (HSD) Test ($P \leq 0.05$: SAS Institute 1990) with linear contrasts of treatments. According to Steel *et al.* (1997), before ANOVA, data of nymphal survival and egg hatch rate were subject to arcsine transformation; data of development time (Table 3.3 and Table 3.5), body weights (Table 3.7) and growth rate (Table 3.12) were subject to natural logarithmic transformation to fit the normal distribution of data residuals.

Based on the development time at constant temperatures and/or three host plants, the linear regression y = *b*T + *a* was fitted to the development rates versus temperatures for each preimaginal stage, where y was the development rate and T was the temperature. The development threshold temperature (T°) for each preimaginal stage was found by extrapolating the regression line and noting where it cut the temperature axis (i.e. T° = -*a*/b). The degree-days (DD) above the T° required to complete a stage of development was estimated by the reciprocal of the slope (*b*) of the fitted regression line (i.e. DD = 1/*b*). Formulae for calculating the standard errors of T° and DD were given by Campbell *et al.* (1974). The standard error of T° was approximately: $(\bar{y}/b)\sqrt{(S^2/n\bar{y}^2)+(SE_b/b)^2}$ and that of DD was approximately: SE_b/b^2, where $\bar{y}$ was the mean of samples, s^2 was the residual mean square of y, n was the number of samples, SE_b was the standard error of *b*. In addition, a

linear regression was also fitted to the growth rates on temperatures. At a given temperature, the DD requirement for *N. huttoni* to complete development was calculated using the methods described by Braman *et al.* (1984).

To find the relationship between development time and body weight of newly hatched nymphs (or adults), a linear regression was fitted after natural logarithmic transformation was applied to the data.

The pooled sex ratio of emerged adults was tested for conformation to a 1:1 ratio using the Chi-square (χ^2) test ($\chi^2_{0.05}$ = 3.841, df = 1).

3.2.2 Results

3.2.2.1 Development time

No eggs hatched at 10°C in 100 d. Freshly laid eggs were pale or yellowish orange in colour. After approximately 50 d of incubation at 10°C, most eggs turned orange throughout and the embryos had developed to some extent with eyes developed as two deep orange spots. However, all became dry after approximately 100 d. These eggs transferred to 15 and 20 °C conditions after the fiftieth day also failed to hatch.

The mean development time of *N. huttoni* eggs was significantly different for all temperatures tested from 15 to 30°C, decreasing as temperature increased. Egg hatch rate was significant higher at 20°C and body weights of first instar nymphs were significantly higher at 20 and 25°C (Table 3.1).

Table 3.1: Mean (± SE) egg development time, hatch rate and body weight (Wt) of 1st instar nymphs *

Temp. (°C)	n	Time (d)	n	Hatch (%)	n	Wt/nymph (10 μg)
30	60	5.20 ± 0.02d	60	48.50 ± 1.46b	28	2.67 ± 0.05b
25	60	7.13 ± 0.02c	60	53.00 ± 1.43b	32	2.89 ± 0.05a
20	60	11.60 ± 0.04b	60	68.17 ± 2.11a	40	2.93 ± 0.03a
15	60	26.13 ± 0.02a	60	49.33 ± 1.97b	28	2.50 ± 0.06c
F		699.99		29.08		17.72
df		3		3		3
P		0.0001		0.0001		0.0001

*Means followed by the same letters in columns were not significantly ($P > 0.05$).
n is the number of replicates.

At 10°C, all newly hatched or moulted nymphal instars that were transferred from the breeding colony failed to develop to the next instar or adult, although they grew (see section 3.2.2.2). The surviving time for nymphs at 10°C was significantly different between instars (Table 3.2). Fourth and fifth instar nymphs lived for significantly longer time than second and third instar nymphs when 50% nymphal mortality occurred; and the survival period for fifth instar nymphs was significantly longer than that for second, third and fourth instar nymphs until 100% of them died.

Table 3.2: Mean (± SE) survival period for nymphs of different instars at 10°C until 50 and 100% of them died *

		Survival period (d)	
Instar	n	50% death	100% death
2nd	7	48.43±2.52b	92.43±6.84b
3rd	6	50.67±7.54b	102.17±5.12b
4th	6	80.50±4.54a	111.50±1.89b
5th	5	97.20±9.14a	145.60±15.20a
F		17.21	7.90
df		3	3
P		0.0001	0.0011

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).

Nysius huttoni nymphs were found to pass through five nymphal instars by observing moulting and counting shed skins. Temperatures and host plants

significantly influenced the development time for each instar. The mean development time of nymphs fed with any of the three host plants significantly decreased when temperature increased from 15 to 30°C (Tables 7 and 8).

At 20, 25 and 30°C, nymphs reared with TC and SP successfully developed through to the adult stage. Development times for successive stages generally increased as nymphs developed from the first to the fifth instar; the time for the fifth instar was longest at all temperatures. Nymphs fed with TC generally spent a shorter time in completing development to the next instar (or adult) than did those fed with SP, and nymphs fed with TC or SP grew faster than those fed with CW (Table 3.3). A significantly shorter time was required for *N. huttoni* to complete the preimaginal development (from egg to adult) when fed with TC than with SP, except at 30°C (Table 3.4). Nymphs reared with CW failed to develop beyond the third, fifth, fourth and fifth instar at 15, 20, 25 and 30°C, respectively. At 15°C, nymphs reared with TC and SP also failed to develop beyond the fifth and fourth instar, respectively. In all treatments, the shortest development time occurred at 30°C for fourth instar fed with TC, and the longest one occurred at 15°C for first instar fed with SP.

Table 3.3: Mean (± SE) development time (d) of *N. huttoni* nymphs at various combinations of temperature and host plant *

Plant	Temp. (°C)	Instar n	1st	n	2nd	n	3rd	n	4th	n	5th
TC	30	15	5.93±0.33f	15	4.13±0.22d	15	5.53±0.27f	15	5.20±0.43f	15	7.55±0.25d
	25	15	7.53±0.34e	15	7.60±0.40d	15	7.80±0.63dec	15	8.53±0.53de	14	11.29±0.16c
	20	15	14.60±0.29d	15	13.40±0.40bc	15	10.93±0.49de	15	13.47±0.27bcd	15	16.01±0.70ab
	15	15	28.00±0.90b	15	22.20±0.94ab	15	24.50±2.09abc	4	23.33±1.45a		---
SP	30	15	6.13±0.29f	15	6.00±0.40d	15	6.73±0.55ef	14	7.07±0.49ef	2	8.00±4.00d
	25	15	9.00±0.24e	14	7.33±0.50d	13	10.50±1.00def	12	11.08±0.09cde	5	12.40±1.63bc
	20	15	13.87±0.26d	15	13.47±0.59bc	15	12.73±0.75cd	15	15.87±1.01abc	14	18.14±2.71a
	15	13	57.23±2.21a	5	26.20±3.50a	1	25ab		---		---
CW	30	15	8.50±0.40e	11	5.55±0.53d	11	10.55±1.83def	6	15.83±0.21abc		---
	25	15	14.60±0.46d	12	9.08±1.18cd	3	14.67±4.06bcd		---		---
	20	15	21.23±1.42c	13	19.44±1.83ab	11	10.55±1.83def	2	19.00±6.00ab		---
	15	3	31.20±2.63b	1	27a		---		---		---
	F		300.30		47.09		25.94		29.60		38.63
	df		11		11		10		8		5
	P		0.0001		0.0001		0.0001		0.0001		0.0001

* Means followed by the same letters in columns were not significantly different ($P > 0.05$). Before ANOVA, data were subject to natural logarithmic transformations.

Table 3.4: Mean (± SE) development time (d) of preimaginal stages of *N. huttoni* reared from eggs with TC or SP under three temperature conditions*

Temp. (°C)	Plant n	TC	n	SP	*F*	df	*P*
30	15	35.42±0.42cα	2	38.70±6.50cα	1.72	1	0.2100
25	14	49.49±0.81bβ	5	60.13±2.90bα	25.16	1	0.0001
20	15	80.01±0.95aβ	14	85.24±1.05aα	14.04	1	0.0001
F		962.15		88.79			
df		2		2			
P		0.0001		0.0001			

* Means followed by the same English letters in columns and Greek letters in rows were not significantly different ($P > 0.05$).

No significant difference in time required to complete development was found between sexes at each temperature and host plant, but both sexes required longer time to complete development (from egg to adult) reared with SP than with TC (Table 3.5).

Table 3.5: Mean (± SE) development time (d) of *N. huttoni* females and males reared from eggs with TC or SP under three temperature conditions *

Temp. (°C)	Plant	n	Female	n	Male	*F*	df	*P*
30	TC	13	35.46 ± 0.53dα	12	35.20 ± 0.64dα	0.10	1	0.7549
	SP	1	37.20①	1	40.20①			
25	TC	14	49.81 ± 0.84cα	12	45.36 ± 1.43cα	2.75	1	0.1030
	SP	4	60.88 ± 3.64bα	2	56.13 ± 1.00bα	0.76	1	0.4331
20	TC	15	79.11 ± 0.92aα	14	75.51 ± 0.61aα	3.33	1	0.0634
	SP	10	87.77 ± 3.10aα	9	82.52 ± 1.40aα	2.55	1	0.1285
	F		278.38		248.60			
	df		1		1			
	P		0.0001		0.0001			

*Means followed by the same English letters in columns and the same Greek letters in rows were not significantly different ($P > 0.05$).
Before ANOVA, data were subject to natural logarithmic transformation.
①Only one female and one male emerged at 30°C when fed with SP, the means were not compared.

The thermal requirement for development varied with temperature and host plant (Table 3.6). Estimates of T° and DD required to complete each stage of development are summarised in Table 3.6. The coefficients of determination (R^2) were high (> 0.92). A T° of 11.88°C was obtained for development from egg to adult when reared with TC, which was slightly lower than that when reared with SP. When fed with TC, *N. huttoni* required 638.28, 642.88 and 637.21 DD at temperatures of 20, 25 and 30°C, respectively; when fed with SP, 657.20, 764.25 and 685.38 DD were required for temperatures of 20, 25 and 30°C, respectively.

Table 3.6: Regression of development rate (y) on temperature (T) and estimated development thresholds (T°) and degree-days (DD) for each stage of *N. huttoni*

Plant	Stage	Equation	R^2	*P*	T° (± SE)	DD (± SE)
	Egg	y = 0.0103T – 0.1179	0.9994	0.0003	11.45 ± 0.21	97.09 ± 1.64
TC	1st instar	y = 0.0093T – 0.1070	0.9833	0.0024	11.51 ± 1.13	107.53 ± 9.96
	2nd instar	y = 0.0084T – 0.0853	0.9602	0.0042	10.15 ± 1.16	119.05 ± 10.08
	3rd instar	y = 0.0091T – 0.0950	0.9954	0.0003	10.43 ± 0.64	109.89 ± 5.24
	4th instar	y = 0.0098T – 0.1143	0.9960	0.0021	11.66 ± 1.76	102.04 ± 14.56
	5th instar	y = 0.0070T – 0.0800	0.9799	0.0033	11.43 ± 2.08	142.86 ± 21.09
	1st-5th instar	y = 0.0017T – 0.0170	0.9950	0.0005	10.00 ± 1.09	588.24 ± 42.99
	Egg to adult	y = 0.0016T – 0.0190	0.9999	0.0001	11.88 ± 0.801	625.00 ± 7.44
SP	1st instar	y = 0.0095T – 0.1232	0.9966	0.0019	12.97 ± 0.48	105.26 ± 4.56
	2nd instar	y = 0.0090T – 0.0977	0.9824	0.0011	10.78 ± 1.22	111.11 ± 10.61
	3rd instar	y = 0.0070T – 0.0668	0.9802	0.0028	9.54 ± 1.42	142.86 ± 14.28
	4th instar	y = 0.0078T – 0.0978	0.9699	0.0038	12.54 ± 2.23	128.21 ± 22.48
	5th instar	y = 0.0070T – 0.0874	0.9760	0.0099	12.49 ± 2.05	142.86 ± 22.50
	1st-5th instar	y = 0.0016T – 0.0199	0.9608	0.0041	12.44 ± 2.71	625.00 ±124.18
	Egg to adult	y = 0.0014T – 0.0172	0.9698	0.0043	12.29 ± 2.37	714.29 ±125.02
CW	1st instar	y = 0.0056T – 0.0589	0.9250	0.0098	10.52 ± 2.65	178.57 ± 36.19
	2nd instar	y = 0.0098T – 0.1249	0.9353	0.0092	12.74 ± 1.18	102.04 ± 19.06
	3rd instar	y = 0.0057T – 0.0743	0.9989	0.0002	13.04 ± 0.42	175.44 ± 5.94

3.2.2.2 Nymphal growth

At 10°C, nymphs transferred from the breeding colony failed to develop, but body weight increased (Table 3.7). There were significant differences in body weights of nymphs after rearing for 50 and 100 d, body weights increased as time developed, but they were similar for the third, fourth and fifth instars after the fiftieth day. The growth rates were faster in the first growth period (0—50 d) than in second growth period (51—100 d), but they were similar for second and fifth instars (Table 3.8).

Table 3.7: Mean (± SE) body weights of *N. huttoni* nymphs reared with TC at 10°C *

	Body weight/nymph (10 µg)								
Instar	n	0 d	n	50 d	n	100 d	*F*	df	*P*
2nd	6	11.26±0.23c	6	17.39±1.01b	4	21.67±0.67a	48.85	2	0.0001
3rd	6	21.00±0.98b	6	33.31±1.93a	3	35.13±4.08a	15.74	2	0.0010
4th	6	49.30±0.32b	6	70.58±21.9a	5	72.43±3.74a	34.84	2	0.0001
5th	5	95.58±1.47b	5	115.62±4.68a	5	122.66±3.13a	23.08	2	0.0001

* Means followed by the same letters in rows were not significantly different ($P > 0.05$). One hundred percent of first instar died within 40 d, the data were not included here. Before ANOVA, data were subject to natural logarithmic transformation.

Table 3.8: Mean (± SE) growth rate of *N. huttoni* nymphs reared with TC at 10°C *

	Growth rate (10 µg/d)						
Instar	n	0-50 d	n	51-100 d	*F*	df	*P*
2nd	7	0.1226±0.0237a	3	0.1127±0.0359a	0.05	1	0.8241
3rd	6	0.2473±0.0384a	3	0.0704±0.0256b	13.41	1	0.0052
4th	6	0.4257±0.0464a	5	0.0370±0.0714b	20.84	1	0.0001
5th	5	0.4008±0.1054a	5	0.2208±0.0871a	1.73	1	0.2245

* Means followed by the same letters in rows were not significantly different ($P > 0.05$).

There were significant temperature-host plant interactions for body weights of each instar and adults, and for growth rates of nymphs.

When grouped by instar, body weights of nymphs reared with TC were usually greater than those reared with SP, and both were greater than with CW at a given temperature, except at 25°C. When grouped by host plant, greater body weights of nymphs and adults were usually obtained at 15 and 20°C on TC, at 20 and 25°C on SP and CW (Table 3.9).

Regardless of sex, at 20, 25 and 30°C, no significant differences could be detected in adult body weight when preimaginal stages were reared with TC or SP (Table 3.9). Similarly, there was no significant difference in body weight between females and between males at combinations of temperatures and

host plants, but body weight of females was significantly greater than that of males (Table 3.10).

Table 3.9: Mean (± SE) body weight of *N. huttoni* nymphs at various combinations of temperature and host plant *

Plant	Temp. (°C)	Body weight/individual (10 μg) n	2nd	n	3rd	n	4th	n	5th	n	Adult
TC	30	15	10.95±0.61ab	15	23.64±1.46abc	15	42.53±2.60ab	15	72.47±3.67bc	15	129.9±4.91a
	25	15	10.93±0.32ab	15	21.85±0.84abc	15	40.69±1.77ab	15	80.96±3.41b	14	136.1±1.95a
	20	15	11.69±0.36a	15	27.45±1.10a	15	51.91±2.20a	15	103.3±3.28a	15	138.6±2.61a
	15	15	10.83±0.44ab	15	25.13±0.71ab	15	44.28±2.31ab	4	91.13±3.49ab		----------
SP	30	15	9.27±0.34abc	15	21.97±0.82acd	15	36.21±1.87ab	14	73.92±3.05b	2	125.0±7.00a
	25	15	11.54±0.39a	14	21.15±0.83abc	13	38.93±2.71ab	12	76.27±4.32b	5	132.41±9.13a
	20	15	11.06±0.31ab	15	24.25±1.30abc	15	46.26±1.98ab	15	81.45±2.63b	14	122.3±4.27a
	15	13	9.91±0.61abc	5	17.54±0.88bc	1	35b		----------		----------
CW	30	14	8.84±0.77bc	11	18.57±1.91bc	11	32.32±1.59b	6	71.05±4.48bc		----------
	25	15	10.79±0.38ab	12	22.03±1.30abc	3	39.67±8.67ab		----------		----------
	20	15	9.76±0.90abc	3	19.70±1.40bc	7	34.50±2.15b	2	51.00±4.00c		----------
	15	3	8.24±0.56c	1	17c		----------		----------		----------
	F		3.57		5.31		5.82		9.69		2.27
	df		11		11		10		8		5
	P		0.0001		0.0001		0.0001		0.0001		0.0591

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).

Table 3.10: Mean (± SE) body weight of females and males reared from eggs with TC or SP under three temperature conditions *

Plant	Temp. (°C)	Body weight/individual (10 μg) n	Female	n	Male	*F*	df	*P*
TC	30	13	142.69±4.00aα	12	112.18±4.69aβ	24.50	1	0.0001
	25	14	146.86±4.27aα	12	128.23±2.91aβ	13.65	1	0.0011
	20	15	148.35±3.08aα	14	125.15±3.37aβ	25.94	1	0.0002
SP	30	1	132①	1	118①			
	25	4	139.25±7.79aα	2	122.00±0.17aβ	5.63	1	0.0421
	20	10	136.18±6.55aα	9	116.42±3.03aβ	8.02	1	0.0115
	F		1.07		2.53			
	df		4		4			
	P		0.3865		0.0518			

*Means followed by the same English letters in columns and the same Greek letters in rows were not significantly different ($P > 0.05$). ①Only one female and one male emerged at 30°C when fed with SP, the means were not compared.

Body weight of newly moulted nymphs or adults increased with development time at test temperatures and host plants and the data fitted a linear regression. The equations and coefficients of determination (R^2) are summarised in Table 3.11. The R^2 values were high (> 0.91). At a given temperature, the slopes (*b*) of the fitted regression lines were higher on TC than on SP, which were higher than on CW. That is, the body weight increased faster on TC than on SP, and it increased faster on SP than on CW.

Table 3.11: Regression of body weight (y) of newly moulted nymphs or adults of *N. huttoni* against development time (x) at four temperatures and three host plants

Temp. (°C)	Host plant		
	TC	SP	CW
30	logy = 1.9901logx – 1.0378	logy = 1.7822logx – 0.8625	logy = 1.5075logx – 0.6867
range	1st—adult	1st—adult	1st—5th instar
R^2	0.9681	0.9749	0.9317
P	0.0001	0.0001	0.0001
25	logy = 1.9441logx – 1.2176	logy = 1.7337logx – 1.0325	logy = 1.32421logx – 0.6801
range	1st—adult	1st—adult	1st—4th instar
R^2	0.9736	0.9649	0.9456
P	0.0001	0.0001	0.0001
20	logy = 2.0409logx – 1.7561	logy = 1.8870logx – 1.5752	logy = 1.3057logx – 0.9543
range	1st—adult	1st—adult	1st—5th instar
R^2	0.9812	0.9836	0.9618
P	0.0001	0.0001	0.0001
15	logy = 2.1260logx – 2.6073	logy = 1.3354logx – 1.5154	----------
range	1st—5th instar	1st—4th instar	
R^2	0.9587	0.9194	
P	0.0001	0.0001	

With all host plants tested, growth rates of different instars significantly increased as temperature increased from 15 to 30°C, and increased with the development of nymphs from first instar to later instars (Table 3.12). At a given temperature, growth rates of instar nymphs reared with TC were usually greater than those reared with SP, and growth rates on CW were lowest.

Linear regression of growth rate against temperature was fitted for each nymphal instar of *N. huttoni* (Table 3.13). The R^2 values were high (> 0.92).

Table 3.12: Mean (± SE) growth rate of *N. huttoni* nymphs at various combinations of temperature and host plant *

Plant	Temp. (°C)	Growth rate (10 µg/d) n	1st	n	2nd	n	3rd	n	4th	n	5th
TC	30	15	1.35±0.16a	15	2.12±0.30a	15	3.49±0.48a	15	6.67±1.35a	2	7.63±0.94a
	25	15	1.11±0.09a	15	1.46±0.11ab	15	2.72±0.34ab	15	4.90±0.59abc	14	5.04±0.23ab
	20	15	0.60±0.02c	15	1.15±0.08abc	15	2.32±0.21ab	15	3.84±0.22abc	15	2.28±0.32b
	15	15	0.29±0.01de	15	0.66±0.40cde	15	0.79±0.11bc	4	2.00±0.33c		----------
SP	30	15	1.08±0.06a	15	2.23±0.18a	15	2.36±0.29ab	14	5.41±0.39a	2	5.32±2.07ab
	25	15	0.98±0.06ab	14	1.37±0.11abc	13	1.89±0.37abc	12	3.63±0.56abc	5	4.57±1.11ab
	20	15	0.59±0.22c	15	0.83±0.06bcd	15	1.50±0.12abc	15	2.20±0.16bc	14	2.26±0.32b
	15	13	0.21±0.08f	5	0.36±0.08e	1	0.40cdbc		----------		----------
CW	30	14	0.74± 0.08bc	11	1.78 ± 0.18ab	11	1.70 ± 0.38abc	6	2.55 ± 0.26abc		----------
	25	15	0.55± 0.03c	12	1.44 ± 0.26abc	3	1.21 ± 0.27abc		----------		----------
	20	15	0.31± 0.04d	13	0.49 ± 0.09de	7	0.59 ± 0.06c	2	0.68 ± 0.36d		----------
	15	3	0.20± 0.02ef	1	0.33e		----------		----------		----------
	F		63.58		19.40		8.24		11.40		10.52
	df		11		11		10		8		5
	P		0.0001		0.0001		0.0001		0.0001		0.0260

* Means followed by the same letters in columns were not significantly different ($P > 0.05$). Before ANOVA, data were subject to natural logarithmic transformation.

Table 3.13: Regression of growth rate (y) against temperature (T) for *N. huttoni* nymphs reared with three host plants

Plant	Stage	Equation	R^2	*P*
TC	1st instar	y = 0.0736T – 0.8160	0.9836	0.0082
	2nd instar	y = 0.0992T – 0.8620	0.9715	0.0144
	3rd instar	y = 0.1700T – 1.4950	0.9336	0.0338
	4th instar	y = 0.3014T – 2.4290	0.9902	0.0049
	5th instar	y = 0.5350T – 8.3917	0.9997	0.0012
SP	1st instar	y = 0.0600T – 0.6350	0.9492	0.0257
	2nd instar	y = 0.1230T – 1.5700	0.9787	0.0107
	3rd instar	y = 0.1254T – 1.2840	0.9377	0.0316
	4th instar	y = 0.3210T – 4.2783	0.9921	0.0040
	5th instar	y = 0.3060T – 3.600	0.9203	0.0182
CW	1st instar	y = 0.0372T – 0.3970	0.9817	0.0090
	2nd instar	y = 0.1060T – 1.3750	0.9230	0.0360
	3rd instar	y = 0.1110T – 1.6083	0.9954	0.0043

3.2.2.3 Nymphal survival

Nymphal survival depended on environmental temperature and host plant (Table 3.14). When nymphs were reared with any of the three host plants tested, their survival was higher at 20, 25 and 30°C than at 15°C. For a given host plant, there was no significant difference in survival between 20, 25 and 30°C, except that significantly lower survival was detected for first instar on TC at 30°C, and third instar on SP at 25°C. At 15°C, lowest survival was 13.5% for fourth instar on TC, 2.0% for third instar on SP and second instar on CW, and none of them completed the fifth instar. At a given temperature, nymphal survival was usually higher when fed with TC than with SP, and both were higher than with CW. Fed with CW, nymphs failed to develop through all instar stages at any of the test temperatures. The overall survival was significantly higher on TC at 20°C.

Temperature and host plant affected the colour of nymph's body, which was related to nymphal survival. In contrast to the dark grey body colour of nymphs reared with TC at 20°C, the body colour of fourth and fifth instars was usually pale grey when reared with SP or CW at all test temperatures. Pale grey insects could also be observed at 25 and 30°C when reared with TC. In such cases, higher mortality occurred and most of the nymphs died just before or in eclosion.

Table 3.14: Mean (± SE) survival rate (%) of *N. huttoni* nymphs reared with three host plants under four temperature conditions *

Plant	Temp. (°C)	Instar										Total survival	
		n	1st	n	2nd	n	3rd	n	4th	n	5th		
TC	30	15	80.00±5.69b	15	66.88±5.44abc	15	88.38±3.45a	15	88.69±3.45ab	15	79.00±5.67a	15	30.67±3.00bc
	25	15	96.00±1.90a	15	86.71±2.64a	15	81.25±5.18a	15	63.70±6.39abcd	15	75.48±7.27a	14	34.67±5.06b
	20	15	90.67±2.67ab	15	84.90±2.80ab	15	90.19±2.78a	15	93.07±2.94a	15	84.33±5.61a	15	55.33±4.24a
	15	15	61.33±3.63c	15	45.33±4.24cde	15	48.14±8.09abc	15	13.47±6.37cd	4	0b		--------
SP	30	15	86.00±3.35ab	15	69.76±2.88abc	15	63.91±6.15abc	14	55.57±8.40abcd	14	5.95±4.14b	2	1.33±0.91d
	25	15	86.67±2.70ab	15	70.69±5.54abc	15	55.75±7.15abc	14	49.16±7.22abcd	12	31.94±12.72ab	5	4.00±1.36d
	20	15	92.00±2.00ab	15	73.65±4.86abc	15	81.92±3.25a	15	79.04±4.44abc	15	42.55±5.39ab	14	19.33±3.71c
	15	15	27.33±5.02e	13	6.15±2.67ef	5	2.00±2.00c	1	0d		---------		--------
CW	30	14	33.33±4.85de	11	50.95±9.58bcd	11	75.76±7.23a	11	42.43±13.36abcd	6	0b		---------
	25	15	56.00±3.21cd	15	28.88±5.42def	12	12.50±6.53bc	3	0d		---------		---------
	20	15	32.00±5.63de	15	37.82±9.74cde	13	61.11±14.1ab	7	28.57±18.40bcd	2	0b		---------
	15	15	10.00±4.25e	3	2.00±2.00f	1	0c		----------		---------		---------
	F		45.13		14.61		9.58		9.11		13.22		45.52
	df		11		11		11		10		8		11
	P		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).

Temperature and host plant significantly influenced the survival rate of resulting adults (Table 3.14). Of four temperatures and three host plants tested, *N. huttoni* could complete development only at 20, 25 and 30°C on TC and SP. Because only two females successfully emerged at 30°C, and two females and four males at 25°C on SP, no statistical comparison was made.

However, there was no significant difference in sex ratio in surviving adults in each combination of temperature and host plant. The total number of females and males in surviving adults was 15:13 (χ^2 = 0.143, $P > 0.05$) at 20° C on SP, and 48:36 (χ^2 = 1.714, $P > 0.05$), 25:27 (χ^2 = 0.077, $P > 0.05$) and 27:19 (χ^2 = 1.391, $P > 0.05$) at 20, 25 and 30°C on TC, respectively, which conformed to a 1:1 ratio.

3.3 Effect of temperature and host plant on adult reproduction and longevity

3.3.1 Materials and methods

Newly moulted females and males reared at test temperatures and on the experimental host plants (TC and SP) were sexed according to Eyles (1963b) and weighed individually and separated after emergence. Fifteen pairs were then set up on TC for each of three temperature treatments and 12 pairs on SP at 20°C. Each pair was maintained together every day for one hour. Once they mated, they were allowed to be together permanently, and a small cotton ball (0.5 cm diameter) was placed as oviposition substrate. The dates of emergence, mating and oviposition were recorded.

The premating period (between the final moult and copulation), preoviposition period (from emergence until initial oviposition), oviposition period (between first and final oviposition), postoviposition period (from final oviposition until death), fecundity (total number of eggs laid by a female during her lifetime), daily egg production (number of eggs laid by a female per day)

and longevity (between final moult and death of adults) were recorded. The hatch rates of eggs were calculated.

Nymphs could not complete development to adults at 10 and 15°C. Therefore, 15 pairs were transferred from the breeding colony, and pairs that did not lay eggs in 100 d were then transferred to 20°C condition. TC was used as the host plant.

Statistics: The data of this experiment were analysed using ANOVA (General Linear Models procedure) on SAS (SAS Institute 1990). Where significant differences in variables occurred, means were separated using Tukey's Studentized Range (HSD) Test with linear contrasts of treatments. According to Steel *et al.* (1997), before ANOVA, data of egg hatch rate were subject to arcsine transformation and other data were subject to natural logarithmic transformation to fit normal distribution of data residuals.

Linear regressions were fitted to the reciprocals of premating and preoviposition period (1/day) versus temperatures.

3.3.2 Results

No mating or oviposition occurred in adults reared with SP at 25 and 30°C, and those adults died within 8 d. Twelve pairs were set up from the resulting adults on SP at 20°C, but only 5 pairs mated. The mean premating period was 14.6 ± 0.75 d (n = 5), which was significantly longer than that on TC at 20°C (9.47 ± 0.98 d) (F = 8.28, df = 1, P = 0.0100). Adults laid eggs when reared with TC but did not when reared with SP. There was no significant difference in longevity between females and males (23.92 ± 3.67 d and 17.25 ± 2.65 d, respectively; F = 2.13, df = 1, P = 0.1551) on SP (n = 12 pairs). Longevity of females and males was significantly shorter on SP than TC (F = 28.15, df = 1, P = 0.0001 for females; F = 37.77, df = 1, P = 0.0001 for males).

The influence of temperature on reproduction of adults is summarised in Table 3.15. Temperature significantly influenced premating, preoviposition and oviposition periods, which significantly decreased as temperature increased from 20 to 30°C. However, there was no significant difference in postoviposition periods between the test temperatures. There was no significant difference in fecundity and egg hatch rate between test temperatures, but daily egg production was significantly higher at 30°C than at 20°C. The longevity of both sexes significantly decreased as temperature increased, but there was no significant difference in longevity between sexes at a given temperature ($P > 0.05$) (Table 3.16).

Linear regressions fitted to reciprocals of premating and preoviposition period (1/day) against temperature are shown in Table 3.17. T° and DD required for adult maturation and oviposition were estimated. The coefficients of determination (R^2) were high (> 0.94).

Table 3.15: Reproduction parameters (mean ± SE) of *N. huttoni* adults reared with TC from eggs at three temperature conditions *

Temp.		Reproductive phase (d)								Daily egg				Nymphs/	
(°C)	n	Premat.	n	Preovi.	n	Ovi.	n	Postovi.	n	fecundity	n	production	n	Hatch (%)	n	female
30	15	4.20±0.39c	14	7.21±0.49c	14	9.36±0.46b	14	2.79±1.14a	14	12.93±1.71a	14	1.65±0.21a	14	43.93±8.96a	14	5.79±1.42a
25	15	6.20±0.47b	12	14.83±0.96b	12	13.17±2.43b	12	3.18±0.51a	12	11.00±1.90a	12	1.09±0.12ab	12	41.49±8.17a	12	4.67±0.79a
20	15	9.47±0.98a	12	25.67±3.04a	12	23.50±5.15a	12	4.24±1.28a	12	14.50±4.58a	12	0.83±0.22b	12	45.63±9.54a	12	8.73±3.17a
F		18.31		53.78		5.28		0.24		0.26		7.80		0.08		0.15
df		2		2		2		2		2		2		2		2
P		0.0001		0.0001		0.0106		0.7869		0.7763		0.0016		0.9221		0.8602

*Means followed by the same letters in columns were not significantly different ($P > 0.05$).
Data were subject to natural logarithmic transformation before ANOVA except those of hatch rate (%) which were subject to arcsine transformation.

Table 3.16: Longevity (mean ± SE) of *N. huttoni* adults at three temperature conditions*

Temperature	Sex	
(°C)	n F	n M
30	15 18.47±1.85c	15 18.13±1.33c
25	15 26.73±3.05b	15 24.07±2.33b
20	15 50.80±3.45a	15 47.80±3.90a
F	22.86	30.10
df	2	2
P	0.0001	0.0001

*Means followed by the same letters in columns were not significantly different ($P > 0.05$).

Table 3.17: Regression for reciprocals (y) of premating and preoviposition periods (1/day) against temperatures (T) and estimated T° and DD for *N. huttoni* adults

	Equation	R^2	*P*	T°	DD
Premat.	y = 0.0132T – 0.1629	0.9916	0.0058	12.34 ± 1.21	75.76 ± 6.94
Preovi.	y = 0.0100T – 0.1677	0.9422	0.0155	16.77 ± 0.11	101.01 ± 24.83

Females experienced significantly longer reproductive phases at 15°C than at 20°C (i.e. significantly longer premating period (14.13 ± 0.82 d, F = 36.76, df = 1, P = 0.0001), preoviposition period (25.21 ± 2.49 d, F = 12.85, df = 1, P = 0.0001), oviposition period (34.86 ± 4.95 d, F = 15.5, df = 1, P = 0.0003) and postoviposition period (31.43 ± 5.36 d, F = 7.1, df = 1, P = 0.011)). Furthermore, females had significantly lower mean total egg number (15.36 ± 2.17, F = 25.67, df = 1, P = 0.0001), daily egg output (0.49 ± 0.07, F = 7.722, df = 1, P = 0.0083) and egg hatch rate (2.13 ± 7.3, F = 10.58, df = 1, P = 0.0024) at 15°C than at 20°C. There was no significant difference in longevity of both sexes between 15 and 20°C (F = 0.126, df = 1, P = 0.7252 for female; F = 1.178, df = 1, P = 0.2842 for male), and between females and males at 15°C (89.47 ± 6.56 and 81.07 ± 5.96 d, respectively; F = 0.612, df = 1, P = 0.4405).

3.4 Effect of sunflower seeds and mating on female reproduction and longevity

3.4.1 Materials and methods

To examine the effect of food on female reproduction and fecundity, four treatments were carried out at 20°C. TC was used in all treatments but access to sunflower seeds varied. Treatment A, sunflower seeds were supplied for both nymphs and adults; treatment B, sunflower seeds were supplied for all instars of nymphs; treatment C, sunflower seeds were supplied for first to fourth instar nymphs, and treatment D, no sunflower seeds were supplied. There were 20, 30 20 and 15 pairs for treatments A, B, C and D, respectively.

To determine the effect of mating on female reproduction, fecundity and longevity, two treatments were performed at 20°C on TC: treatment 1, 30 pairs were maintained permanently and treatment 2, 20 females were allowed to mate once. Methods of measurements and statistics were described in section 3.3.1.

3.4.2 Results

3.4.2.1 Sunflower seeds

Food for nymphs significantly influenced the body weight of adults (Table 3.18). Females had significantly higher body weights when sunflower seeds were available for all instar nymphs. When nymphs were fed only with TC, the body weight of both females and males was significantly lower than that of those being supplied with sunflower seeds.

Table 3.18: Effect of food supply on *N. huttoni* adult body weight (mean ± SE)*

Treat.	n	Female (10 µg)	n	Male (10 µg)	*F*	df	*P*
A	20	190.05±4.17aα	20	147.25±3.96aβ	59.04	1	0.0001
B	30	190.97±5.38aα	30	152.63±4.97aβ	27.37	1	0.0001
C	20	168.40±3.28bα	20	145.35±3.83aβ	20.90	1	0.0001
D	15	151.00±4.90cα	15	123.40±4.59bβ	17.65	1	0.0002
F		14.51		6.54			
df		3		3			
P		0.0001		0.0005			

* Means followed by the same English letters in columns and the same Greek letters in rows were not significantly different ($P > 0.05$).
Before ANOVA, data were subject to natural logarithmic transformation.

When both nymphs and adults were fed with TC and sunflower seeds, females had significantly shorter premating and preoviposition periods than in other three treatments (Table 3.19). In this case, the fecundity and daily egg production were significantly higher, but the egg hatch rate was significantly lower. However, the number of viable offspring per female was highest in those females fed with sunflower seeds. Fed with only TC in immature and adult stage, females had significantly longer preoviposition periods, shorter oviposition periods, lower fecundity (Table 3.19) and shorter lives (Table 3.20). Access to sunflower seeds by all instars or only first four instars of nymphs did not significantly affect the reproductive phases, fecundity, daily egg production (Table 3.19) and longevity (Table 3.20). Supply of sunflower seeds to both nymphs and adults significantly influenced female fecundity. The fecundity significantly decreased if sunflower seeds were absent in adult, fifth instar or complete life stage (Table 3.19).

3.4.2.2 Mating

The effect of mating on female reproduction, fecundity and longevity is summarised in Table 3.21. There was no significant difference in reproductive phases, fecundity, daily egg production and longevity between mated-once and permanently paired females. However, the hatch rate of eggs laid by permanently paired females was significantly higher; those females produced five times as many offspring as mated-once females.

Table 3.19: Effect of food on *N. huttoni* reproductive parameters (mean ± SE) *

Treat.	Reproduction phase (d)								Fecundity		Daily egg		Hatch (%)		Nymphs	
	n	Premat.	n	Preovi.	n	Ovi.	n	Postovi.	n		n	production	n		n	per female
A	20	6.25±0.27b	18	13.27±1.13c	18	54.33±5.11a	18	8.39±3.25a	18	101.70±13.28a	18	1.83±0.14a	18	28.19±5.33b	18	28.67±7.29a
B	28	10.39±0.36a	27	18.11±0.70b	27	61.89±4.23a	27	10.48±1.67a	27	41.48±3.77b	27	0.66±0.04b	27	48.93±4.14a	27	20.10±2.46a
C	20	11.45±0.98a	17	18.76±1.10b	17	48.53±4.41a	17	10.06±2.52a	17	27.18±3.07b	17	0.59±0.06b	17	39.60±5.49ab	17	10.70±2.02ab
D	15	9.47±0.98a	12	25.67±3.04a	12	23.50±5.15b	12	4.24±1.28a	12	14.50±4.58c	12	0.83±0.22b	12	45.63±9.54a	11	8.00±2.98b
F		17.18		11.97		11.70		1.27		23.85		24.54		2.82		4.67
df		3		3		3		3		3		3		3		3
P		0.0001		0.0001		0.0001		0.2915		0.0001		0.0001		0.0454		0.0051

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).
Data were subject to natural logarithmic transformation before ANOVA except those of hatch rate (%) which were subject to arcsine transformation.

Table 3.20: Effect of food on *N. huttoni* adult longevity (mean ± SE)*

Treat.	n	Female	n	Male
A	20	71.30±7.10bc	20	76.95±8.53ab
B	28	91.57±4.23a	28	89.04±4.21a
C	20	76.95±3.33ab	20	70.90±6.24ab
D	15	50.80±3.45c	15	47.80±3.90b
F		9.28		6.35
df		3		3
P		0.0001		0.0006

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).
Before ANOVA, data were subject to natural logarithmic transformation.

Table 3.21: Effect of mating on *N. huttoni* female reproductive parameters and longevity (mean ± SE)*

	Reproduction phase (d)								Fecundity		Daily egg		Hatch (%)		Nymphs		Longevity (d)	
Treat.	n	Premat.	n	Preovi.	n	Ovi.	n	Postovi.	n		n	production	n		n	per female	n	Female
Permanently paired	28	10.39±0.36a	27	18.11±0.70a	27	61.89±4.23a	27	10.48±1.67a	27	41.48±3.77a	27	0.66±0.04a	27	48.93±4.23a	27	20.15±2.46a	28	91.57±4.23a
Mated once	20	9.11±0.85a	18	18.65±0.82a	18	61.50±4.81a	18	10.67±3.63a	18	38.11±3.86a	18	0.63±0.04a	18	9.89±3.62b	18	3.47±1.82b	20	89.70±3.75a
F		3.49		0.59		0.01		0.69		0.22		0.63		36.68		23.67		0.12
df		1		1		1		1		1		1		1		1		1
P		0.0683		0.4460		0.9716		0.4103		0.6385		0.552		0.0001		0.0001		0.7257

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).
Data were subject to natural logarithmic transformation before ANOVA except those of hatch rate (%) which were subject to arcsine transformation.

3.5 Discussion

3.5.1 Effect of temperature and host plant on preimaginal development, growth and survival

3.5.1.1 Temperature

For insects, development rate increases almost linearly with temperature over the optimal range of operating temperatures for a species (Gilbert and Raworth 1996).

In the present study, the mean incubation period of *N. huttoni* eggs that were transferred from the breeding colony was 5.20, 7.13, 11.60 and 26.13 d at 30, 25, 20 and 15°C, respectively. In *N. vinitor* Bergroth, 4.5, 6.5, 12.5 and 36.8 d were required for egg incubation at 30, 25, 20 and 15°C, respectively (Kehat and Wyndham 1972a). Incubation period was 5 d at 30.65°C for *N. inconspicus* Distant (Kakakhel and Amjad 1997) and 8 d at 23 ± 2°C for *N. ericae* (Schilling) (Burgess and Weegar 1986). Egg hatch rate of *N. huttoni* was significantly higher at 20°C (68.2%) than at 30, 25 and 15°C (48.5, 53.0 and 49.3%, respectively); while in *N. vinitor,* egg hatch rate was 80-100% in the range 20-30°C, but only 9-18% at 15°C (Kehat and Wyndham 1972a). The result shows that *N. huttoni* eggs were more tolerant to lower temperatures than *N. vinitor* eggs. The development times for successive stages generally increased as nymphs developed from the first through to the fifth instar with the longest time for fifth instar at all temperatures in this study. A similar case was reported by James (1990) for Austrian stink bug, *Biprorulus bibax.*

The relationship of development rate of *N. huttoni* with temperature was nearly linear over the range of temperatures (from 15 to 30°C) in this study. The estimated lower temperature thresholds for preimaginal stage development were all above 10°C except the third instar fed with SP. At 10°C embryonic development occurred but eggs failed to hatch and nymphs failed to moult. The egg hatch rate and nymph development were also poor at 15°C, which agrees with the experimental estimation of lower temperature thresholds. These results agree with the observation in the field by Eyles

(1965b) that overwintered females commenced oviposition during late September and the first and second instar nymphs were found in early October in Palmerston North. Obviously, eggs successfully hatched and nymphs moulted between 11.4 and 13.2°C (the mean temperature for September and October, respectively). In *N. ericae,* eggs could hatch at 10°C (Milliken 1918), indicating that the low temperature threshold for egg stage is lower than that of *N. huttoni.* In *N. vinitor*, the low temperature threshold was estimated as 14.5° C for eggs and 15°C for nymphs (Kehat and Wyndham 1972a), which are higher than that in *N. huttoni.* It may be possible that regions where *N. huttoni* is distributed in New Zealand are colder than for *N. vinitor* in Australia.

A low temperature threshold of 12.3°C was estimated for mating in this study, supporting observations by Eyles (1965b). He stated that no mating was found on 8 May. The mean temperature of this month was 11.8°C (Anon 1995-1999) which was lower than the low temperature threshold for mating. The estimated low temperature threshold for oviposition was 16.8°C in this study, but *N. huttoni* females that were transferred from the laboratory breeding colony started oviposition at 15°C. It is possible that supplying extra food in the form of sunflower seeds to nymphs was conducive to egg formation and accelerated sexual maturation. Females initiated oviposition significantly earlier when the nymphs or both nymphs and adults were fed with additional sunflower seeds compared with that were fed with only TC (in Table 3.19, treatment A, B and D).

The egg incubation period and nymph development of *N. huttoni* were studied by Eyles (1963b) in an unheated greenhouse, but lower temperature thresholds and thermal requirement for development of *N. huttoni* were not reported. The thermal requirements for development of this species in greenhouse conditions may be calculated from Eyles' (1963a) data. According to Eyles (1963a), the development of *N. huttoni* in a greenhouse was expected to be faster than normal, because temperatures within the greenhouse were 5.6°C higher than those occurring in the field in Palmerston North. The total

development time from egg to adult was 54 d for the first generation (from October to November, mean temperature 13.6°C), 56 d for the second (from December to January, mean temperature 17.3°C) and 45 d for the third (February, mean temperature 18.4°C) (Eyles 1963a, Anon 1995-1999). Thus, the greenhouse temperature was estimated by adding 5.6 to the mean temperature a generation experienced. The thermal requirement above the lower temperature threshold of 11.88°C for a generation was estimated as 530.28, 510.72 and 509.04 DD, respectively, all of which were lower than the 625.00 DD estimated by linear equation in this experiment.

However, for some species, it has been demonstrated that development rates (or development times) under fluctuating temperatures are not equivalent to those at constant temperatures (Messenger and Flitters 1959, Hagstrum and Hagstrum 1970, Hagstrum and Leach 1973). For example, in the squash bug, *Anasa tristis*, fluctuating temperatures accelerate development rate in the laboratory (Fielding and Ruesink 1988). In the red flour beetle, *Tribolium castaneum* (Herbst), development is significantly slower at constant temperatures of 22.5, 25, and 27°C and significantly faster at 32.5 and 35°C than at fluctuating temperatures with similar means (Hagstrum and Milliken 1991). In the nettle-feeding nymphalid butterfly larvae of *Aglais urticae* L., *Inachis io* L., *Polygonia c-album* L. and *Vanessa atalanta* L., development is faster at 20/10°C (day/night) than at 15°C, but it is slower at 30/20°C than at 25°C (Bryant *et al.* 1999). There are not enough data to determine if this is the case with *N. huttoni*.

Moreover, in *N. vinitor*, Kehat and Wyndham (1972a) demonstrated a 10-20% increase in development rate when it was reared under fluctuating temperatures. McDonald and Smith (1988) reported that the rate of development in spring in south-eastern Australia was more rapid than that predicted by laboratory-derived degree-days estimation. They suggested that this was attributable to increased body temperature through absorption of solar radiation, and the increased development rate was adjusted by increasing daily

minimum and maximum temperatures by 1.3°C for the first and second instar and 5.5°C for older instars. In addition, *N. vinitor* was regularly observed basking in direct sunlight on flowers, seeds or leaf surfaces to increase body temperature through solar radiation (Digby 1955). At night, it could maintain its body temperature above ambient by remaining in warmer micro-niches, such as the lower vegetative canopies (Willmer 1982). As with *N. vinitor, N. huttoni* is also diurnally active. The activity of *N. huttoni* is accelerated or slowed down with temperature fluctuation. Gurr (1957) stated that *N. huttoni* thrived best under hot and dry conditions and preferred situations where the direct sunlight struck through to the ground. *Nysius huttoni* individuals concealed themselves under clods or debris on the ground as soon as the temperature began to fall in the evening and became active when day was advanced and the temperature had risen (Gurr 1957). Although the body temperature of *N. huttoni* has not been measured directly, it is highly likely that it increases during the day through basking as with *N. vinitor*. Therefore, because of the possible different effect of constant and fluctuating temperatures on *N. huttoni* development, predictive formulae obtained under constant temperatures may not be used directly for accurate prediction of development time in the field but can represent an approximation of the range for field development time for *N. huttoni*.

Low temperature retards insect growth and development and results in lower survival. Eyles (1963b) reported that four of 16 fourth instar nymphs moulted to the fifth instar but those fifth instar nymphs failed to moult to adult at 6°C when nymphs fed on wireweed (*Polygonum aviculare* L.). Unfortunately, the age of the fourth instar nymphs was not mentioned; it is important because the moulted fourth instar nymphs may have had some growth and development before transferring to low temperature. While eggs and nymphs of *N. huttoni* failed to hatch or moult, nymphs grew at 10°C in this study. It is possible that low temperature might retard or prevent moulting but permit size increase (Eyles 1963b). Eggs transferred to 15 and 20°C conditions after 50 d incubation at 10°C failed to hatch in this laboratory study. Kehat and Wyndham (1974) reported that exposure of eggs of *N. Vinitor* to low temperatures (from –

6 to 1°C) for short period (6-24 h) did not affect survival; however, continuous exposure to a temperature of 15°C (which was near the threshold for development) caused considerable mortality. In other cases, the eggs of the milkweed bug, *Oncopeltus fasciatus* (Dallas), hatched at 15°C in a mean of 33.8 d, but would not hatch at 30°C if the periods of exposure to 14°C or 13°C exceeded 17 or 15 d, respectively (Hodson and Al Rawy 1958). Similarly, a small proportion of eggs of the confused flour beetle, *Tribolium confusum*, hatched at 17°C in 33.6 d but exposure to either 16°C or 15°C for 30 d prevented hatching (Hodson and Al Rawy 1958).

Complete development of immature stages at 15°C was theoretically possible (as predicted by calculated lower temperature threshold) but the long development time and substantial high mortality at this temperature resulted in nymphs failing to reach the adult stage. Eyles (1965b) recorded all five instar nymphs at the end of March in Palmerston North but very few second instar nymphs in mid April, third instar nymphs at the end of April and fourth instar nymphs in late May. Fifth instar nymphs collected in early June and transferred to an unheated greenhouse moulted in 16 d. In the present study, the failure of eggs and instar nymphs to develop at 10°C suggests that prolonged cold conditions (especially at low night temperatures) during late April to May could reduce the population of *N. huttoni* by preventing egg hatching and nymph eclosion in field conditions. It is suggested that in most parts of New Zealand eggs and young nymphs of *N. huttoni* cannot survival the winter. The tolerance of the fifth instar to low temperatures with a significantly longer surviving time to low temperature in this experiment supports the suggestion by Eyles (1963a) that a few fifth instar nymphs overwinter. Overwintering fifth instar nymphs were also recorded in *N. vinitor* and *N. clevelandensis* Evans (Attia 1982), and *N. plebejus* Distant (Kim *et al.* 1994). In *N. plebejus*, 10% of fifth instar nymphs were found in the overwintering populations (Kim *et al.* 1994).

3.5.1.2 Host plant

The experimental results show that host plants play a role in *N. huttoni* development, growth, and mortality.

Nysius huttoni nymphs generally developed faster when fed with TC than with SP, with both of which they developed faster than with CW. Thus, low temperature thresholds for nymphs decreased in the order of CW → SP → TC. At a given temperature, development from eggs to adult was significantly faster on TC than on SP; and the thermal requirement was lower on TC (625.00 DD) than on SP (714.29 DD). On the other hand, the longer development time, lower body weight and lower survival of nymphs on SP and CW indicated the unsuitability of these two plant species for nymphal development and growth. Similarly, that host plants (or plant parts) affect nymphal development and female reproduction was also reported for other *Nysius* species. For example, under laboratory conditions, nymphs of *N. clevelandensis* and *N. vinitor* failed to reach adulthood when fed only on cotton tips or squares, and adults of both species were unable to reproduce on such a diet if no alternative food was supplied (Chinajariyawong *et al.* 1989). Moreover, *N. inconspicus* nymphs could not survive on sunflower florets but reached adulthood on sunflower heads (Kakakhel and Amjad 1997).

The physical or chemical factors of TC, SP and CW that affect *N. huttoni* growth, development and survival are unknown. Benepal and Hall (1966) reported that mineral nutrition of the host plants influenced the feeding response of squash bug, *Anasa tristis*, whose growth and reproduction required a large amount of nitrogenous food. Although nutritional levels of *N. huttoni* host plants were not determined in this study, food supply had a significant effect on *N. huttoni*, indicating that differences in nutritional composition of host plants may influence *N. huttoni* growth, development and survival. Therefore, detailed physical and nutritional studies are needed to determine the critical factors affecting *N. huttoni* growth, development and survival.

Based on the knowledge of the effect of host plants on *N. huttoni*, the population of *N. huttoni* would be expected to increase faster on TC than on SP, but would not be able to sustain itself on CW. Thus, when larger populations were present on SP (Gurr 1957) or other unsuitable host plants such as CW, a possible explanation may be that *N. huttoni* moved from overpopulated TC or other suitable host plants. It may explain, to some extent, the outbreak of *N. huttoni* on crops that this insect does not particularly prefer and, therefore, producers can use this information to determine the critical time to apply insecticides. Furthermore, the effect of host plant on *N. huttoni* growth, development and survival can be taken into account when developing control strategies for this pest. Successive plantings of suitable host plants can attract and support *N. huttoni* populations, and concentrating populations on those preferred hosts will delay or reduce economic losses in adjacent commercial fields of less-preferred crops. In addition, pesticides can be used on those trap-crops.

3.5.2 Effect of temperature and host plant on reproduction and adult longevity

Nysius huttoni females and males required similar times to complete development under laboratory conditions. This was also reported under greenhouse conditions (Eyles 1963b). The sex ratio in adults was 1:1 in this study. Therefore, neither females nor males had a survival advantage under laboratory conditions. Eyles (1963b) also reported that the total number of females and males was 49:50 over a complete breeding season (from October to April), but there were more females (female:male = 20:11) in February and more males (female : male = 6:12) in November. However, the sex ratios in those samples were still 1:1 (X^2 = 2.065 and 2 in February and November, respectively; $P > 0.05$). The sex ratio of 1:1 was also reported in other species, such as the *N. plebejus* (Kim *et al.* 1994) and the *Anasa tristis* (Fargo *et al.* 1988, Bonjour and Fargo 1989).

3.5.2.1 Temperature

Although *N. huttoni* reproductive phases, fecundity and adult longevity of a few individuals were observed under greenhouse conditions by Eyles (1963a), it is difficult to compare them with the results obtained under laboratory conditions because of the different environmental conditions (i.e., temperature, humidity or even photoperiod).

As previously mentioned, premating and preoviposition periods decreased linearly as temperature increased; oviposition period decreased as temperature increased. A similar result was obtained for *N. vinitor* (Kehat and Wyndham 1972a). In both this study and Eyles (1963a), most *N. huttoni* females oviposited until the day of death. This may explain the short mean postoviposition period of *N. huttoni*.

Temperature affects fecundity in many insect species. For example, in *N. vinitor*, females laid significantly more eggs at 25 (578 eggs) and 30°C (542 eggs) than at 22 (371 eggs) and 35°C (255 eggs) (Kehat and Wyndham 1972a). In the sycamore lace bug, *Corythucha ciliata* (Say), the number of eggs per females was greatest at 25 and 28°C within 15-35°C (Kim *et al.* 1999). In the present study, *N. huttoni* females laid a similar number of eggs at 20, 25 and 30°C, indicating that temperature had little effect on fecundity in this range. Gurr (1957) also stated that no correlation existed between air temperature and the number of eggs laid. However, when fed with TC, females laid a smaller number of eggs at constant temperatures than under fluctuating temperatures (Eyles 1963b). The reason why *N. huttoni* fecundity is different under constant and fluctuating temperatures is unknown. Daily egg production increased as temperature increased, with twice as many eggs being laid at 30°C as at 20°C, because there was a significantly shorter oviposition period at 30°C. A similar case was reported in *N. vinitor* (Kehat and Wyndham 1972a).

At 15°C, *N. huttoni* had long reproductive phases, low fecundity and poor hatch rate, showing that this temperature is not conducive to *N. huttoni* reproduction. Eyles (1963b) stated that low temperatures in the shade in a greenhouse during October increased the incubation period and reduced the hatch rate; females caught in the field during October produced eggs that had a reduced hatch rate. This might be a reflection of the adverse effect of winter on overwintered females (Eyles 1963b).

Longevity of both sexes of *N. huttoni* significantly decreased with the increased temperatures from 20 to 30°C. In *N. vinitor,* longevity of both sexes was also negatively correlated with temperature (Kehat and Wyndham 1972a). This is probably attributed to the greater energetic requirement for the faster reproductive development (faster sexual maturation) of both sexes and higher daily egg output of females at higher temperatures.

3.5.2.2 Host plant

SP was sufficient for nymphs and allowed some development to adult stage, and was sufficient for sexual maturation, but not for successful oviposition under laboratory conditions. However, when adults were fed with fresh stems of SP, females laid eggs in an open insectary (Gurr 1957). Food suited for nymphal growth is not always necessarily adequate for egg reproduction. Nutritional requirements for *N. vinitor* fecundity appeared to be more specific than for nymphal development (Kehat and Wyndham 1972b). However, *N. huttoni* is a polyphagous insect that can live on many culitivated plants as well as a variety of weeds (Gurr 1957), which is an important factor in supporting nymphs and adults to complete development during the growth and reproductive season. In field conditions, it is impossible that *N. huttoni* is confined to one host plant (or habitat) such as SP or CW. The dispersion of *N. huttoni* from grass on golf links after min-January was noted by Eyles (1965b). He suggested it was possible that *N. huttoni* moved away in search for food.

When fed with TC, both sexes of *N. huttoni* lived longer than when fed with SP. The longevity of many other insects was also influenced by host plants, such as the green stink bug, *Nezara viridula* (L.) (Panizzi *et al.* 1996), and bober *Mussidia nigrivenella* Ragonot (Setamou *et al.* 1999).

The longevity of both sexes of *N. huttoni* was similar at a given temperature or host plant in this study. Eyles (1963a) reported that males lived as long as females in an unheated greenhouse. The same longevity of both sexes was also recorded for *N. inconspicus* (Kakakhel and Amjad 1997).

3.5.3 Effect of sunflower seeds and mating on reproduction and longevity

3.5.3.1 Sunflower seeds

Additional food supply had striking effects on *N. huttoni* reproduction. Females fed with additional sunflower seeds (treatment A) had significantly shorter premating and preoviposition periods, suggesting that nutrients in sunflower seeds could accelerate sexual maturation and egg production. Moreover, those females laid significantly more eggs than that fed with only TC. Insects that feed on more protein either as immature instars, adults, or both, produced many more eggs than those that take in little (Huffaker and Rabb 1984).

Females in treatment B (first to fifth instars were supplied with sunflower seeds) laid significantly more eggs than those in treatment C (first to fourth instars were supplied with sunflower seeds), and both treatments produced more eggs than treatment D (preimaginal stages were only fed with TC). It is suggested that nutritional supplies in both nymphal and adult stages had significant effect on reproduction of *N. huttoni*. In the green stink bug, *Nezara viridula*, reproductive performance and percentage gain in adult body weight were greater for insects fed with immature soybean (*Glycine max* (L.)) pods than with immature fruits of radish (*Raphanus raphanistrum* L.) (Panizzi and Saraiva 1993).

Although females laid a significantly greater number of eggs in treatment A than treatment B, hatch rate was significantly lower than in treatment B. The influence of nutrients on egg fertility is unknown. However, although hatch rate was low in treatment A, the benefit from additional food was the greater number of offspring produced. Therefore, populations of *N. huttoni* respond positively to increased nutrient quality of food. The shorter longevity of females in treatment A than in treatment B, is probably attributable to the greater energy requirement for the faster reproductive development (faster sexual maturity) and greater egg production and deposition.

3.5.3.2 Mating

In this study, presence or absence of males after one mating did not significantly influence oviposition and postoviposition periods, fecundity and daily egg production and female longevity. However, significantly lower hatch rate of eggs laid by mated-once females than that laid by permanently paired females in the present study does not support Eyles' (1963a) statement that one copulation was sufficient to fertilise a female for life. It is suggested that the females could not carry viable sperm over a complete oviposition period (61.50 ± 4.81 d) in this study. Khalifa (1950) studied oviposition in the triatomine bug, *Rhodnius prolixus* Stål, and showed that one mating fertilised a female for only part of her oviposition period, and that remating was necessary. However, the influence of surrounding environmental conditions (such as fluctuating temperatures and humidity) on egg fertilisation is unknown.

Chapter four
Effect of photoperiod on preimaginal development and adult reproductive diapause

4.1 Introduction

Knowledge of the influence of photoperiod on the preimaginal development and adult reproduction is essential for an understanding of reproductive diapause, which could allow increased precision in the implementation of pest management strategies for production pests, and for development of risk management strategies for quarantine pests.

Nysius huttoni adults usually start to overwinter at the end of April and become active again at the beginning of August in Nelson (Gurr 1952). In Manawatu, overwintering fifth instar nymphs were recorded by Eyles (1963b). Farrell and Stufkens (1993) indicated that reproductive diapause (the absence of oviposition for 30 d after emergence) was induced by the shortening day length of late summer. The influence of long (16:8, L:D) and short photoperiods (12:12, L:D) on adult reproduction was observed under laboratory conditions by Farrell and Stufkens (1993). However, adult reproduction has not been studied at photoperiods of 14:10 (L:D) (between 16:8 and 12:12, L:D) and 10:14 (L:D) (shorter than 12:12, L:D). The critical photoperiod for reproductive diapause (50% population enter diapause) and the influence of photoperiod on development and growth of preimaginal stages are also unknown.

The aim of the research presented in this chapter is to provide a better understanding of the biology of *N. huttoni* with special emphasis on the photoperiodic effect on development and growth of preimaginal stages and the effect of photoperiodic history that preimaginal stages experience on adult reproduction.

4.2 Effect of photoperiod on preimaginal development

4.2.1 Materials and methods

4.2.1.1 Photoperiods

This experiment was carried out at constant temperature of 20 ± 1°C in the Massey University Entomology and IPM Laboratory. Four treatments were set up under four constant photoperiods (16:8, 14:10, 12:12 and 10:14, L:D).

4.2.1.2 Eggs

Newly laid eggs (< 24 h old) were collected from the breeding colony (see section 3.2.1.1) and immediately transferred to one of the four photoperiods for incubation: 16:8, 14:10, 12:12 and 10:14 (L:D). Fifteen replicates were set up for each photoperiod with 20 eggs per replicate in a glass vial (2.5 × 8.0 cm height) with 1.5 cm mesh covered hole in lips. The duration and hatch rate of eggs were recorded. Ten newly hatched nymphs (as one replicate) from the same photoperiod treatment were weighed together using a Dual Range Balance (Mettler AE163, Switzerland) with a readability of 10 µg.

4.2.1.3 Nymphs

Two experiments were set up to determine the influence of photoperiod on nymphal development and growth:

Experiment I

Newly hatched nymphs under four different photoperiods were transferred to the above-mentioned glass vials (10 individuals each) and reared with TC under the same photoperiod at which their eggs were incubated. Fifteen replicates for each treatment were set up with each vial as a replicate.

Experiment II

Newly hatched or moulted nymphs of each instar were immediately transferred from the breeding colony to 20 glass vials (10 individuals each), and maintained under four different photoperiods (16:8, 14:10, 12:12 and 10:14, L:D) until they moulted to the next instar when nymphs were discarded after being weighed. Twenty replicates for each treatment were set up with each vial as a replicate. TC was used as food.

Nymphs were checked every 24 hours and insects in each replicate were weighed together when 50% or more individuals in each vial moulted. Newly emerged adults were sexed according to Eyles (1963b) and weighed individually. The dates of weighting were recorded as the moulting dates; the number of live nymphs was also recorded for those dates. Nymph mortality and growth rate of each instar were calculated (see section 3.2.1.3).

Statistical methods used for this experiment were the same as in section 3.2.1.4. Linear regression analysis was used to test the relationship between development time and body weights of nymphs and adults for each treatment. According to Steel *et al.* (1997), before ANOVA, data of nymphal survival and egg hatch rate were subject to arcsine transformation; other data were subjected to natural logarithmic transformation to fit normal distribution of data residuals. A Chi-square (χ^2) test ($P \leq 0.05$) was used to detect the sex ratio of newly emerged adults.

4.2.2 Results

4.2.2.1 Development time

Development time of eggs increased as photoperiod decreased (11.50 ± 0.14, 13.25 ± 0.10, 13.45 ± 0.11 and 14.80 ± 0.09 d for 16:8, 14:10, 12:12 and 10:14 (L:D), respectively; F = 144.30, df = 3, P = 0.0001), but no significant difference was found between 14:10 and 12:12 (L:D). Egg hatch rate was not influenced by photoperiod (68.52 ± 7.39, 65.50 ± 4.07, 70.51 ± 2.56 and 68.53

± 2.84% for 16:8, 14:10, 12:12 and 10:14 (L:D), respectively; $F = 0.11$, df = 3, $P = 0.9529$).

For the preimaginal stages held at the constant photoperiod conditions (experiment I) or directly transferred from breeding colony (experiment II), their development was significantly influenced by photoperiod (Table 4.1). Among photoperiods, development time of each nymphal instar usually increased as photoperiod decreased. At 14:10, 12:12 and 10:14 (L:D), development time for the nymphs of experiment I was significantly shorter than that of experiment II, except for the first instar at 12:12 and 10:14 (L:D), where no significant difference was detected. At 16:8 (L:D), no significant difference in development time was found between treatment I and II.

In experiment I, regardless of sex, *N. huttoni* took significantly longer time to complete development (from egg to adult) at 10:14 (L:D) (96.68 ± 1.59 d) and 12:12 (L:D) (95.19 ± 1.16 d) than at 14:10 (L:D) (87.36 ± 1.68 d) and 16:8 (L:D) (79.81 ± 1.02 d) ($F = 31.76$, df = 3, $P = 0.0001$).

Table 4.1: Mean (±SE) development time (d) of *N. huttoni* nymphs of different instars under four photoperiod conditions *

	Photo.	Instar									
	(L:D)	n	1st	n	2nd	n	3rd	n	4th	n	5th
I	16:8	15	14.60±0.29b	15	13.27±0.40b	15	10.87±0.48de	15	13.47±0.27c	15	16.01±0.70f
	14:10	15	16.20±0.61b	15	13.13±0.64b	15	12.27±0.55cde	15	12.73±0.08c	15	19.76±0.95de
	12:12	15	19.93±0.42a	15	13.07±0.45b	15	12.87±0.76cd	15	13.93±1.09c	15	21.81±1.28cd
	10:14	15	19.60±0.84a	15	13.13±0.72b	14	13.13±0.77bc	14	15.43±1.23bc	14	20.54±1.63de
II	16:8	20	14.85±0.24b	20	13.40±0.25b	20	10.40±0.19e	20	13.05±0.41c	20	16.43±0.46ef
	14:10	20	19.10±0.67a	20	17.00±0.30a	20	13.75±0.33abc	20	16.75±0.47b	20	26.11±0.96bc
	12:12	20	20.35±0.56a	20	16.70±0.35a	20	14.50±0.30ab	20	21.85±0.53a	20	33.37±0.78a
	10:14	20	19.95±0.65a	20	17.40±0.30a	20	16.15±0.35a	20	22.55±0.72a	20	29.86±0.78ab
	F		25.57		19.73		16.00		28.17		40.32
	df		7		7		7		7		7
	P		0.0001		0.0001		0.0001		0.0001		0.0001

* Means followed by the same letters in columns were not significantly different ($P > 0.05$). Before ANOVA, data were subject to natural logarithmic transformations.

For both experiments I and II, no significant difference in time required to complete development was found between females and males at a given photoperiod (*P > 0.05*) (Table 4.2). In both experiments I and II, development time of both sexes was significantly longer at 12:12 and 10:14 (L:D) than that at 14:10 (L:D); a significantly shorter time was detected at 16:8 (L:D). There was no significant difference between 12:12 and 10:14 (L:D).

Table 4.2: Mean (±SE) development time (d) of *N. huttoni* female and male under four photoperiod conditions *

Photo.	I				II			
(L:D)	n	Female	n	Male	n	Female	n	Male
16:8	15	79.63±1.11c	15	80.36±2.00c	20	15.91±0.56c	20	16.96±0.65c
14:10	12	87.22±2.20b	15	88.66±2.60b	20	24.97±1.03b	18	26.34±1.02b
12:12	11	94.54±1.62a	15	94.73±1.08a	19	35.18±1.20a	20	32.33±1.07a
10:14	13	97.23±3.43a	14	95.14±1.87a	20	30.90±1.20a	19	29.52±1.06a
F		14.15		13.25		76.89		63.17
df		3		3		3		3
P		0.0001		0.0001		0.0001		0.0001

* Means followed by the same English letters in columns were not significantly different (*P > 0.05*). Before ANOVA, data were subject to natural logarithmic transformations.

4.2.2.2 Nymphal growth

For newly hatched nymphs and adults maintained at a constant photoperiod from the egg stage, the body weight in later instars was significantly influenced by photoperiod (Table 4.3). At 16:8 and 14:10 (L:D), fifth instar nymphs and adults had significantly greater body weight than at 12:12 and 10:14 (L:D); but no significant difference was found between 16:8 and 14:10 (L:D), and between 12:12 and 10:14 (L:D). Body weight of third and fourth instar nymphs was significantly greater at 16:8 (L:D) than at 12:12 (L:D). Newly emerged females were significantly heavier than males at all photoperiod treatments (Table 4.4). The body weight of both females and males was significantly greater at 16:8 and 14:10 (L:D) than at 12:12 and 10:14 (L:D), but there was no significant difference between 16:8 and 14:10 (L:D) and between 12:12 and 10:14 (L:D).

Table 4.3: Mean (± SE) body weight of newly moulted nymphs and adults of *N. huttoni* under four photoperiod conditions *

Photo.	Body weight/individual (10 µg)											
(L:D)	n	1st	n	2nd	n	3rd	n	4th	n	5th	n	Adult
16:8	15	2.92±0.06a	15	11.69±0.36a	15	27.45±1.10a	15	51.91±2.20a	15	103.30±3.28a	15	138.55±2.60a
14:10	15	3.05±0.04a	15	10.65±0.30a	15	24.21±0.83ab	15	48.76±1.61a	15	94.25±3.45a	15	139.36±2.31a
12:12	15	2.97±0.03a	15	11.17±0.30a	15	22.28±0.82b	15	42.67±1.69b	15	64.96±2.21b	15	121.35±2.42b
10:14	15	3.07±0.07a	15	11.39±0.48a	15	23.97±0.93ab	15	44.03±2.72b	15	60.59±2.93b	15	120.4±2.29b
F		0.07		1.36		5.07		4.61		49.39		16.80
df		3		3		3		3		3		3
P		0.9777		0.2657		0.0036		0.0059		0.0001		0.0001

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).
Before ANOVA, data were subject to natural logarithmic transformation.

Table 4.4: Mean (± SE) body weight of newly moulted females and males reared from eggs under four photoperiod conditions *

Photo.	Body weight/individual (10 µg)						
(L:D)	n	Female	n	Male	*F*	df	P
16:8	15	148.32±3.08aα	15	122.59±2.67aβ	39.92	1	0.0001
14:10	13	155.98±3.73aα	15	131.52±2.98aβ	26.47	1	0.0001
12:12	13	136.23±4.78bα	15	114.29±3.60bβ	12.10	1	0.0018
10:14	12	135.71±3.66bα	13	109.24±3.20bβ	29.35	1	0.0001
F		6.75		9.23			
df		3		3			
P		0.0007		0.0001			

* Means followed by the same English letters in columns and the same Greek letters in rows were not significantly different ($P > 0.05$).
Before ANOVA, data were subject to natural logarithmic transformation.

In both experiments I and II, growth rates of nymphs usually decreased as photoperiod decreased (Table 4.5). At 14:10, 12:12 and 10:14 (L:D), except the first instar, nymphs usually grew faster in experiment I than in experiment II. At 16:8 (L:D), except for the fourth instar, growth rates of nymphs were similar in both experiments I and II. In experiment I, growth rates from egg to adult were significantly faster at 16:8 and 14:10 (L:D) than at 12:12 and 10:14 (L:D), but no difference was found between 16:8 and 14:10 (L:D) and between 12:12 and 10:14 (L:D).

At a given photoperiod, body weight of nymphs and adults was significantly positively correlated with development time ($P < 0.001$) (Fig. 4.1).

Table 4.5: Mean (±SE) growth rate (10 μg/d) of *N. huttoni* nymphs under four photoperiod conditions*

Photo.	Instar											
(L:D)	n	1st	n	2nd	n	3rd	n	4th	n	5th	n	Total
A												
16:8	15	0.60±0.02a	15	1.17±0.07a	15	2.32±0.20a	15	3.84±0.21a	15	2.28±0.30bc	15	2.00±0.06a
14:10	15	0.48±0.02bc	15	1.05±0.07ab	15	2.02±0.09ab	15	3.68±0.26ab	15	2.38±0.23abcb	15	1.85±0.05a
12:12	15	0.41±0.02c	15	0.85±0.06bc	15	1.57±0.11c	15	1.68±0.18cd	15	2.67±0.19abc	15	1.45±0.03b
10:14	15	0.43±0.17c	15	0.96±0.06ab	15	1.50±0.12c	14	1.11±0.22d	14	3.30±0.43a	14	1.44±0.04b
B												
16:8	20	0.58±0.02a	20	1.14±0.05a	20	2.04±0.06ab	20	2.99±0.10b	20	3.08±0.18ab		
14:10	20	0.52±0.02ab	20	0.59±0.02d	20	1.66±0.06bc	20	2.21±0.09c	20	1.68±0.10cd		
12:12	20	0.48±0.02bc	20	0.64±0.03cd	20	1.60±0.05bc	20	1.47±0.08d	20	1.21±0.19d		
10:14	20	0.46±0.15bc	20	0.60±0.03d	20	1.27±0.08c	20	1.60±0.07d	20	1.27±0.09d		
F		11.04		24.85		11.47		38.92		12.47		37.24
df		7		7		7		7		7		3
P		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001

* Means followed by the same letters in columns were not significantly different ($P > 0.05$).
Before ANOVA, data were subject to natural logarithmic transformation.

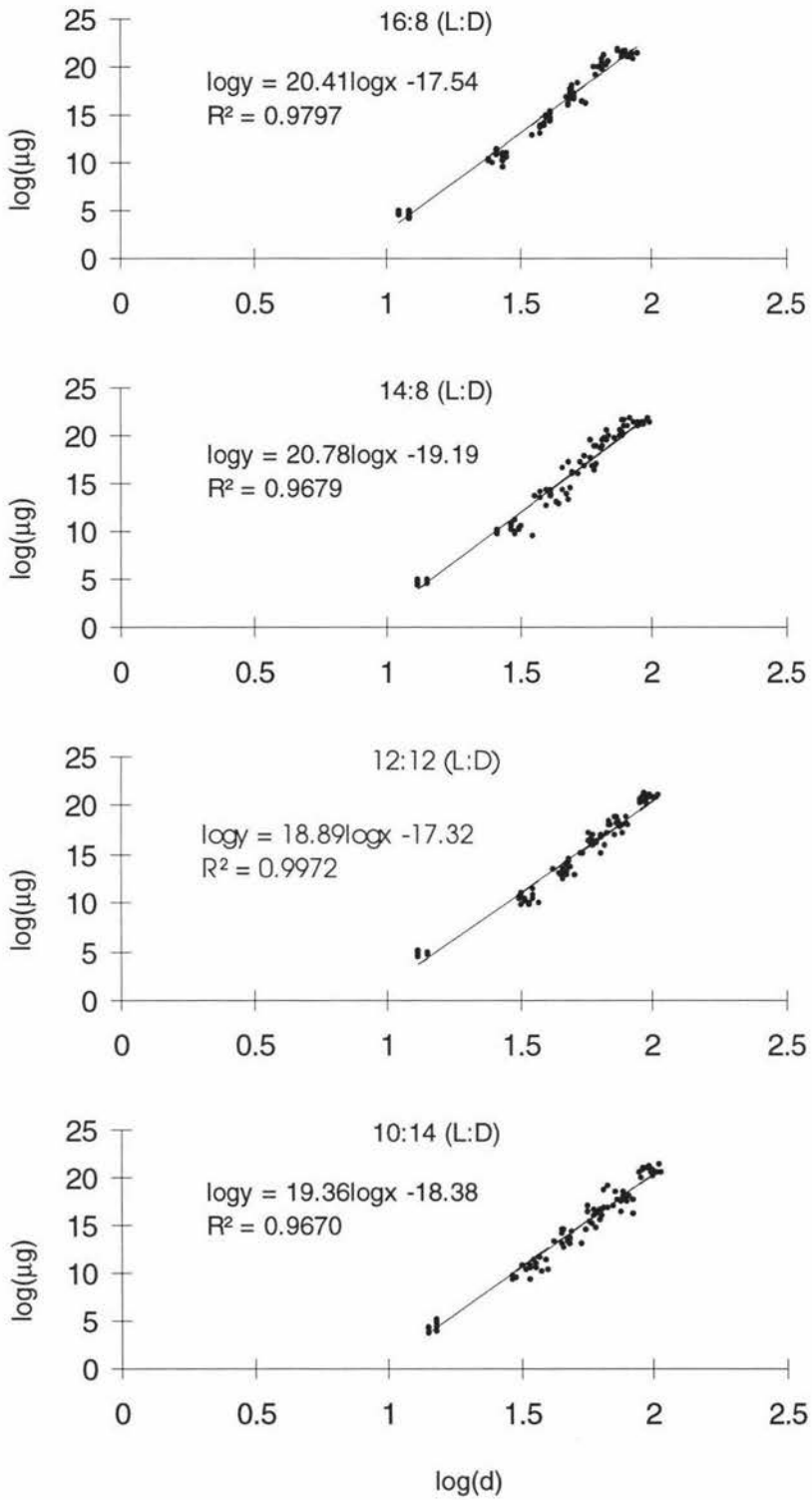

Fig. 4.1: Regression of body weight (y) on development time (x) at each photoperiod.

4.2.2.3 Survival of nymph and adult

Photoperiod significantly influenced nymphal survival (Table 4.6). At a given photoperiod, nymphal survival rate was usually higher in experiment II than in experiment I, except first and fifth instars at 12:12 and 14:10 (L:D) in experiment II, where lower survival rate was detected. For second, third and fourth instars, no significant difference was detected between photoperiods in both experiments I and II.

In experiment I, no significant difference in nymphal survival was found among nymphal instars at a given photoperiod, but the overall survival rate (from egg to adult) was significantly higher at 16:8 (L:D). In experiment II, lower survival usually occurred at first and fifth instars at each photoperiodic condition.

Table 4.6: Mean (±SE) survival rate (%) of *N. huttoni* nymphs under four photoperiod conditions *

Photo. (L:D)	Instar																			
	n	1st	n	2nd	n	3rd	n	4th	n	5th	*F*	df	*P*	n	Total survival
I															
16:8	15	90.67±2.67aα	15	84.90±2.80cα	15	90.17±2.78abcα	15	93.07±2.94abα	15	84.33±5.61abα	1.29	4	0.2813	15	55.33±4.24a
14:10	15	79.33±3.71abα	15	83.45±4.39bcα	15	84.23±3.09cα	15	86.14±3.34abα	15	82.83±5.76abα	0.79	4	0.5354	15	39.33±3.84b
12:12	15	78.67±3.07bcα	15	89.41±2.75abcα	15	88.70±2.97bcα	15	83.52±4.03abα	15	82.71±4.90abα	1.50	4	0.2106	15	42.00±3.27b
10:14	15	72.00±3.11cα	15	85.05±3.21bcα	15	87.27±3.78bcα	14	80.13±8.15bα	14	67.84±7.76bα	2.36	4	0.0613	14	30.00±3.27b
II															
16:8	20	90.50±2.11aγ	20	93.50±1.96abcβγ	20	98.50±1.09aα	20	96.50±1.50abαβ	20	91.15±2.20aγ	5.03	4	0.0010		
14:10	20	77.94±2.96bcβ	20	95.94±1.14abα	20	93.50±1.50abcα	20	97.00±1.28aα	20	73.30±3.95bβ	21.16	4	0.0001		
12:12	20	74.75±3.51cβ	20	93.94±1.14abcα	20	96.00±1.52abα	20	92.94±1.65abα	20	73.66±3.61bβ	22.22	4	0.0001		
10:14	20	74.24±3.50cγ	20	97.94±0.94aα	20	92.50±2.39abcαβ	20	94.50±1.35abαβ	20	86.45±3.24abβ	11.86	4	0.0001		
F		5.87		4.38		3.73		2.74		3.21					7.45
df		7		7		7		7		7					3
P		0.0001		0.0002		0.0010		0.0109		0.0036					0.0003

* Means followed by the same English letters in columns and the same Greek letters in rows were not significantly different ($P > 0.05$).

Photoperiod significantly influenced the sex ratio of newly emerged adults in experiment I (Table 4.7). The sex ratio of newly emerged adults was significantly more male-biased at 12:12 (L:D) while at 16:8, 14:10 and 10:14 (L:D) the sex ratio was about 1:1. In experiment II, the sex ratio was 1:1 at all photoperiods.

Table 4.7: Sex ratio in newly emerged adults under four photoperiods *

Photo. (L:D)		Female : Male	χ^2
I	16:8	47 : 37	1.190
	14:10	22 : 37	3.814
	12:12	23 : 40	4.587
	10:14	19 : 23	0.381
II	16:8	80 : 99	2.017
	14:10	70 : 67	0.066
	12:12	70 : 85	1.452
	10:14	75 : 92	1.731

* Ratio with a $\chi^2 \leq \chi^2_{0.05} = 3.841$ (df = 1) conformed to 1:1 ($P > 0.05$).

4.3 Effects of photoperiod on female reproductive diapause

4.3.1 Materials and methods

The methods of Farrell and Stufkens (1993) were modified and used to examine the effects of photoperiod on adult reproductive diapause. Three experiments were carried out at four photoperiods (16:8, 14:10, 12:12 and 10:14, L:D):

Experiment A

This started from the egg stage. Newly laid eggs (< 24 h old) were transferred from the breeding colony (sunflower seeds and TC were used as food) to the test photoperiods (see section 4.2.1.2). Resulting nymphs and adults were maintained in the same photoperiods. Fifteen pairs were set up. One pair was held in each vial with TC as food.

Experiment B

This started with fifth instar nymphs. Newly moulted fifth instar nymphs from the breeding colony were transferred to the test photoperiods with 20 replicates of 10 individuals per replicate in a vial; then 20 pairs of the newly emerged adults were held in the same photoperiod where they moulted. One pair was held in each vial with TC as food.

Experiment C

Adults for this experiment were transferred to the test photoperiods from the breeding colony directly; 30 pairs were set up. One pair was held in each vial with TC as food.

For experiment A, adults were maintained in the same test photoperiod until death. For experiments B and C, to determine if long photoperiod helped terminate reproductive diapause, females that failed to lay eggs within 50 d at 10:14, 12:12 and 14:10 (L:D) were transferred to 16:8 (L:D). If all females failed to lay eggs, then 50% of the surviving pairs were randomly selected and transferred to 16:8 (L:D). The measuring methods and statistics were described in the section 3.3.1.

To determine whether the differences in adult diapause incidence within 30 and 50 d between different photoperiods and between different experiments were significant, a Chi-square (χ^2) test ($P \leq 0.05$) was used. The critical photoperiod was estimated by plotting the diapause incidence (%) against photophase (hours).

4.3.2 Results

4.3.2.1 Induction of diapause

Photoperiod had significant effect on the incidence of reproductive diapause. The numbers of females of different age that failed to oviposit under four constant photoperiods and combinations of long (16:8, L:D) and short

photoperiods were shown in Table 4.8. The percentages of female diapause incidence at test photoperiods within 30 and 50 d were also plotted (Fig. 4.2).

Table 4.8: Number of females of different age that laid (Lay) and did not lay (DNL) eggs under different photoperiodic conditions

Photo.	Exp. A					Exp. B					Exp. C				
	30d*		50d		>50d	30d		50d		>50d	30d		50d		>50d
(L:D)	n	DNL	DNL	n	Lay	n	DNL	DNL	n	Lay	n	DNL	DNL	n	Lay
16:8	15	6	3	3	0	20	3	3			28	1	1		
14:10	15	7	5	5	0	20	11	2①			28	16	7		
12:12	15	9	6	6	0	20	20	20	10	7	29	23	23③		
10:14	15	11	10	10	2	20	20	20	8	5②	30	14	14		
14:10/16:8														7	6
12:12/16:8									10	8				22	19
10:14/16:8									10	9				14	12
χ^2		3.97	3.73		3.06		45.81	62.32		2.18		34.51	37.58		0.0038
P**		0.2644	0.2919		0.3830		0.0001	0.0001		0.5366		0.0001	0.0001		0.9999

* Age of the female.
** Chi-square significant differences test ($P \leq 0.05$).
① Two females died at the age of 38 and 50 d, respectively.
② Two females died at the age of 18 and 38 d, respectively.
③ One female died at the age of 50 d.

For females held at the same photoperiod as their preimaginal stages in experiment A, there was an increasing tendency to enter diapause as photoperiod decreased (Fig. 4.2). More than 50% of females failed to oviposit within 30 d at 12:12 and 10:14 (L:D) and within 50 d at 10:14 (L:D), but there was no significant difference in the number of females that failed to oviposit between test photoperiods (Table 4.8).

However, for females exposed to the same photoperiod as their fifth instars in experiment B, there was a rapid increase in incidence of diapause within 50 d between 14:10 and 12:12 (L:D) (Fig. 4.2). All females entered diapause under photoperiods shorter than 14:10 (L:D). The percentage of females that failed to oviposit within 30 d was greater at 14:10 than at 16:8 (L:D) (Table 4.8).

In experiment C, where adults were transferred to test photoperiods from a long photoperiod of 16:8 (L:D) immediately after emergence, the

incidence of diapause increased between 10:14 and 12:12 (L:D) and decreased between 12:12 and 14:10 (L:D). No females initiated oviposition after 30 d at 12:12 and 10:14 (L:D). Exposed to 12:12 (L:D), more females (79.4%) entered diapause. The percentages of females that terminated diapause at long photoperiod (16:8, L:D) were very similar (85.71% at 10:14/16:8 (L:D), 86.36% at 12:12/16:8 (L:D) and 85.71% at 14:10/16:8 (L:D)).

As Fig. 4.2 shows, if diapause is defined as the absence of oviposition for 30 or 50 d after emergence, then the critical photoperiod fell between 14:10 to 14.5:9.5 or 13:11 to 13.5:10.5 (L:D), respectively.

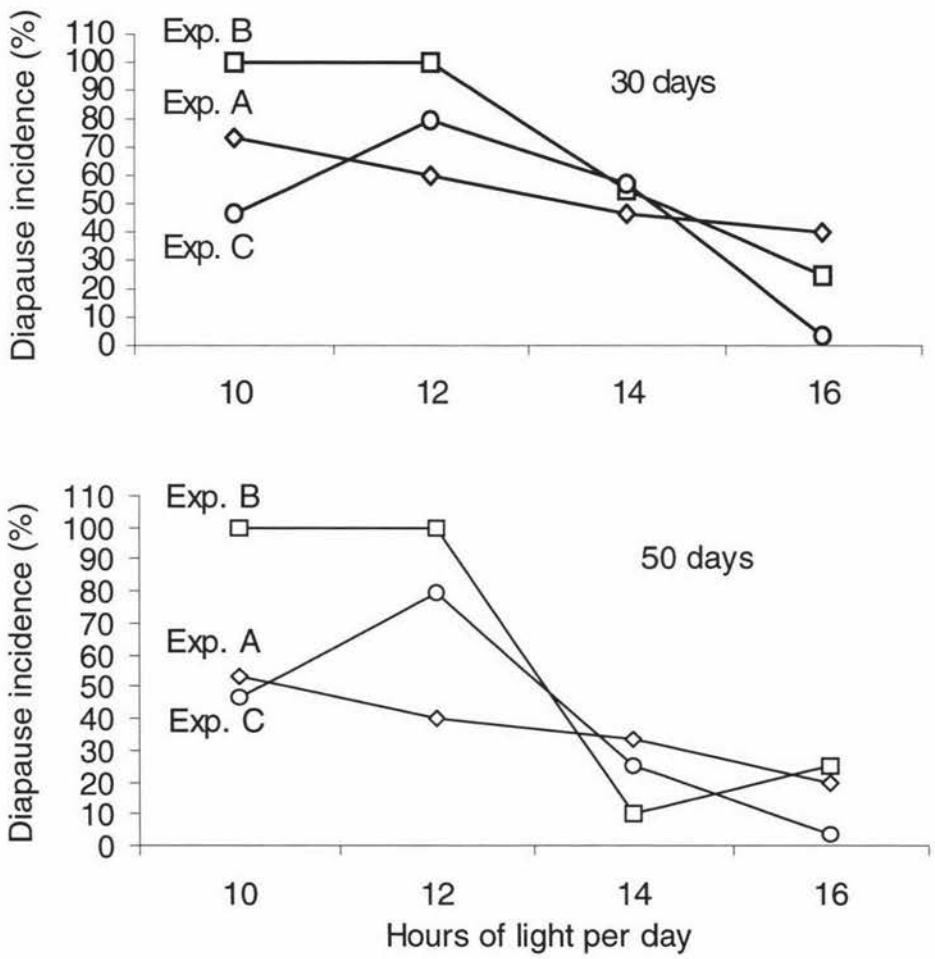

Fig. 4.2: Female diapause incidence within 30 and 50 d after emergence.

4.3.2.2 Continuation and termination of diapause

Photoperiod and photoperiodic history of the preimaginal stages significantly influenced female reproductive parameters (Tables 4.9) and adult longevity (Table 4.10).

For experiment A, where adults were held at the same photoperiod as the perimaginal stages, photoperiod was found to significantly affect the premating and postoviposition periods, and the fecundity (Table 4.9) and longevity (Table 4.10). Premating period usually increased as photoperiod decreased, but no significant difference was found between 16:8 and 14:10 (L:D). However, there was no significant difference in preoviposition and oviposition periods, daily egg production and hatch rate between test photoperiods (Table 4.9). But preoviposition period was longer at 10:14 (L:D) than at 12:12, 14:10 and 16:8 (L:D). Longevity was not significantly different between sexes at a given photoperiod, and the adults lived significantly longer at 12:12 and 10:14 (L:D) than at 16:8 and 14:10 (L:D) (Table 4.10).

For experiment B, where adults were held at the same photoperiod as the fifth instar nymphs, the premating and preoviposition periods of ovipositing females significantly increased as photoperiod decreased (Table 4.9). However, there was no significant difference in premating period between 16:8 and 14:10 (L:D) and in preoviposition period between 12:12 and 10:14 (L:D). The oviposition periods were significantly longer at 16:8 and 14:10 (L:D) than at 12:12 and 10:14 (L:D). Females laid significantly greater numbers of eggs at 16:8 and 14:10 (L:D) than at 12:12 and 10:14 (L:D), with a significantly lower egg hatch rate detected at 10:14 (L:D). Although the longevity between sexes was not significantly different, adults held at 12:12 and 10:14 (L:D) lived significantly longer than that at 16:8 and 14:10 (L:D) with the exception of males at 10:14 (L:D) (Table 4.10).

As Table 4.9 shows, reproductive diapause was terminated about 14 d after females were transferred from short photoperiods (12:12 and 10:14, L:D) to a long photoperiod (16:8, L:D) in experiment B. There was no significant

difference in female reproductive phases, fecundity, daily egg production, and hatch rate between between 12:12/16:8 and 10:14/16:8 (L:D), but the number of offspring produced per female was significantly higher at 12:12/16:8 (L:D). The oviposition period for transferred females was about twice as long as that for females at constant photoperiod conditions (Table 4.9). The longevity between sexes and between 12:12/16:8 and 10:14/16:8 (L:D) was not significantly different (Table 4.10).

In experiment C, where adults were transferred from the breeding colony to test photoperiods, photoperiod significantly influenced the ovipositing female's premating, preoviposition and postoviposition periods, and fecundity, daily egg production and number of offspring (Table 4.9) and adult longevity (Table 4.10). The premating period was significantly longer at 12:12 (L:D) than at 16:8 (L:D), while preoviposition period was significantly longer at 14:10 (L:D). Fecundity and daily egg production were significantly higher at 16:8 and 14:10 (L:D) and the number of offspring produced per pemale was significantly higher at 16:8 (L:D). No significant difference in oviposition period and hatch rate was detected between test photoperiods. The longevity of females at 10:14 (L:D) and males at 12:12 (L:D) were significantly longer than at 16:8 and 14:10 (L:D), respectively. No differences in longevity were found between sexes at all photoperiods.

In experiment C, reproductive diapause was broken between 10 and 20 d after females were transferred from short (10:14, 12:12 and 14:10, L:D) to long (16:8, L:D) photoperiods (Table 4.9). There was no significant difference in female reproductive phases, fecundity, daily egg production, egg hatch rate, number of offspring (Table 4.9) and longevity of both sexes (Table 4.10) between those fluctuating photoperiods. Compared with those females held under continous short (10:14, 12:12 and 14:10, L:D) photoperiods, females had significantly shorter oviposition and postovposition periods under changing photoperiod conditions except at 14:10/16:8 (L:D). However, there was no significant difference in fecundity, egg hatch rate and number of offspring except for a significantly lower fecundity detected at 14:10/16:8 (L:D) than at continous photoperiod 14:10 (L:D).

Table 4.9: Reproductive parameters (mean ± SE) of *N. huttoni* under four photoperiod conditions *

Phot. (L:D)	Reproductive phase (d)								Fecundity		Daily egg production		Hatch (%)		Nymphs per female	
	n	Premat.	n	Preovi.	n	Ovi.	n	Postovi.	n		n		n		n	
Exp. A 16:8	15	9.47±0.98f	12	25.67±3.04bc	12	23.50±5.15cd	12	4.24±1.28d	12	14.50±4.58cd	12	0.83±0.22ab	12	45.63±9.54ab	12	8.00±2.98b
14:10	14	11.07±1.35ef	10	26.10±2.66b	10	15.10±3.37d	10	14.70±4.25bc	10	9.00±1.99de	10	0.88±0.25ab	10	32.06±9.17abc	10	3.70±1.54cd
12:12	15	17.60±1.57d	9	27.11±2.24b	9	25.11±4.86cd	9	9.56±2.55cd	9	8.89±2.29de	9	0.58±0.20bc	9	46.48±11.00ab	9	4.11±1.03cd
10:14**	11	26.45±2.88c	7	36.00±9.20b	7	11.86±4.77d	7	16.71±5.13bc	7	4.43±1.11e	7	0.91±0.30ab	7	27.98±7.96bc	7	1.43±0.43d
Exp. B 16:8	20	11.45±0.98def	17	18.76±1.10d	17	48.53±4.41ab	17	10.06±2.52bc	17	27.18±3.07ab	17	0.59±0.06b	17	39.60±5.49ab	17	10.70±2.02b
14:10	18	16.85±1.46de	18	29.39±2.19b	18	35.56±5.06bc	18	8.44±2.25cd	18	18.89±3.1bc	18	0.55±0.05bc	18	17.93±5.30bc	18	4.44±1.34cd
12:12	16	40.13±4.23b	7	72.43±1.96a	7	12.71±3.83d	7	28.43±8.28a	7	7.57±1.46de	7	0.77±0.11ab	7	29.54±9.73abc	7	3.00±1.18d
10:14	15	48.50±3.60a	5	86.00±9.79a	5	12.80±2.44d	5	8.20±3.07cd	5	7.80±2.08de	5	0.78±0.26ab	5	5.71±3.50c	5	0.40±0.24d
12:12/16:8			8	64.63±1.75a	8	27.38±6.58cd	8	7.25±7.50cd	8	13.88±2.80cd	8	0.56±0.09bc	8	25.35±10.25bc	8	5.00±2.29bc
10:14/16:8			8	64.33±1.83a	8	24.22±5.63cd	8	12.56±3.05bc	8	10.44±1.96cd	8	0.62±0.13ab	8	12.84±8.14c	8	1.13±0.46d
Exp. C 16:8	28	10.39±0.36f	27	18.11±0.70d	27	61.89±4.23a	27	10.48±1.67bc	27	41.48±3.77a	27	0.66±0.04ab	27	48.93±4.14a	27	20.10±2.46a
14:10	28	13.25±1.54def	21	28.76±2.97b	21	45.50±2.92ab	21	18.62±5.01ab	21	28.86±2.79ab	21	0.67±0.07ab	21	33.90±5.45abc	21	9.25±1.97b
12:12	26	17.57±1.91d	6	16.33±1.69d	6	60.50±12.68a	6	31.50±14.83a	6	15.50±3.71cd	6	0.26±0.02c	6	41.13±14.90ab	6	5.50±2.51bc
10:14	28	12.75±1.45def	13	19.31±1.20cd	13	66.77±9.35a	13	39.15±9.20a	13	17.54±2.27c	13	0.35±0.07c	13	39.02±7.77ab	13	6.08±1.05bc
14:10/16:8			6	60.50±3.77a	6	21.00±8.48cd	6	18.20±4.77ab	6	16.00±7.24cd	6	0.99±0.24a	6	35.40±15.70abc	6	5.00±1.71bc
12:12/16:8			19	68.80±1.41a	19	25.80±3.50cd	19	11.10±2.70bc	19	18.90±2.32bc	19	0.89±0.12ab	19	24.50±4.97bc	19	5.32±1.38bc
10:14/16:8			12	69.50±1.03a	12	29.40±3.03c	12	11.90±2.69bc	12	18.30±2.62bc	12	0.75±0.14ab	12	31.40±8.06abc	12	6.83±2.45bc
F		36.40		43.30		6.31		3.83		7.47		3.30		2.38		4.26
df		16		16		16		16		16		16		16		16
P		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.0030		0.0001

* Means (±SE) followed by the same letters in columns were not significantly different ($P > 0.05$).
**Two females commenced diapause at 10:14 (L:D) with mean preoviposition period of 67.50 ± 9.50 d and longevity of 74.50 ± 1.50 d.
Except daily egg production and hatch rate (%), data were subject to natural logarithmic transformation before ANOVA.
Data of daily egg production was subject to square root transformation.

Table 4.10: Longevity (mean ± SE) of *N. huttoni* under four photoperiod conditions *

Phot. (L:D)	n	Female (d)	n	Male (d)
Exp. A 16:8	15	50.80±3.45f	15	47.80±3.90f
14:10	15	47.80±4.04f	15	45.27±4.41f
12:12	15	61.87±2.27e	15	66.80±4.98e
10:14	15	65.87±2.84e	15	67.13±2.67e
Exp. B 16:8	20	76.95±3.33de	20	70.90±6.24de
14:10	20	72.20±3.64e	20	79.70±2.97cde
12:12	10	110.30±7.34ab	10	97.50±6.45abc
10:14	10	90.38±8.88bcd	10	101.40±9.61ab
12:12/16:8	10	96.10±5.54bc	10	89.40±9.01bcd
10:14/16:8	10	98.90±4.87ab	9	97.20±4.33abc
Exp. C 16:8	28	91.57±4.23bcd	28	89.04±4.21bcd
14:10	21	94.29±4.87bcd	21	89.95±4.18bcd
12:12	7	100.00±10.35ab	7	117.70±12.18a
10:14	14	117.64±11.71a	14	108.80±12.83ab
14:10/16:8	7	103.57±7.03ab	7	100.00±10.20abc
12:12/16:8	22	103.64±4.88ab	22	99.86±4.35abc
10:14/16:8	14	106.00±5.55ab	14	99.21±3.87abc
F		28.10		71.77
df		16		16
P		0.0001		0.0001

* Means (±SE) followed by the same letters in columns were not significantly different ($P > 0.05$). The longevity was not significantly different between sexes at a given photoperiod in each experiment.
Before ANOVA, data were subject to natural logarithmic transformation.

4.3.2.3 Oviposition patterns

At a given photoperiod, for females that were held at the same photoperiod as their preimaginal stages (Fig. 4.3) and the fifth instar nymphs (Fig. 4.4), or directly transferred from the breeding colony (Fig. 4.5), daily egg production was the highest in the first day of the oviposition period.

As Fig. 4.3 shows, at 16:8 (L:D), the daily oviposition pattern was quite steady after the peak of the first oviposition day, and then gradually tailed away to zero at the end of the female live. The oviposition patterns were very similar between 14:10 and 12:12 (L:D). Between the fifth and twentieth days of the oviposition period, daily egg production was relatively low. It had a peak around the twenty-first day and a small peak just before the end of the oviposition period (Fig. 4.3).

At 10:14 (L:D), the distribution of daily egg production became uneven with no eggs laid for many days of a lengthy oviposition period (Fig. 4.3)

For females held at the same photoperiod as the fifth instar nymphs, the daily egg production patterns were very similar at 16:8 and 14:10 (L:D) with relatively steady oviposition for 50 to 60 d and then tapering off to the end. Uneven daily egg production patterns were found at both 12:12 and 10:14 (L:D) with frequent and lengthy breaks (Fig. 4.4).

When females were directly transferred from the breeding colony, the daily egg production patterns were very similar at 16:8 and 14:10 (L:D), and the peaks tapered off to the end after a relative long oviposition. The frequent and lengthy breaks were also found at 12:12 and 10:14 (L:D) (Fig. 4.5). About 45% of females (3 of 7 at 12:12 (L:D) and 6 of 13 at 10:10 (L:D)) had a oviposition break of over 50 d.

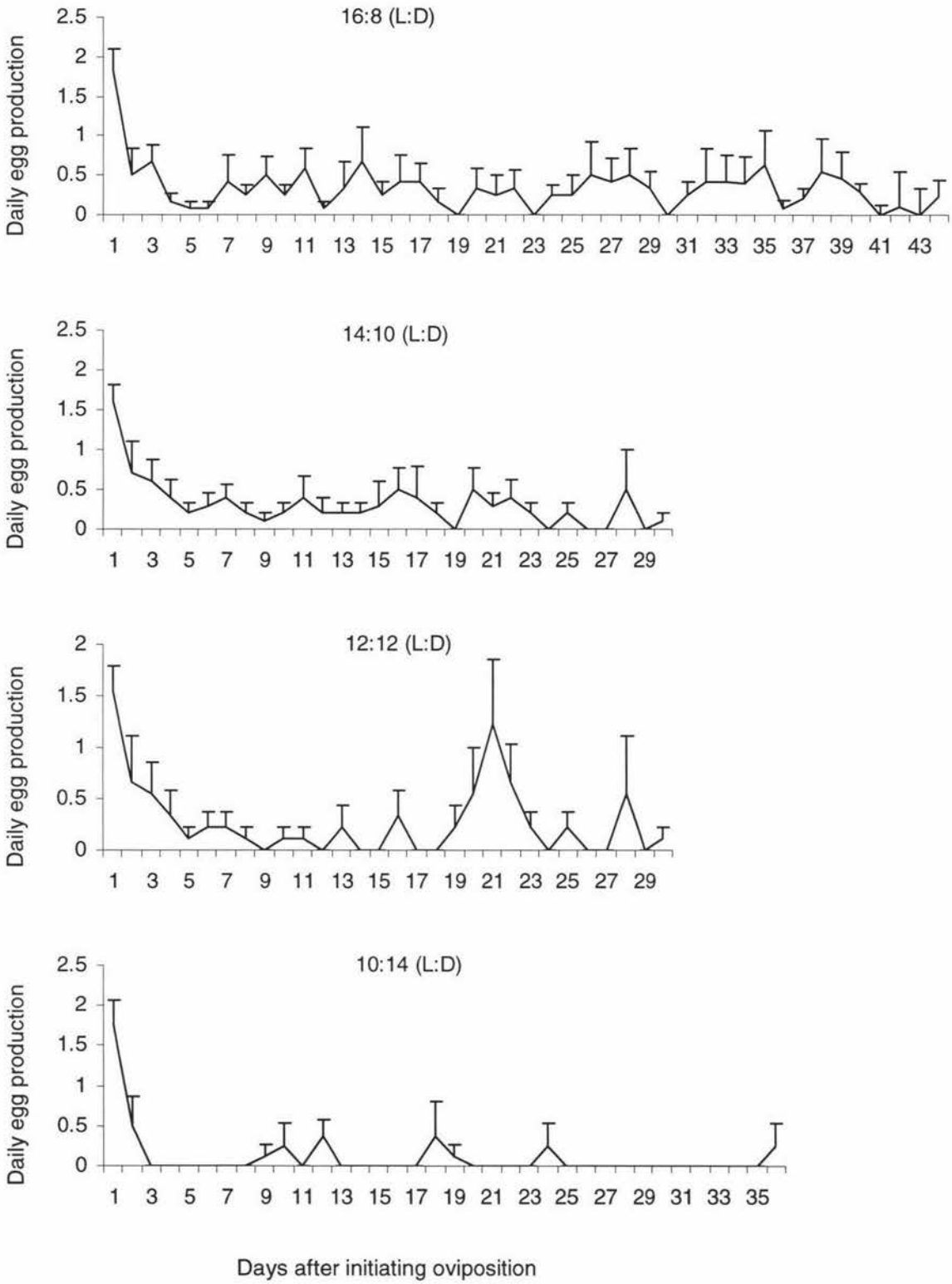

Fig. 4.3: Mean (± SE) daily egg production by females held at the same photoperiod as their preimaginal stages.

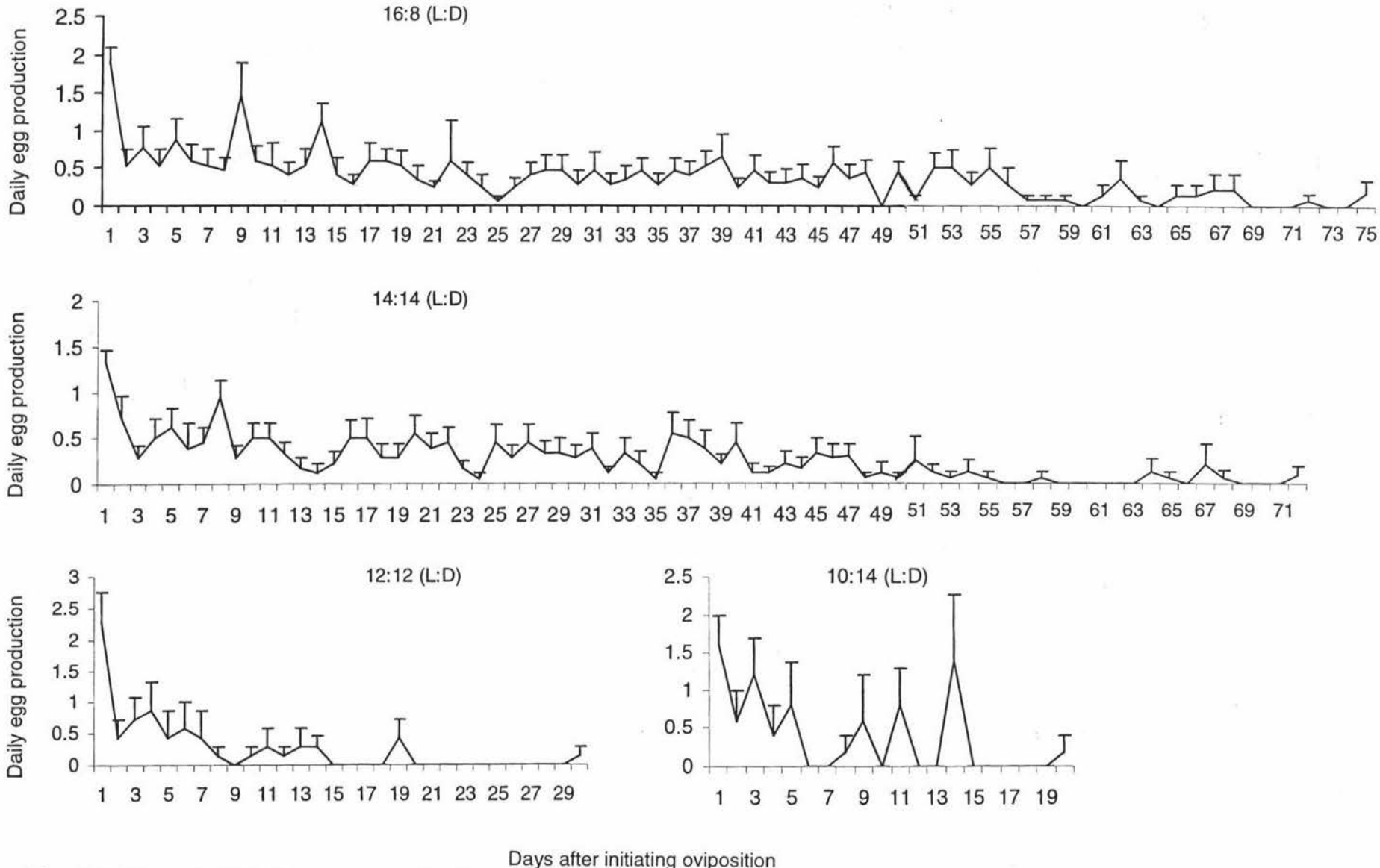

Fig. 4.4: Mean (± SE) daily egg production by females held at the same photoperiod as their fifth instar nymphs.

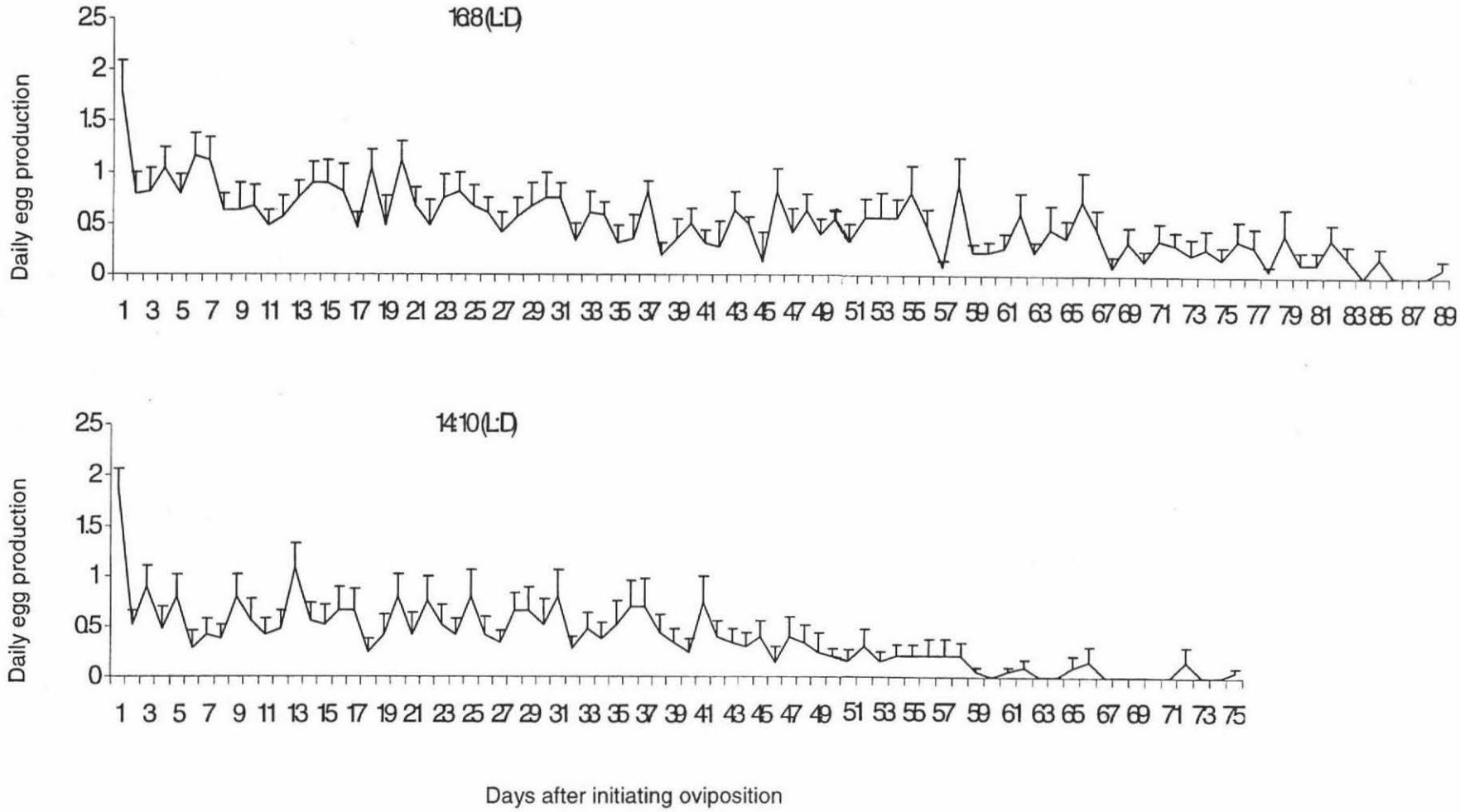

Fig. 4.5: Mean (± SE) daily egg production by females transferred from breeding colony.

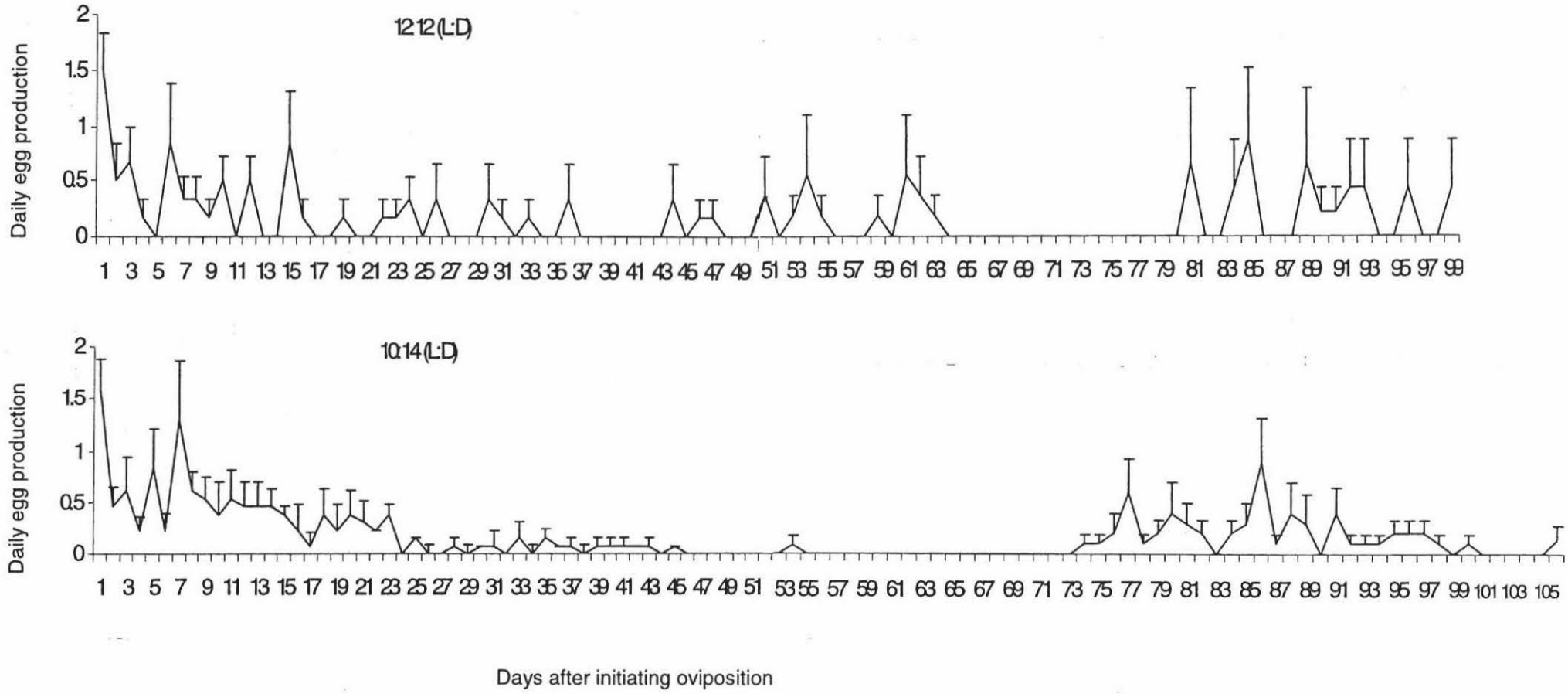

Fig. 4.5 (continued): Mean (± SE) daily egg production by females transferred from breeding colony.

4.4 Discussion

4.4.1 Effect of photoperiod on preimaginal development

In this study, development and growth of preimaginal stages of *N. huttoni* slowed as photoperiod decreased. Beck (1968) stated that species in which diapause was induced by short photoperiod also displayed slower growth in response to short photoperiods, e.g., the cricket *Velarifictorus parvus* (Seiji *et al.* 1999). Obviously, *N. huttoni* fits this case. However, the influence of photoperiod on insect development is species specific. In *N. vinitor*, there was no significant difference in nymphal periods between 10:14 and 14:10 (L:D) (Allsopp 1984); in the predatory bug, *Podisus maculiventris*, nymphal development was faster under the diapause-inducing short photoperiod of 8:16 (L:D) than under the diapause-averting long photoperiod of 16:8 (L:D) (Chloridis *et al.* 1997).

Nysius huttoni nymphs developed more slowly at changing photoperiods (10:14/16:8, 12:12/16:8 and 14:10/16:8, L:D) than at constant short photoperiod. It is possible that this species is more sensitive to a changing photoperiod than a constant one. Moreover, fifth instar nymphs directly transferred from 16:8 (L:D) to changing photoperiods (10:14/16:8, 12:12/16:8 and 14:10/16:8, L:D) took significantly more time to reach the adult stage, where 100% of females entered diapause. It has been suggested that diapause in some species has been associated with a decrease in growth rate of the prediapause stage (Andrewartha 1952).

The survival of *N. huttoni* nymphs was also related to photoperiods with shorter light resulting lower survival rate. However, Allsopp (1984) reported that there was no significant difference in *N. vinitor* nymphal survival between 10:14 and 14:10 (L:D).

4.4.2 Effects of photoperiod on female reproductive diapause

4.4.2.1 Induction of diapause

In many insect species, diapause incidence increases with exposure of increased numbers of prediapause stages to diapause-inducing condition. For example, in the tufted apple bud moth, *Platynota idaeusalis* (Walker), the whole immature period is sensitive to photoperiod; a maximum of 39% diapause occurs when four of the five larval instars are exposed to short photoperiods, whereas 100% diapause occurs when all instars experience short photoperiods (Rock *et al.* 1983). However, this is not the case with *N. huttoni*, because, on the one hand, exposure of the entire life cycle to 10:14 and 12:12 (L:D) gave a lower percentage of diapausing females; and on the other hand, transfer of fifth instar or adult stage to 10:14 and 12:12 (L:D) produced a higher percentage of diapausing females. In many insects that undergo reproductive diapause the adult is the sensitive stage that responds to photoperiod (Tauber and Tauber 1970; Tauber *et al.* 1986), for example in the bean bug, *Riptortus clavatus* Thunberg (Nunata and Hidaka 1982, 1983) and square bug, *Anasa tristis* (Nechols 1988). Transfer of adult *N. huttoni* to 10:14 and 12:12 (L:D) resulted in a high percentage of females entering diapause and this finding suggests that the adult stage is very sensitive to photoperiod. Exposure of fifth instar to a short photoperiod (10:14 and 12:12, L:D) resulted in all of the females entering diapause at the same photoperiod, indicating that the fifth instar is also highly sensitive to photoperiod. Increased exposure of late stage instars to short photoperiod increases the incidence of diapause in females for some Heteroptera species. The incidence of diapause in females increased as insects were transferred from 16:8 (L:D) to experimental 12:12 (L:D) photoperiod as third and fourth instars of the true bug, *Neacoryphus bicrucis* (Say) (Solbreck 1979), or as newly moulted adults, fifth, and fourth instar nymphs of *Anasa tristis* (Fielding 1988). Farrell and Stufkens (1993) reported that after the transfer of third and fourth instars of *N. huttoni* from field (photoperiod 14.4:9.6 (L:D) on 17 January 1991) to 12:12 (L:D), no oviposition occurred in 30 d. Thus, in addition to adult and fifth instar stages, the later immature stages (third and fourth instars) of *N. huttoni* are also sensitive to diapause-inducing photoperiods.

However, the exposure of entire life cycle of *N. huttoni* to 10:14 and 12:12 (L:D) resulted in low percentage of females entering diapause, whether this is result of the loss of diapause photoperiodic response at constant photoperiods is unknown. It has been reported in the green lacewing, *Chrysopa carnea* Strains, when the entire life cycle was maintained in a constant short photoperiod of 12:12 (L:D), the adults underwent a much less intense diapause than those that experienced photoperiod of 16:8 (L:D) before being placed in the diapause-inducing photoperiod (Tauber and Tauber 1970, Tauber *et al.* 1970).

4.4.2.2 Diapause and critical photoperiod definition

Nysius huttoni is a Type I (Beck 1968) species in which a long photoperiod promotes continuous development while short photoperiod induces diapause. Danilevskii (1965) stated that the long-day type response is a typical of temperate zone, multivoltine species that enter a reproductive winter diapause; since *N. huttoni* has at least three generations per year (Eyles 1965b), it falls into this category.

The critical photoperiod has not been previously reported for *N. huttoni.* The absence of oviposition for 30 d after emergence was used to define *N. huttoni* female diapause by Farrell and Stufkens (1993). If this definition is accepted, then the critical photoperiod for female diapause induction would be between 14:10 to 14.5:9.5 (L:D). However, this "critical photoperiod" occurs in January in Palmerston North (which is similar to the whole range of New Zealand), which is the rapid growth and development season for *N. huttoni.* If diapause is defined as the absence of oviposition for 50 d after emergence, then the critical photoperiod falls between 13:11 to 13.5:10.5 (L:D) which is the photoperiod in late February and early March. Farrell and Stufkens (1993) reported that a high proportion of second generation females in the Lincoln weed area and in trap catches did not develop eggs during February and March 1991 and 1992. At 14:10 (L:D), between 90 and 75% of females laid eggs in 50 d after emergence in the present study. Therefore, *N. huttoni* reproductive diapause ought to be defined as the absence of oviposition

around 50 d after the emergence or during oviposition period, and the critical photoperiod is between 13:11 to 13.5:10.5 (L:D). However, this estimated critical photoperiod is based on constant photoperiods and does not take any account of any effect of the changes in photoperiod, temperature and/or humidity to which *N. huttoni* may also respond. For example, temperature exerted a modifying effect on the critical photoperiod in the weevils *Listronotus oregonensis* (LeConte) (Stevenson and Boivin 1990) and *L. bonariensis* (Kuschel) (Kalvelage 1999).

4.4.2.3 Diapause termination

The difference in preoviposition periods between the photoperiods may have been related to diapause intensification (Ruberson 1991). My study indicates that a weak diapause resulted when the entire life cycle of *N. huttoni* was maintained at a constant photoperiod, and strong diapause occurs under changing photoperiods. This may be due either to a "long-day short-day effect" as found in the bollworm *Heliothis zea* (Boddie) (Wellso and Adkisson 1966) and the red locust, *Nomadacris septemfasciata* (Serville). (Norris 1965), or to the insects perceiving the indication of changing photoperiods as in the green lacewing, *Chrysopa carnea* (Tauber and Tauber 1970). Bell and Adkisson (1964) stated that conditions giving the most intense diapause also caused the greatest percentage of insects to enter diapause, such as in pink bollworm, *Pectinophora gossypiella*.

In both experiments B and C, females required a similar time to terminate diapause when transferred from 10:14 and 12:12 (L:D) to 16:8 (L:D), indicating that females underwent a diapause with similar intensity when those photoperiods were shorter than the critical photoperiod. However, when the adult stage was held at 10:14 and 12:12 (L:D) (experiment C), females probably underwent a more intense diapause with 4-5 d more being required to terminate diapause at 16:8 (L:D), than when females in their fifth instar and adult stages were exposed to 10:14 and 12:12 (L:D) (experiment B). Unfortunately, as the time requirement for females to commence oviposition at

10:14 and 12:12 (L:D) in experiment C was not detected, the comparison of diapause intensity to experiment B could not be made.

In experiment B, females held at constant photoperiods of 10:14 and 12:12 (L:D) had much shorter oviposition periods than those at changing photoperiods of 10:14/16:8 and 12:12/16:8 (L:D), suggesting that the photoperiodic activation did not result in the loss of photoperiodic response, although insects usually underwent a gradual diminution of sensitivity to photoperiod during photoperiodically maintained diapause (Tauber *et al.* 1986). Nunata and Hidaka (1982) reported that in the bean bug, *Riptortus clavatus*, sensitivity to photoperiod remains even after diapause had ended.

Females took 13.5 d more to commence oviposition at 10:14 (L:D) than at 12:12 (L:D), indicating that females underwent a more intense diapause at 10:14 (L:D) than at 12:12 (L:D). Tauber *et al.* (1986) stated that for some species, very short photoperiods are capable of inducing a more intense diapause than those just below the critical photoperiod. Moreover, *N. huttoni* females took longer (preoviposition period) to commence oviposition at 10:14 and 12:12 (L:D) and shorter time to terminate diapause at 16:8 (L:D) after 50 d diapause. In this case, photoperiodic sensitivity may persist throughout diapause or it may become important as a diapause-maintaining factor during a second phase of diapause (Tauber *et al.* 1986). Conversely, in experiment C, these females initiating oviposition at 10:14 and 12:12 (L:D) had significantly longer oviposition periods than those terminating diapause at 16:8 (L:D), indicating the loss of the photoperiodic response at the experimental conditions favourable for spontaneous completion of diapause.

In experiment B, *N. huttoni* females required 86.00 ± 9.79 and 72.43 ± 1.96 d to commence oviposition at 10:14 and 12:12 (L:D), respectively. Similarly, Farrell and Stufkens (1993) also reported that females failed to oviposit over 80 d when maintained under 12:12 (L:D).

4.4.2.4 Effects of photoperiod on fecundity and longevity

In each experiment, there was a tendency for fecundity to decrease with the decrease of photoperiod, i.e., fecundity was greater at 16:8 (L:D) than at 14:10 (L:D), and in turn, it was greater than at 12:12 and 10:14 (L:D), and also fecundity was greater at 16:8 (L:D) than it was when diapause terminated at changing photoperiods (14:10/16:8, 12:12/16:8 and 10:14/16:8 (L:D)). Farrell and Stufkens (1993) reported that females of *N. huttoni* laid 98.6 ± 10.6 eggs at 16:8 (L:D) and 43.0 ± 11.9 eggs at 12:12/16:8 (L:D), but only one female laid one egg at 12:12 (L:D). Moreover, females laid more eggs after winter (under increased photoperiods) than before winter (under decreased photoperiods) (Eyles 1963b). In *N. vinitor*, females laid similar number of eggs at 10:14 (L:D) and 14:10 (L:D) (Allsopp 1984).

Farrell and Stufkens (1993) stated that when females terminated diapause at 16:8 (L:D), the daily egg production was similar to that in constant photoperiod of 16:8 (L:D). It was also the case in experiments B and C in this study.

Longevity of *N. huttoni* adults was longer at 10:14 and 12:12 (L:D) than at 14:10 and 16:8 (L:D) in each experiment and there was no significant difference between both sexes. Allsopp (1984) also reported that *N. vinitor* adults survived significantly longer at 10:14 (L:D) than at 14:10 (L:D) but males survived longer than females at 10:14 (L:D).

4.4.2.5 Effects of photoperiod on oviposition patterns

The influence of photoperiod on oviposition in some species of Heteroptera has been studied, for example, in *N. vinitor* (Allsopp 1984), *Anasa tristis* (Fielding 1988) and *N. huttoni* (Farrell and Stufkens 1993), but few studies have looked at oviposition patterns during oviposition period under different photoperiods. Under unheated greenhouse conditions, Eyles (1963a) reported that there was a short period of low production, followed by a fairly steady peak level of production which tailed away to zero towards the end of the females'

lives, and most intervals were short. This pattern is similar to that found in the experiments A, B and C in this study when females were held at 16:8 and 14:10 (L:D), but not at 12:12 and 10:14 (L:D) where frequent and lengthy breaks were found. Especially in experiment C, where females had oviposition breaks over 50 d at 10:14 and 12:12 (L:D) apparently entered diapause. Fielding (1988) found that in the species of *Anasa tristis,* some females transferred from 17:7 (L:D) to 12:12 (L:D) as newly moulted adults produced one or two clusters of eggs before entering diapause. The decrease in daily egg production at the close of oviposition period at 16:8 and 14:10 (L:D) is possible because of the age of the females.

Chapter five
Summary and conclusion

The aim of this study was to investigate aspects of the biology of *N. huttoni*, with special reference to the preimaginal growth and development, and reproduction under known environmental conditions. This would provide basic knowledge that could assist the establishment of both pest management and risk management strategies for this species which, although can be a production pest, has its most dramatic effects as a quarantine pest.

The first objective was to determine the influence of temperature and host plant on preimaginal and imaginal development in the laboratory; and the effect of food supply and mating on reproduction. It is clear that temperature and host plant play a major role in *N. huttoni* development. Generally, the results for preimaginal development times, and premating and preoviposition periods show a significant trend with temperature, being shorter as temperature increases; while growth rate is higher as temperature increases. At each temperature, the time for both sexes required to complete development is similar and sex ratio is 1:1. Eggs and nymphs fail to develop at 10°C. Few nymphs grow and develop through to the adult stage on SP and CW. The overall survival is significantly higher on TC at 20°C. Based on these results, potential population increase would be expected to faster on TC. The larger populations present on SP (Gurr 1957) or other unsuitable host plants such as CW, may result from the spread from overpopulated TC or other suitable host plants. This may explain to some extent outbreak of *N. huttoni* on crops that this insect does not particularly prefer and, therefore, producers may use this information to determine the critical time to apply insecticides.

Based on the variables of development time at different temperatures, the low threshold temperature and degree-days (DD) requirements for *N. huttoni* growth and development were estimated. The low threshold temperature for each instar stage and premating and preoviposition were first

reported under constant temperature conditions. However, for some species, development times are known to differ between constant and fluctuating temperatures with the same mean (Messenger and Flitters 1959, Hagstrum and Hagstrum 1970, Hagstrum and Leach 1973), such as the squash bug, *Anasa tristis* (Fielding and Ruesink 1988), red flour beetle, *Tribolium castaneum* (Hagstrum and Milliken 1991) and the nettle-feeding nymphalid butterfly larvae of *Aglais urticae*, *Inachis io*, *Polygonia c-album* and *Vanessa atalanta* (Bryant *et al.* 1999). Therefore, development time data collected at constant temperatures in the laboratory can be expected to provide only rough estimates of development times in the field. However, several investigators have shown that the differences in development times between constant and fluctuating temperatures can be partially resolved by integration of constant temperature development times over the fluctuating temperature cycle to predict development times at fluctuating temperatures (Eubank *et al.* 1973, Stinner *et al.* 1974, Ables *et al.* 1976, Butler *et al.* 1976, Hilbert and Logan 1983, and Dallwitz 1984). Although there are not enough data to determine if this is the case with *N. huttoni*, the observation of activity of *N. huttoni* that accelerated or slowed down with temperature (Gurr 1957) suggests that body temperature of *N. huttoni* may increase during the day through basking. In addition, the fecundity and hatch rate were lower at constant temperatures in this study than under fluctuating temperatures observed by Eyles (1963b) in an unheated greenhouse. Therefore, future observation under fluctuating temperatures in the laboratory or field conditions is needed in order to estimate the precise DD requirements for *N. huttoni* development and potential populations.

Obviously, temperature, food, and mating influence *N. huttoni* fecundity. Few or no eggs were laid at 10 and 15°C or when adults were fed with SP. Females fed with TC and sunflower seeds had significantly higher fecundity and daily egg production. Although the egg hatch rate was lower in this case, additional nutrients conducive to *N. huttoni* reproduction increased the number of offspring. Eyles (1963a) reported that the presence of males was not necessary for the formation of eggs. However, in the present study, the hatch

rate of eggs laid by mated-once females was significantly lower than that of eggs laid by permanently paired females, suggesting that repeated copulation was needed to realise maximun fertile eggs.

Temperature and food also strongly influenced the longevity, with shorter adult life at higher temperatures or fed with TC and sunflower seeds. It is probably due to: (1) the greater energy requirement for the faster reproductive development of both sexes, and higher daily egg output of females at higher temperatures and (2) the greater energy requirement for greater egg production and deposition when fed with TC and sunflower seeds.

The second objective of this study was to determine the influence of photoperiod on preimaginal development and reproductive diapause in the laboratory. The development rate of preimaginal stages in the laboratory at various photoperiods was determined for the first time. *Nysius huttoni* required a longer period to complete development from egg to adult, and the body weight and growth rate (from first instar to adult) were also lower, at 10:14, and 12:12 (L:D) than at 14:10 and 16:8 (L:D). Reproductive diapause was induced by short photoperiods 10:14 and 12:12 (L:D) in this study. This result supports Beck's (1968) finding that species in which diapause was induced by short photoperiod also displayed slower growth in response to short photoperiods. Nymphs developed more slowly at changing photoperiods (10:14/16:8, 12:12/16:8 and 14:10/16:8 (L:D)) than under constant photoperiods, suggesting that *N. huttoni* was more sensitive to a changing photoperiod than a constant one.

Nysius huttoni reproductive diapause was defined as the absence of oviposition within about 50 d after the emergence or during oviposition period, and the critical photoperiod was between 13:11 and 13.5:10.5 (L:D). The definition of critical photoperiod was supported by the observation of Farrell and Stufkens (1993) that second generation females did not develop eggs during February and March 1991 and 1992 at Lincoln, Canterbury.

The photoperiodic history that preimaginal stages experienced significantly influenced the incidence of diapause. Exposure of the entire life cycle to 10:14 and 12:12 (L:D) gave a low diapause incidence. This may be the result of the loss of the photoperiodic response at constant photoperiods. Therefore, the incidence of diapause did not increase with exposure of increased numbers of prediapause stages to diapause-inducing condition, as was found in the tufted apple bud moth, *Platynota idaeusalis* (Rock *et al.* 1983). Conversely, a very high percentage of reproductive diapause was induced by transferring fifth instar nymphs or adults from 16:8 to 10:14 and 12:12 (L:D). It appears that, in terms of diapause induction, *N. huttoni* is more sensitive to changing photoperiods than to constant photoperiods. Moreover, exposure of third, fourth and fifth instar nymphs (Farrell and Stufkens 1993) or just fifth instar nymphs (in this study) induces 100% of females to enter diapause. Thus, in addition to adult stage, the later immature stages (third, fourth and fifth instars) of *N. huttoni* are also sensitive to diapause-inducing photoperiods, and exposure of later immature stages to short photoperiod will accelerate the incidence of reproductive diapause.

The photoperiodic history that preimaginal insects experienced also strongly influenced the diapause intensity. A longer time was required to commence oviposition under changing photoperiods than at constant photoperiods, suggesting that females under the former conditions underwent a more intense diapause, as was found in bollworm *Heliothis zea* (Wellso and Adkisson 1966), red locust, *Nomadacris septemfasciata* (Norris 1965) and green lacewing, *Chrysopa carnea* (Tauber and Tauber 1970). Changing photoperiods that induce the intense diapause also caused a higher percentage of *N. huttoni* females to enter diapause, as was found in pink bollworm, *Pectinophora gossypiella* (Bell and Adkisson 1964).

Regardless of the photoperiodic history that preimaginal *N. huttoni* experienced, different photoperiods were found to influence the intensity of diapause. When *N. huttoni* females were held in the same photoperiod as fifth instar nymphs, they underwent a more intense diapause at 10:14 (L:D) than at

12:12 (L:D), supporting Tauber *et al.*'s (1986) finding that very short photoperiods are capable of inducing a more intense diapause than that just below the critical photoperiod. Moreover, *N. huttoni* females had longer preoviposition period, shorter oviposition period, and lower fecundity at 10:14 and 12:12 (L:D) than those at 10:14/16:8 and 12:12/16:8 (L:D). Tauber *et al.* (1986) suggested that the photoperiodic sensitivity might persist throughout diapause or it might become important as a diapause-maintaining factor during a second phase of diapause.

Although the initiation of oviposition at 10:14 and 12:12 (L:D) suggests the loss of the diapause-photoperiodic response to some extent, the apparent long breaks over the oviposition period indicate that the photoperiodic response was not completely gone and some females apparently re-entered diapause. Similar cases were found in the squash bug, *Anasa tristis* (Fielding 1988) and Argentine stem weevil, *Listronotus bonariensis* (Goldson 1981, Kalvelage 1999).

The interaction between photoperiod and temperature may play a role in preimaginal development and reproductive diapause. Temperature may also influence growth rates and therefore exert a strong effect on the proportion of individuals completing their photosensitive stages before or after the occurrence of diapause-inducing photoperiods (Tauber *et al.* 1986). In long-day insects, low temperatures tend to promote diapause, whereas high temperatures tend to prevent it (Tauber *et al.* 1986). Thus, temperature may modify the photoperiodic response and in turn modify the critical photoperiod. For example, in the noctuid moth, *Acronycta rumicis*, the critical photoperiod increases by 1.5 hours as temperature decreases by 5°C (Danilevsky 1965). Similar cases are found in the carrot weevil, *Listronotus oregonensis* (Stevenson and Boivin 1990) and Argentine stem weevil, *Listronotus bonariensis* (Kalvelage 1999). Therefore, future experiments on the interaction between photoperiod and temperature in laboratory conditions are essential to

precisely predict preimaginal development and reproductive diapause in field conditions.

Reproductive diapause in insects is marked by developmental arrest of reproductive organs (Tauber *et al.* 1986). Diapausing females fail to develop eggs because the corpora allata produce greatly reduced amounts of juvenile hormone (Tauber *et al.* 1986). A good example that short photoperiod induces reproductive diapause and arrests the development of reproductive organs and corpora allata is reported by Kotaki and Yagi (1989). They found that the adult of brown-winged green bug, *Plautia stali* Scott, entered diapause under short photoperiod condition, and the development of the ectadene reservoir in males, ovary in females, and corpora allata in both sexes were all inhibited. Thus, future experiments examining the development of reproductive organs and corpora allata will be useful to confirm *N. huttoni* reproductive diapause under short photoperiods.

This study is a step towards a better understanding of preimaginal development, growth and survival and reproduction of *N. huttoni* in known environmental conditions. Besides a better understanding of *N. huttoni* biology and ecology, control action thresholds and strategy need to be developed considering the various field (or crop) management systems, with a more detailed knowledge of the interactions of *N. huttoni* with its host plants and environmental conditions (such as temperature, photoperiod and humidity) in future research. These, in turn, it will lead to more sustainable management of this pest.

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