WAITE INSTITUTE
10-12-73
LIBRARY

# TRANSMISSION OF LETTUCE NECROTIC YELLOWS VIRUS BY *HYPEROMYZUS LACTUCAE* (L.) (HOMOPTERA:APHIDIDAE): WITH SPECIAL REFERENCE TO APHID BEHAVIOUR

by

D.B. Boakye

B.Sc. (Agric.) London, M.S. (Entomology) Arizona

Department of Entomology
Waite Agricultural Research Institute

May, 1973

A thesis submitted for the degree of Doctor of Philosophy in the University of Adelaide

CONTENTS

page

SUMMARY v

DECLARATION viii

ACKNOWLEDGEMENTS ix

GLOSSARY x

1. INTRODUCTION 1

1.1 Mechanism of aphid transmission of plant viruses 1

1.2 Economic importance of lettuce necrotic yellows virus 3

1.3 Vectors of LNYV and their distribution 4

1.4 Host plants of *H. lactucae* 4

1.5 Origin of LNYV in Australia 5

1.6 Relationship between LNYV and other Rhabdoviruses 5

1.7 Mode of LNYV transmission by *H. lactucae* 7

2. GENERAL MATERIALS AND METHODS 10

2.1 LNYV isolate 10

2.2 Raising and maintenance of sowthistle plants 11

2.3 Transmission studies 11

2.4 Establishment and maintenance of aphid colonies 12

2.41 Establishment of first virus-free colony 13

2.42 Establishment of first viruliferous colony 14

2.43 Second virus-free and viruliferous colonies 14

2.5 Behavioural studies 16

2.51 Desiccation and starvation of *H. lactucae* 16

2.52 Labelling of seedlings with $^{32}P$ 17

2.6 Field studies 18

2.61 Establishment of lettuce crop in the field 18

2.62 Establishment of aphid colonies on infected sowthistle plants 18

2.63 Spread of LNYV from virus-free sowthistle seedlings colonized by progeny of infected alate *H. lactucae* at the time of introduction into the crop 19

2.64 Determination of the period taken for LNYV in the inoculated sowthistle seedlings in section 2.63 to become available to the progeny of alate viruliferous *H. lactucae* 19

2.65 Trapping of apterous *H. lactucae* in lettuce plots (Alverstoke orchard) 20

2.66 Trapping of alate *H. lactucae* in lettuce plots (Alverstoke and Claremont orchards) 20

2.67 Confirmation of LNYV in diseased lettuce plants 21

3. CHARACTERISTICS OF THE TRANSMISSION OF LNYV BY *H. LACTUCAE* 22

3.1 Introduction 22

3.2 Latent period of LNYV in *H. lactucae* and its dependence on temperature 23

3.3 Transmission of LNYV by some stages and morphs of *H. lactucae* 26

3.4 Transovarial transmission of LNYV by *H. lactucae* 28

3.5 Inoculation threshold of LNYV in lettuce seedlings 36

3.6 Inoculation threshold of LNYV in sowthistle seedlings 36

3.7 Discussion 37

4. STUDIES ON THE PROBING BEHAVIOUR OF *H. LACTUCAE* 45

4.1 Investigations into the settling behaviour of *H. lactucae* on lettuce 45

4.2 Saliva production by probing *H. lactucae* 48

4.3 Depth of stylet penetration of *H. lactucae* on lettuce and sowthistle seedlings. 49

4.4 Uptake of plant sap by probing *H. lactucae* 51

4.5 Discussion 53

5. BIOLOGICAL STUDIES ON H. LACTUCAE 57

5.1 Comparison of rates of development of *H. lactucae* on seedlings and leaf discs of sowthistle plants 57

5.2 Effect of temperature on the development of *H. lactucae* 59

5.3 Results 59

5.31 Developmental periods of the life stages of the aphid 59

5.32 Developmental zero 60

5.4 Influence of population density on dispersal of *H. lactucae* 62

5.5 Results 65

5.51 Temperature in the cages 65

5.52 Influence of population density on reproduction of *H. lactucae* 66

5.53 Influence of population density on emigration of *H. lactucae* from sowthistle plants 69

5.6 Discussion 72

6. FIELD TRANSMISSION OF LETTUCE NECROTIC YELLOWS VIRUS BY H. LACTUCAE 77

6.1 Introduction 77

6.2 Layout of experimental plots 83

6.3 Treatments 83

6.4 Measurement of effects of treatment 84

6.5 Results 86

6.51 Confirmation of LNYV in diseased lettuce plants 86

6.52 Availability of LNYV to *H. lactucae* in treatment C 87

6.53 Alate H. *lactucae* caught in water traps 87

6.54 The effects of treatments 88

6.6 Discussion 92

7. THE EPIDEMIOLOGY OF LNYV - A GENERAL DISCUSSION 98

8. BIBLIOGRAPHY 105

WAITE INSTITUTE
LIBRARY



SUMMARY

Lettuce necrotic yellows virus (LNYV) which is transmitted by the sowthistle aphid, Hyperomyzus lactucae (L.) causes major losses in lettuce crops in South Australia and in most Australian states. Because lettuce is not a host to the aphid a number of factors were investigated to help understand why the disease is so important in lettuce crops. The transmission characteristics of the virus in the vector; probing behaviour of the aphid particularly on lettuce; dispersal of the aphid from sowthistle plants; and the importance of this dispersal in relation to spread of the disease in the field are reported.

When H. lactucae are given acquisition feeds of 24 hr on LNYV-infected sowthistle plants, a temperature-dependent latent period must be completed before the virus can be transmitted. The mean duration of latent period is 18.0, 9.2 and 5.4 days at 15°, 20° and 28°C respectively. On completion of the latent period H. lactucae transmit LNYV consistently except in some instances when they fail to transmit the virus just prior to death. There is an apparent reduction of longevity in viruliferous aphids. Within 24 hr of reaching the adult stage, apterae and alatae which have developed on virus-infected sowthistle plants are capable of transmitting the virus. The efficiency of transmission is similar for both forms of the aphid. The longer the inoculation feed by viruliferous H. lactucae the greater are the chances for successful transmission of the virus to either sowthistle or lettuce seedlings. Inoculation thresholds are between 5 and 30 min on sowthistle and 1 to 5 min on

lettuce. Transovarial transmission has been demonstrated in viruliferous viviparae apterae but the rate of passage of virus to progeny is low. Out of 73 sets of progeny 5 sets acquired LNYV maternally. It appears that nymphs which acquire the virus complete the latent period at or shortly after birth.

When apterous H. lactucae, freshly removed from sowthistle, are allowed to probe on lettuce they generally walk off the plant; however, the probing threshold is considerably reduced when they are pretreated by starving them in continuous light at a relative humidity of 65-70% for 6 to 24 hr. The site of introduction of LNYV into lettuce appears to be outside the vascular tissue and it is probably introduced via salivary secretions.

The developmental zero for H. lactucae is 10°C and the upper limit of development appears to be 28°C. The reproductive rate of H. lactucae on sowthistle is influenced by population density and it is higher at low than at high densities. Conversely more aphids (adult apterae and alatae) leave the host plant at higher population densities. More alatae than apterae leave the sowthistle plant as the population grows probably because more alates are produced. Dispersal of nymphs is negligible.

In the field, spread of LNYV is effected predominantly by migrant alate H. lactucae. Short range dispersal of alatae is probably unimportant because alate aphids which were put in lettuce plots made no significant contribution to LNYV incidence in these plots. There is an association between peak trap catches of alate aphids and high disease

incidence which occurs 24 to 33 days after the peak of aphid catches. This probably represents the incubation period of the virus in lettuce in the field. Apterous aphids which develop on old infected sowthistle plants near a lettuce crop may play an important role in close range transmission of the disease although this may be eclipsed by the activity of infected migrant alates. Sowthistle seedlings which occur in a lettuce crop and which germinate at or after the germination of the lettuce and which become colonized by progeny of infected migrant alatae are unimportant in LNYV spread.

## Declaration

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been published or submitted to any university for the award of any degree.

May, 1973 (D.B. Boakye)

## ACKNOWLEDGEMENTS

This study was carried out during the tenure of a University of Adelaide Research Grant.

It is a pleasure to thank Professor T.O. Browning for allowing me to study in the Department of Entomology and to the University of Science and Technology (Ghana) for granting me study leave.

I wish to express my gratitude to Drs. D.A. Maelzer and J.W. Randles for supervising and encouraging this study. The help of Drs. R. Laughlin and R.I. Francki is also gratefully acknowledged.

I would like to thank the various members of the Library, Biometry Section and the Farm Staff for their co-operation.

My thanks are also due to Mr. B. Palk for printing the photographs at short notice, to Mrs. Danuta Slowiak for preparing the drawings and to Mrs. Pam Anderson for typing the thesis.

To fellow students and the staff of the Departments of Entomology and Plant Pathology, it was a great pleasure working with them.

Finally a word of gratitude to my wife Comfort, for being a constant source of inspiration.

# GLOSSARY

BMYV: beet mild yellows virus

BNYV: broccoli necrotic yellows virus

BYV: beet yellows virus

LNYV: lettuce necrotic yellows virus

PEMV: pea enation mosaic virus

PLRV: potato leaf roll virus

PSTV: potato spindle tuber virus

PVY: potato virus Y

PYDV: potato yellow dwarf virus

SCV: strawberry crinkle virus

SYVV: sowthistle yellow vein virus

TSWV: tomato spotted wilt virus

VSV: vesicular stomatitis virus

WSMV: wheat striate mosaic virus

WTV: wound tumor virus

# 1. INTRODUCTION

Arthropod-borne viruses are among the most important, complex and widely distributed plant disease agents in the world. The economic importance of the diseases they cause, coupled with the poorly understood mechanism by which they survive and propagate has attracted many workers to this field of research (Maramorosch, 1963).

Aphids are the most important vectors of plant virus diseases. Of the 249 plant viruses listed by Kennedy et al., (1962), 159 are stated to be transmitted by 190 species of aphids. The actual number of vectors is probably much higher, since only 9% of the world aphid fauna has so far been tested for their ability to transmit viruses. The mouthparts of aphids are the direct means of acquisition and transmission of plant viruses and because of their structure and function they are particularly suited for this role.

## 1.1 Mechanism of aphid transmission of plant viruses

The mode of virus acquisition, retention and inoculation by aphids have been used to classify aphid-borne plant viruses as non-persistent, semi-persistent and persistent (Watson and Roberts, 1939; Day and Irzykiewicz, 1954; Sylvester, 1958). More recently Black (1959) has introduced the term 'circulative' for the persistent viruses to emphasise inferred route of transport whereas Kennedy et al., (1962) have suggested the term 'stylet-borne' to include all non-persistent and semi-persistent viruses.

Stylet-borne (or non-persistent) viruses are, by definition (Kennedy et al., 1962), those viruses which are carried at the tips of the aphids' stylets. The differences in the efficiency and specificity with which these viruses are transmitted indicate that the process may be a complex one (Pirone, 1969). To explain the mechanism of transmission, several hypotheses have been proposed which include specific inactivation of viruses by aphid secretions (Day and Irzykiewicz, 1954), differences in aphid behaviour (Day and Irzykiewicz, 1954; Sylvester, 1954) and differences in the surfaces of stylets resulting in differential adsorption of viruses (van der Want, 1954). Sylvester (1954) suggested that specificity was dependent upon the compatible combination of virus, saliva and inoculated host cell. Recently, Garrett (1971) suggested that non-persistent viruses are carried in the cibarium and transmission is effected by the ejection of the viruses from the cibarium. All these hypotheses suggest that the transmission process is essentially mechanical. Stylet-borne viruses are transmitted (acquired and inoculated) within minutes, and usually survive in the vector for less than one hour (Watson and Plumb, 1972).

Circulative (or persistent) viruses are those which have a cycle in their vectors. The virus is ingested, absorbed, translocated and finally reaches the salivary glands from where it is injected into the plant tissue via salivary secretions during feeding (Black, 1959). Circulative viruses are also characterized by a high virus-vector specificity, long retention of the virus in the vector and the presence

of a latent period which must be completed in the vector before the virus can be transmitted (Sylvester, 1969a). Some circulative viruses multiply in their vectors (Stegwee and Ponsen, 1958; Sylvester, 1969b) but others apparently do not (Sylvester and Richardson, 1966).

Semi-persistent viruses are intermediates between the non-persistent and persistent viruses. They have no latent period and are probably held more tenaceously by the stylets than the non-persistent viruses, for they are not lost or inactivated as readily (Sylvester, 1962).

Lettuce necrotic yellows virus (LNYV), whose transmission by aphids is the subject of this thesis, appears to be both circulative and propagative (Stubbs and Grogan, 1963; O'Loughlin and Chambers, 1967).

## 1.2 Economic importance of lettuce necrotic yellows virus

Lettuce necrotic yellows virus is a very destructive virus disease of lettuce (*Lactuca sativa* L.) which was first recognised by Stubbs and Grogan (1963). No varieties of commercially cultivated lettuce are known to be resistant to infection. Infected lettuce plants become chlorotic and show varying degrees of necrosis. Mortality is high; chronically infected survivors have small, distorted and unmarketable heads. The disease is widespread in Australia and it is also found in New Zealand (Randles and Carver, 1971).

LNYV is the most serious disease of lettuce in South Australia. Losses of over 50% have been reported in some crops. The disease

infects most plants during the spring and autumn. Crops sown in mid-September and between early February and late March stand the greatest risk of infection (Randles and Crowley, 1970).

## 1.3 Vectors of LNYV and their distribution

Symptoms shown by lettuce infected with either tomato spotted wilt virus (TSWV) or LNYV are similar. Early outbreaks of LNYV were probably all attributed to TSWV until the specific vector, *Hyperomyzus lactucae* (L.) of LNYV was discovered (Stubbs and Grogan, 1963). Randles and Carver, (1971) have reported that *H. carduellinus* (Theob.) (a closely related species) also transmits LNYV. They, however, stated that because of its limited distribution, *H. carduellinus* may be unimportant in the spread of the disease. Eastop (1961) regards *H. carduellinus* 'as only a tropical anholocyclic form of *lactucae*' and states further that 'typical *lactucae* has a holartic distribution and also occurs in Australia'. *H. lactucae* appears to be cosmopolitan (Hille Ris Lambers, 1949, Cottier, 1953; Eastop, 1958). The absence of parasites of *H. lactucae* in Australia suggests that it has migrated to or has been introduced into Australia without its attendant parasites (Starý and Schlinger, 1967; Starý, 1970).

## 1.4 Host plants of H. lactucae

In Europe *H. lactucae* overwinters in the egg stage on *Ribes* spp. and migrates to *Sonchus* spp. during the spring (Hille Ris Lambers, 1949).

In Australia it breeds throughout the year on sowthistle, *Sonchus oleraceus* (L.). Recently Randles and Carver (1971) observed that *H. lactucae* also breeds on *Embergeria megalocarpa* (Hook. f) Boulos, *Reichardia tingitana* (L.) Roth. and *S. hydrophilus* Boulos which have a more restricted distribution than *S. oleraceus*.

Hille Ris Lambers (1949) and Eastop (1958) agree that *Lactuca* is not a host plant of the aphid. The specific name was apparently given 'because Linné placed *Sonchus oleraceus* in *Lactuca*' (Eastop, 1958).

## 1.5 Origin of LNYV in Australia

Stubbs and Grogan (1963) considered LNYV to be an introduced disease because the only known reservoir plant (at the time), *S. oleraceus*, is presumed to be an introduced species. Randles and Carver (1971) reported natural infection in *S. hydrophilus* and *E. megalocarpa* both of which appear to be indigenous to Australasia. Because LNYV has only been found in Australasia and both *S. hydrophilus* and *E. megalocarpa* support colonies of *H. lactucae*, Randles and Carver (1971) proposed that LNYV was more likely to be endemic to Australasia.

## 1.6 Relationship between LNYV and other Rhabdoviruses

Sixteen groups of plant viruses have been described by Harrison *et al.*, (1971). Some of the plant viruses which do not fit into any of the sixteen groups have been grouped together because of their characteristic bacilliform or bullet-shaped particles. Hull (1970)

referred to these viruses as the Lettuce Necrotic Yellows Virus Group. The particles of this group of viruses show remarkable similarities to the vesicular stomatitis virus (VSV) and its relatives (Howatson, 1970) and are now often described as plant Rhabdoviruses.

About twelve plant viruses belong to the LNYV group (Howatson, 1970). Hull (1970) separated these viruses into two sub-groups on the basis of their biological and physical characteristics. The virus particles of the first sub-group e.g. sowthistle yellow vein virus (SYVV) are associated with the nucleus of the host plant and have a diameter of 75-93 nm. Those of the second sub-group e.g. LNYV have a smaller diameter (54-73 nm) and are restricted to the cytoplasm of the cell of the host plant. Francki (1973) has questioned the validity of Hull's (1970) sub-grouping of the LNYV group.

Most of the negatively stained virus particles viewed in the electron microscope appear to be bullet-shaped; in sections of infected plant or insect material they frequently appear to be bacilliform (MacLeod et al., 1966; Lee, 1967; O'Loughlin and Chambers, 1967; Wolanski et al., 1967; Hills and Campbell, 1968; Richardson and Sylvester, 1968). Several workers have suggested models for some of the viruses in this group. It is generally agreed that the particles consist of several coaxial layers. The outer membranous layer or envelope is made up of hexagonally packed hexagons (hexamers) and frequently has surface projections. Next to the envelope is a helical structure within which is a central channel (Kitajima and Costa, 1966;

Wolanski et al., 1967; Herold and Munz, 1967; Hills and Campbell, 1968; Wolanski and Chambers, 1972). The central channel may contain an inner core (Herold and Munz, 1967). Most members of the plant Rhabdoviruses have insect vectors (Hull, 1970) and aphids are known to be vectors for four of these viruses. These are SYVV, LNYV, strawberry crinkle virus (SCV) and broccoli necrotic yellows virus (BNYV) (Duffus, 1963; Stubbs and Grogan, 1963; Frazier, 1968; Tomlinson et al., 1972).

LNYV and SYVV show remarkable similarities in that they both infect sowthistle and lettuce, have the same vector and are not seed transmissible (Duffus, 1963; Stubbs and Grogan, 1963; Duffus et al., 1970). However, differences exist between them. For example, LNYV is sap transmissible whereas SYVV is not. They also appear to be unrelated serologically. Sowthistle is a symptomless carrier of LNYV but SYVV shows symptoms in this host plant (Francki, 1973). SYVV is known to occur in England and U.S.A. (Duffus, 1963; Duffus and Russell, 1969) and LNYV occurs in Australia and New Zealand (Randles and Carver, 1971).

## 1.7 Mode of LNYV transmission by *H. lactucae*

Following the recognition of the disease (Stubbs and Grogan, 1963), considerable work has been done on the physical and biological properties of the virus and on the physiology of infected *Nicotiana glutinosa* L. (e.g. Wolanski et al., 1967; Randles and Coleman, 1970; Francki and Randles, 1972; Wolanski and Chambers, 1972) but work on the epidemiology of the disease has been less extensive and many aspects have not yet been

studied (Stubbs *et al.*, 1963; Randles and Crowley, 1970; Randles and Carver, 1971).

An understanding of the epidemiology of a virus-vector system requires knowledge of both the transmission characteristics of the virus in the vector and the ecology of the vector. Two attributes of the LNYV system make it a useful one for studying the relationship of vector behaviour to disease epidemiology. The LNYV-host-vector system is a simple one therefore complications arising when several vector species and/or several plant host species are involved are virtually non-existent. One plant species, *S. oleraceus*, acts as the main source of virus for the principal vector (*H. lactucae*)(Randles and Carver, 1971). The LNYV-vector system appears to be unique in that the virus is transmitted in a persistent manner to lettuce, although the vector feeds only reluctantly on lettuce and never breeds on it. Because of this, secondary lettuce-lettuce spread of the virus does not complicate observations on the primary spread of the virus into the crop. Vectors of persistent viruses usually colonize both the economic and non-economic host plants of the viruses (Kennedy *et al.*, 1962).

The objective of the work described in this thesis was to investigate the epidemiology of LNYV in lettuce. The investigations were centred around:

1. The characteristics of transmission of the virus, including the acquisition of the virus from sowthistle, latent period, its subsequent inoculation to lettuce, and perpetuation of the virus in the

vector through transovarial transmission.

2. The feeding behaviour of the vector, particularly on lettuce which is a host plant of the virus but not of the vector.

3. Some of the factors involved in the dispersal of the vector from its host plant (S. oleraceus).

4. The relative importance of the morphological forms of the vector (apterae and alatae) in the spread of the disease in the field.

The results obtained have been used to describe the probable course of the development and build-up of vectors on sowthistle, their dispersal and spread of LNYV, and the conditions which would be expected to favour high disease incidence.

# 2. GENERAL MATERIALS AND METHODS

## 2.1 LNYV isolate

The virus isolate used was obtained from viruliferous aphids by feeding them on virus-free sowthistle seedlings. It was similar to the mild strain of LNYV described by Randles and Carver (1971) in that it produced slight stunting in *N. glutinosa* plants together with downward cupping and yellowing of the margin of young systemically infected leaves.

Partial purification of the virus isolate (McLean and Francki, 1967) was carried out to check the morphology of the virus particle. Infected *N. glutinosa* leaves were homogenized with 1.5 volumes of 0.02M $Na_2HPO_4$ at $0^oC$ and the extract was squeezed through muslin. Bentonite (0.4 gm) was added to the extract; it was shaken and centrifuged at 2000 g for one minute and the supernatant filtered through Celite and centrifuged at 40,000 g for 30 min. The pellet was resuspended in distilled water and a drop was placed on a carbon-coated grid and stained with 2% neutralized phosphotungstic acid (PTA). After removal of excess stain with a filter paper, the grid was air dried and examined in a Philips 100B electron microscope. The virus particles (Figure 2.1a) were similar to those described for LNYV by Wolanski *et al.*, (1967).

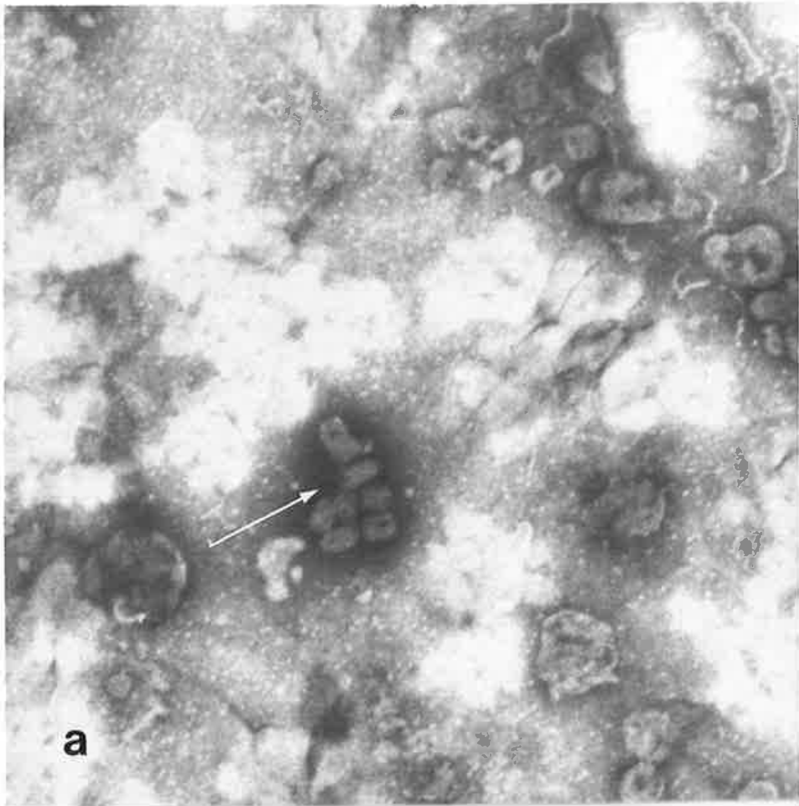
For testing serological relationships the above procedure was carried out except that the Celite filtration step was omitted. LNYV antigens were extracted from *N. glutinosa* plants infected with the

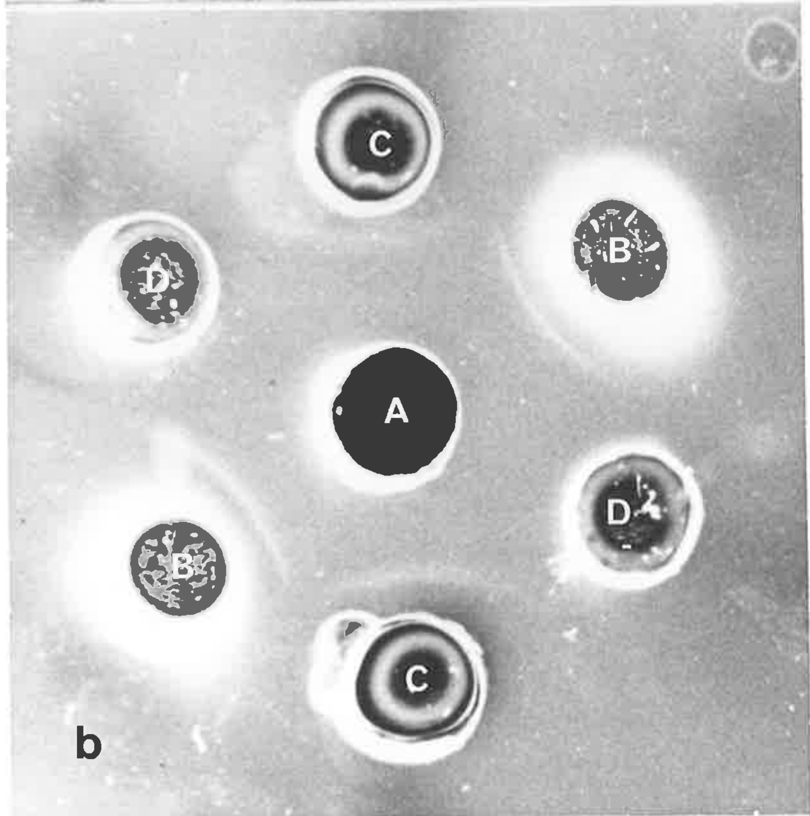
igure 2.1a Electron micrograph showing the typical bullet-shaped particles (arrowed) of the mild strain of LNYV used in this study.

Magnification: x 44,000

igure 2.1b Serological reactions by gel diffusion.

A, antiserum to LNYV (SE3 strain); B, concentrated LNYV (SE3strain); C, concentrated LNYV (mild strain); D, antigen prepared from healthy N. glutinosa.


a


C
D
B
A
D
B
C
b

SE3 isolate (Stubbs and Grogan, 1963) and the mild isolate. Concentrated antiserum (McLean et al., 1971) to the SE3 isolate of LNYV was used to examine the serological relationship between the mild and SE3 isolates. The serological test was carried out by the double-diffusion technique (Crowle, 1961) in 0.75% agar containing 0.01M phosphate buffer (pH 7.6) and 0.02% sodium azide. A positive reaction was observed between antigen concentrated from the mild strain isolate and the LNYV antiserum (Figure 2.1b). The precipitin line formed was confluent with that of the SE3 isolate indicating that the two isolates were closely related serologically. There was no reaction between antigen obtained from healthy N. glutinosa plants and the LNYV antiserum.

## 2.2 Raising and maintenance of sowthistle plants

Sowthistle seeds were germinated in earthen-ware pots and were transplanted into 4" plastic pots. The potted plants were kept in an aphid-proof glasshouse which was fumigated periodically with nicotine sulphate.

## 2.3 Transmission studies

For transmission studies sowthistle seedlings at the 3 to 4 leaf stage were used. Cages made from clear plastic vials measuring 1" in diameter and 3" high were used for confining aphids to sowthistle seedlings. Ventilation was provided by a gauze covered 3/4" diameter hole made in the bottom of the vials. After removal of aphids the

sowthistle seedlings were sprayed with 0.05% 'Hetraphos' (100% commercial hexaethyl tetraphosphate - Lane's Pty Ltd of Sydney) and kept in an aphid-proof glasshouse for incubation of the virus.

The sowthistle seedlings were indexed 1 to 1½ months after the test inoculation feeds. Three leaf discs 10 mm in diameter were cut from each of the six youngest leaves of the sowthistle plant with a cork borer and ground with mortar and pestle previously sterilized in boiling water. The cork borer was disinfected between plant inoculations by shaking it in 30% ethanol and wiping it dry with paper tissue. Two young N. glutinosa seedlings (4 to 5 leaf stage) were mechanically inoculated with sap from each sowthistle. Five to ten minutes after inoculation the seedlings were lightly watered to reduce mechanical damage arising from the inoculation procedure. Symptoms of infection appeared 6 to 10 days after inoculation. All plants were retained and observed for three weeks before they were discarded. The method of testing infection is designated 'indexing' and it is the term used throughout the thesis.

## 2.4 Establishment and maintenance of aphid colonies

A number of apterae viviparae of H. lactucae were collected from a wild sowthistle plant in Alverstoke orchard of the Waite Institute. One aphid was selected from the group and was placed on a sowthistle seedling in a double mesh-lined cage measuring 18" x 18" x 36" high to breed.

A colony of virus-free aphids designated 'first virus-free colony' was established from the progeny of this single apterous aphid. Decline in the transmission ability during preliminary transmission studies in aphids from this colony led to the establishment of another virus-free colony designated 'second virus-free colony'. The procedure used in raising the second virus-free colony will be described later.

### 2.41 Establishment of first virus-free colony

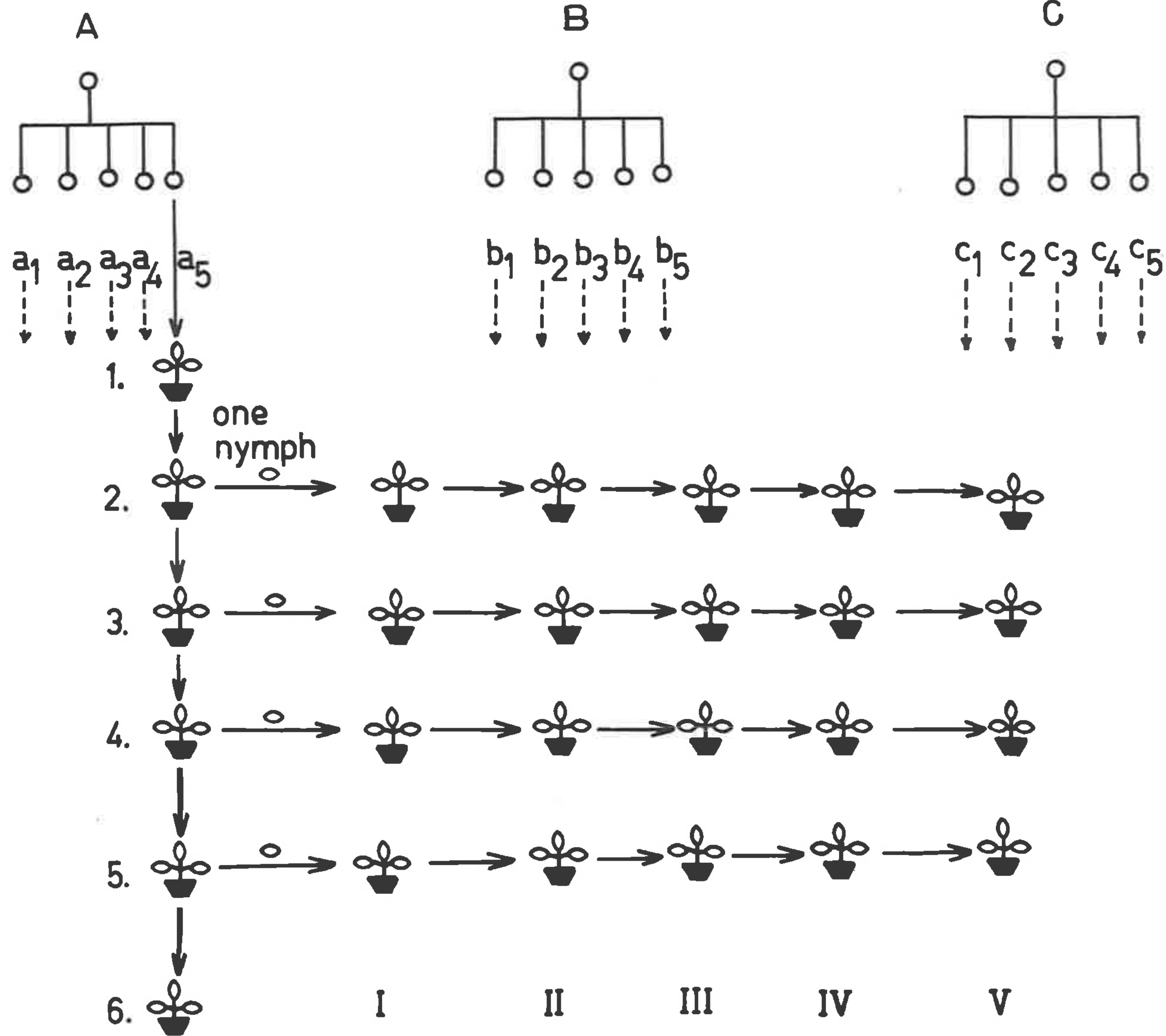
The procedure used to raise this colony is presented diagramatically in Figure 2.2. This method was adopted because reports of transovarial transmission of potato leaf roll virus (PLRV) by *Myzus persicae* (Sulz.) (Miyamoto and Miyamoto, 1966) and SYVV by *H. lactucae* (Sylvester, 1969b) indicated that precautions must be taken to ensure that aphids had a long history of non-transmission when attempting to establish a truly 'virus-free' colony of *H. lactucae*.

Three apterous aphids A, B and C (Figure 2.2) were collected from the aphid colony mentioned above. They were allowed to larviposit by confining each of them separately on a healthy sowthistle leaf in a petri dish for 24 hr. Five nymphs were taken from among the offspring of each of the aphids A, B and C and caged individually on healthy sowthistle seedlings until they started to reproduce. Starting four days later they were each transferred daily to healthy seedlings for six days. At each transfer one nymph was randomly selected from the set of progeny dropped each day, for four days by aphids $a_1$, $b_1$ and $c_1$ etc. These

Figure 2.2 Schematic representation of the production of the virus-free colony of *H. lactucae* from maternal aphids A, B and C.

Legend.

1. A, B and C represent the first generation maternal aphids and five nymphs ($a_1$-$a_5$, $b_1$-$b_5$ and $c_1$-$c_5$) selected from the set of progeny dropped within 24 hr.
2. Plants (designated by Arabic numerals) are sowthistle seedlings to which individual nymphs from the first generation maternal aphids (e.g. $a_1$, $b_1$, $c_1$ etc.) were serially transferred. The aphid remained on seedling 1 for the first 4 days of its reproductive life; on plants 2-5 for 24 hr and on plant 6 aphids were left to reproduce.
3. Plants (designated by Roman numerals) are seedlings to which one nymph out of the set of progeny of each second generation maternal aphid were transferred daily; on plant v the aphids were left to reproduce.



A
B
C
$a_1$ $a_2$ $a_3$ $a_4$ $a_5$
$b_1$ $b_2$ $b_3$ $b_4$ $b_5$
$c_1$ $c_2$ $c_3$ $c_4$ $c_5$
1.
one nymph
2.
3.
4.
5.
6.
I
II
III
IV
V

second generation nymphs were caged singly on healthy sowthistle seedlings and transferred daily to fresh seedlings for five days. The aphids were left to breed on the final seedling in both serial transfers. The aphid colonies were maintained by transferring five apterous aphids to start fresh colonies whenever the seedlings on which the aphids bred began to show signs of deterioration. The sowthistle seedlings used in the serial transfers were indexed. It was observed that maternal aphid B transmitted LNYV to one of its progeny and this in turn transmitted the virus to one of its offspring (see Chapter 3).

A virus-free colony was established by collecting a number of apterous _H. lactucae_ from virus-free populations (as indicated by indexing) and confining them on a single healthy sowthistle plant to larviposit. The maternal aphids were removed after 24 hr.

### 2.42 Establishment of first viruliferous colony

About 30 viruliferous apterous aphids were collected from the progeny of maternal aphid B (Section 2.41) which transmitted LNYV to establish a viruliferous colony by confining them on a virus-free sowthistle seedling. The colony was maintained by transferring apterous aphids periodically and confining them on healthy sowthistle seedling.

### 2.43 Second virus-free and viruliferous aphid colonies

Bjorling and Ossiannilsson (1958) reported that clones of _M. persicae_ obtained from single aphids differed in their ability to transmit PLRV.

However, there was no decline in the frequency of transmission of PLRV by active and moderately active strains. It is inferred that the decline in transmission ability of H. lactucae was not due to the fact that the colony was established from a single maternal aphid; some unknown factors were probably involved in the decline of transmissibility.

A slightly different procedure was used to raise the second virus-free colony because the precise cause of decline of LNYV transmission by the first viruliferous colony was not known. Consequently the number of maternal aphids used was enlarged to increase heterogeneity among the progeny.

Thirty five wild sowthistle plants growing in Alverstoke orchard were selected and labelled. Each plant was indexed and a total of 30 apterae viviparae of H. lactucae were collected (one per plant) from 30 plants which were infected. The aphids were placed into three groups of ten. Each group was caged on a healthy sowthistle seedling and the three colonies were maintained by removing 20 to 30 apterous aphids to fresh seedlings when the seedlings on which the aphids bred started to show signs of deterioration. The first set of sowthistle seedlings was indexed and two were found to be infected with LNYV. Aphids from the two infected colonies were combined and used as a viruliferous colony. To establish a virus-free colony, 12 apterous aphids were collected from the viruliferous colony and were confined singly on healthy sowthistle leaves contained in petri dishes. After 24 hr the offspring (5 to 8 nymphs) of each maternal aphid were collected and

confined as a set on healthy sowthistle seedlings and were transferred daily to fresh seedlings for six days. The aphids were allowed to breed on the sixth set of seedlings and the colonies were maintained as previously described. All the seedlings used in the serial transfers were indexed and none of them was positive for LNYV. A virus-free colony was established by collecting ten apterous aphids from each of the 12 colonies and confining them on a single healthy sowthistle plant for 24 hr to drop progeny. After this period the maternal aphids were removed.

The virus-free and viruliferous colonies were kept in separate compartments in a glasshouse. These colonies were checked periodically for the presence or otherwise of LNYV.

## 2.5 Behavioural studies

### 2.51 Desiccation and starvation of H. lactucae

To improve the settling behaviour of *H. lactucae* on lettuce the aphids were pre-conditioned by starving them in a dry environment. The required humidity was obtained with NaOH solution. Sodium hydroxide solution (SG: 1.507) was prepared and the concentration required for producing a relative humidity of 65 to 70% was made (Madge, 1961). Aphids to be treated were confined in clear plastic vials measuring 3/4" in diameter and 1 9/16" high. Ventilation was provided by ½" gauze covered holes in the bottom and lid of vials. Vials containing aphids were put on a metal gauze placed mid-way in the inside of a clear plastic

container measuring 3½" in diameter and 4½" high. NaOH solution (15 ml) contained in a beaker was placed beneath the metal gauze. The container was covered with a lid and kept in a constant temperature cabinet at 25°C in continuous light (intensity: 150 lux). Aphids which were subjected to such an environment for 24 hr are described hereafter as 'pretreated'. Humidity inside the container was checked by means of cobalt thiocyanate paper in a Lovibond comparator (Solomon, 1957).

2.52 Labelling of seedlings with $^{32}P$

Radioactively labelled sowthistle and lettuce seedlings were used to study sap uptake by *H. lactucae* feeding on sowthistle and lettuce seedlings. The seedlings were labelled by washing the roots free of soil and organic matter, rinsing with distilled water, and adding approximately 400 µCi of carrier free $^{32}P$ (as phosphate in HCl - Australian Atomic Energy Commission) directly to the drained roots (Matthews, 1960). Distilled water was added after 30 min to cover the roots. Aphids were confined on the seedlings 12 hr after labelling. Six leaf discs 2 mm in diameter were cut from the labelled seedlings with a cork borer to determine the amount of radioactivity (c.p.m./µl of sap) in the leaves. The leaf discs were ground and 1 ml distilled water was added to the extract and centrifuged in a low speed MSE bench centrifuge for 15 min at 1000 r.p.m. Half-inch squares were cut from filter paper and an aliquot of 50 µl of the supernatant was dropped on the filter paper squares. All samples were glued onto planchets and radioactivity

was counted in a Nuclear Chicago (Model C115) gas-flow counter.

## 2.6 Field studies

### 2.61 Establishment of lettuce crop in the field

Seeds of 'Gt Lakes' (winter variety) were mixed with blood and bone manure (at the rate of 2 cwt/acre) and drilled into the soil in rows 21" apart; within row spacing was one foot. Thinning and filling of empty spaces was carried out two weeks after the seeds had germinated. Weeds were first controlled with 'Dacthal' (Agchem Pty Ltd., S.A.) at the rate of 3 oz/600 sq ft; rye grass which appeared later was controlled with 'Gramexone' (I.C.I. Australia and New Zealand) at the rate of 1 pint/acre. The lettuce plants were fertilized with a compound fertilizer (NPK, 2:2:1) by sprinkling the fertilizer around each plant. Slugs were controlled with 'Defender' (active ingredient: 1.5% w/w metaldehyde - Defender Co. Pty Ltd, N.S.W.). Owing to the unusually dry autumn (1972) the lettuce plants were sprinkler irrigated. After the introduction of aphid-infested sowthistle plants the field was watered by means of a rose attached to a hose to prevent removal of aphids from the sowthistle plants by washing.

### 2.62 Establishment of aphid colonies on infected sowthistle plants

A number of mature potted sowthistle plants which were known to be infected with LNYV from a previous glasshouse experiment were used to raise viruliferous aphid colonies. The plants were put in a field cage

to acclimatize for a week. Ten to 15 apterous virus-free *H. lactucae* were confined on each plant and left to reproduce. Two weeks before the infested plants were put in the field, aphids on half the plants were thinned by removing alates and alatiform nymphs so that these plants had mostly apterous aphids. These plants were kept in a separate compartment. No thinning was carried out on the remaining plants which were to carry mostly alate aphids.

### 2.63 Spread of LNYV from virus-free sowthistle seedlings colonized by progeny of infected alate *H. lactucae* at the time of introduction into the crop

Healthy potted sowthistle seedlings of approximately the same age as the lettuce plants in the field (see Section 2.61 above) and which had been kept in an aphid-proof glasshouse were transferred to a double net-lined field cage for a week to acclimatize. Groups of 10 to 15 viruliferous alate *H. lactucae* obtained from the infected colony (see section 2.62) were caged on 22 of the seedlings 24 hr before some of these seedlings were put in the lettuce crop. The maternal aphids were removed from 12 of the seedlings after the 24 hr inoculation and larviposition period.

### 2.64 Determination of the period taken for LNYV in the inoculated sowthistle seedlings in section 2.63 to become available to the progeny of alate viruliferous *H. lactucae*.

The inoculation procedure was as above (see Section 2.63) except that

after the 24 hr inoculation feed both the maternal alate aphids and their progeny were removed from 10 of the 22 seedlings (see Section 2.63). The ten seedlings were confined in a cage measuring 6' x 4' x 3' high which was kept in Alverstoke orchard. Twenty four hours after the inoculation five virus-free apterous H. lactucae were caged on each seedling for 48 hr for larviposition. Five, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 days after the seedlings had been inoculated, two aphids were collected from each of the ten seedlings and caged on a single healthy sowthistle and kept in a glasshouse. The aphids were sprayed after two weeks and the seedlings were later indexed.

### 2.65 Trapping of apterous H. lactucae in lettuce plots (Alverstoke orchard)

To prevent the movement of apterous H. lactucae onto lettuce plants in the field, the predominantly alate-infested sowthistle plants (see Section 2.62) were surrounded by a galvanized sheet metal gutter (see Chapter 6) with an outside dimension of 7' 4" by 2' 4" wide. The gutter measured 2" deep and 2" wide. The structure was sunk into the soil with the rim level with the soil. The gutter was filled with water to a depth of 1½" and a few drops of detergent were added to the water.

### 2.66 Trapping of alate H. lactucae in lettuce plots (Alverstoke and Claremont orchards)

Circular metal aphid traps measuring 11" in diameter and 3" deep

were used for trapping immigrant alate H. lactucae (Moericke, 1951). The inside and outside of the traps were painted yellow and brown respectively (Dulux Canary Yellow and Dulux Rich Brown). The traps were filled with water to a depth of 1½" and a few drops of detergent were added to the water. Two traps, one on the soil and the other on a 7½" high brick, were placed in each plot.

2.67 Confirmation of LNYV in diseased lettuce plants

Symptoms of TSWV and LNYV are similar (Stubbs and Grogan, 1963). To confirm the presence of LNYV, diseased lettuce leaves were homogenized in 0.1% sodium sulphite solution containing 1 ml of 0.1M $Na_2HPO_4$ buffer (pH 7) at $0^oC$ (Best, 1968). The extract was inoculated onto N. glutinosa and White Burley tobacco (Bald and Samuel, 1931) which act as differential indicators for the two viruses.

# 3. CHARACTERISTICS OF THE TRANSMISSION OF LNYV BY H. LACTUCAE

## 3.1 Introduction

Knowledge of virus-vector relationships is essential for the understanding of epidemiology of plant virus diseases. Posnette (1960) suggested that with persistent aphid-borne viruses, disease spread is influenced by the latent and retention periods of the viruses in their vectors. Migratory behaviour also determines the distance over which a vector is capable of spreading a disease. The period over which alate aphids may actively spread viruses is restricted to the first few days of adult life, i.e. before the flight muscles autolyse (Johnson, 1957), and there is circumstantial evidence that most aphids migrating are on their first, or early flight (Johnson and Taylor, 1957; Taylor, 1958). Thus transmission of persistent aphid-borne viruses by alates probably depends partly on the ability of aphids that have developed on infected plants being infective on their first flight. This also applies to dispersive apterous aphids which leave infected plants by walking. Perpetuation of insect-borne viruses may depend, at least in part, on being transovarially transmitted by their vectors (Fukushi, 1933 and 1939; Black 1950).

This chapter describes some of the characteristics of the transmission of LNYV including (1) latent period and retention of virus in _H. lactucae_; (2) ability of some morphs and stages of _H. lactucae_ which have developed on infected sowthistle plants to transmit the virus; (3) transovarial transmission and (4) inoculation thresholds of LNYV

in lettuce and sowthistle seedlings when inoculated by viruliferous *H. lactucae*.

## 3.2 Latent period of LNYV in *H. lactucae* and its dependence on temperature

One of the most important characteristics of persistent insect-borne plant viruses is the occurrence of a latent period within their vectors. The latent period is defined as the interval between virus acquisition and the development of inoculativity (Sylvester, 1965). Because of the difficulty of defining these times some German workers (Heinze, 1959) have used the term'celation' which is the interval from the beginning of acquisition to the end of the test access period during which transmission occurs. Recently, Sylvester (1965) has advocated the use of the median latent period ($LP_{50}$) in which a log-probit transformation is used to estimate the time when 50% of vectors that would eventually transmit a virus, had completed their latent period.

Stubbs and Grogan (1963) reported that *H. lactucae* transmit LNYV in a persistent manner, but the duration of the latent period of the virus in its vector was not determined. So an experiment was carried out to find the latent period of LNYV in *H. lactucae* and the effect of ambient temperature on its duration. The latent period was estimated according to the definition of Heinze (1959).

Virus-free apterous *H. lactucae* were caged on a healthy sowthistle seedling for 24 hr to larviposit and the maternal aphids were removed.

Twenty four hours later the nymphs were confined on a single infected sowthistle plant for an acquisition feed of 24 hr in a constant temperature room at 25°C and under continuous light. Three groups of 28 healthy sowthistle seedlings were selected and a nymph was caged on each seedling. Groups of 28 seedlings were put in plant growth chambers maintained at 15°, 20° or 28°C with a 14 hr day-length. The aphids were transferred serially to healthy seedlings at two-day intervals until they died.

The efficiency of transmission of LNYV by the aphids was low; 28 out of the 84 used transmitted the virus. Table 3.1 shows the latent period of the virus in single H. lactucae and the mean latent period (with their standard errors) at the three temperatures. After a 24 hr acquisition feed the aphid could retain the virus for as long as 53 days at 15°C. The latent period is temperature dependent; it ranges from 5 days at 28° to 27 days at 15°C (Table 3.1). On completion of the latent period the aphids transmitted LNYV consistently except in some instances when they failed to transmit the virus just prior to death.

To determine whether LNYV had any effect on the longevity of H. lactucae, longevity (estimated from the time of virus acquisition to death of aphid) of aphids which transmitted and those that failed to transmit LNYV were compared for each of the three temperatures. Aphids which were accidentally lost (Table 3.1) in the course of the experiment were not included in the analysis. A 't-test' was applied to the data to compare differences in longevity. Table 3.2 shows the mean

Table 3.1 Latent period in days of LNYV (at various temperatures) in transmitting *H. lactucae* which were given acquisition feeds of 24 hours commencing 24 hr after birth.

| Temperature (°C) | Days after virus acquisition | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1-3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | 19 | 21 | 23 | 25 | 27 | 29 | 31 | 33 | 35 | 37 | 39 | 41 | 43 | 45 | 47 | 49 | 51 | 53 | 55 | 57 |
| | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | D | | | | |
| | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | D | | |
| 15 | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | D | | |
| | - | - | - | - | - | + | + | + | + | + | + | + | + | L | | | | | | | | | | | | | | |
| | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | o | + | + | + | + | + | + | + | + | - | D |
| | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | D | | | | |
| | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | D | | | | | | | | |
| | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | D | | | | |
| | - | - | - | - | - | + | + | + | + | + | + | o | + | + | + | + | + | + | + | + | + | - | D | | | | | |
| | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | o | + | + | + | + | + | + | D | | | |
| | - | - | - | - | + | + | + | + | + | + | + | + | + | + | D | | | | | | | | | | | | | |
| | - | - | - | - | + | + | + | + | + | + | + | + | - | D | | | | | | | | | | | | | | |
| 20 | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | D | | | | | | | | | | | | |
| | - | - | - | + | + | + | + | + | D | | | | | | | | | | | | | | | | | | | |
| | - | - | - | + | + | + | + | + | + | + | + | + | + | - | D | | | | | | | | | | | | | |
| | - | - | - | + | + | + | + | + | + | + | + | + | + | D | | | | | | | | | | | | | | |
| | - | - | - | + | + | + | + | + | + | + | D | | | | | | | | | | | | | | | | | |
| | - | + | + | + | + | + | + | + | + | + | + | + | + | + | D | | | | | | | | | | | | | |
| | - | + | + | + | + | L | | | | | | | | | | | | | | | | | | | | | | |
| | - | - | + | + | + | D | | | | | | | | | | | | | | | | | | | | | | |
| | - | + | + | + | + | - | D | | | | | | | | | | | | | | | | | | | | | |
| | - | + | + | + | + | - | D | | | | | | | | | | | | | | | | | | | | | |
| 28 | - | + | + | + | + | - | D | | | | | | | | | | | | | | | | | | | | | |
| | - | + | + | + | + | D | | | | | | | | | | | | | | | | | | | | | | |
| | - | + | + | + | D | | | | | | | | | | | | | | | | | | | | | | | |
| | - | + | + | + | + | - | D | | | | | | | | | | | | | | | | | | | | | |
| | - | - | + | + | + | + | + | D | | | | | | | | | | | | | | | | | | | | |
| | - | + | + | + | + | - | D | | | | | | | | | | | | | | | | | | | | | |

o Test seedling died
+ Test seedling infected
- Test seedling not infected
L Aphid lost
D Aphid died

Mean latent period at (a) 15°C = 18.0 ± 5.2 days
(b) 20°C = 9.2 ± 1.8 days
(c) 28°C = 5.4 ± 0.8 days

longevity (with their standard errors) of transmitting and non-transmitting aphids. The results indicate that non-transmitting aphids lived longer than the transmitting ones; at 20° and 28°C the differences were significant ($P < 0.01$). Longevity appears to be inversely proportional to temperature.

## 3.3 Transmission of LNYV by some stages and morphs of *H. lactucae*

An experiment was carried out to find out if 4th instar nymphs, adult apterae and alatae showed any differences in their ability to transmit LNYV when they were allowed to develop on an infected sowthistle plant.

Table 3.2 Comparison of longevity of *H. lactucae* transmitting and not transmitting LNYV in the latent period studies (at 15°, 20° and 28°C) shown in Table 3.1.

| Type of aphid | Longevity (in days) at the three temperatures | | |
|---|---|---|---|
| | 15° | 20° | 28° |
| Transmitting aphids | 49.1 ± 1.5(9)[a] | 27.0 ± 1.6(8)** | 13.2 ± 0.5(9)** |
| Non-transmitting aphids | 51.8 ± 1.8(13) | 36.9 ± 0.7(16) | 16.1 ± 0.4(14) |

[a] Figure in parenthesis is the sample size.

** $P < 0.01$

Forty apterous *H. lactucae* from a virus-free colony were caged on a healthy sowthistle seedling for 24 hr. This seedling was later indexed to confirm that the aphids were virus-free. The aphids were then

caged for 24 hr on an infected sowthistle plant to larviposit. The progeny were left to develop on the infected plant in a constant temperature room at 25°C under continuous light. Within 24 or 48 hr of reaching the 4th instar, and within 24 hr of reaching adult apterous or alate stages, ten aphids were collected and caged individually on healthy seedlings. They were transferred serially to healthy seedlings at daily intervals for four days. All test inoculated seedlings were indexed.

The results (Table 3.3) show that the 24 hr old 4th instar nymphs transmitted LNYV at a low rate. Adult apterae, alatae and 48 hr old 4th instar nymphs transmitted with higher efficiency. It appears that apterous and alate _H. lactucae_ which had developed on an infected sow-thistle plant are capable of transmitting LNYV within 24 hr of reaching this stage. The 4th instar nymphs are more likely to transmit efficiently if left for 48 hr after reaching this stage.

Table 3.3 The effect of stage of development of _H. lactucae_ on the efficiency of transmitting LNYV when aphids had developed on an infected sowthistle plant.

| Stage & morph of aphid | Days after commencement of LNYV acquisition | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 | 10 |
| 4th instar (24 hr)[a] | 1/8 | 1/8 | 2/8 | 3/8 | - | - |
| " (48 hr)[a] | - | 4/10 | 6/10 | 7/10 | 7/10 | - |
| Adult aptera | - | 4/10 | 6/10 | 6/10 | 6/10 | - |
| Adult alate | - | - | 7/10 | 7/10 | 7/10 | 7/10 |

[a] The time in parenthesis represents the age of nymph at commencement of inoculation feed on test sowthistle seedlings.

## 3.4 Transovarial transmission of LNYV by *H. lactucae*

When establishing the first virus-free colony from the three maternal aphids (see Chapter 2), it was observed that maternal aphid B transmitted LNYV to one of its progeny and this in turn transmitted the virus to one of its offspring (Figure 3.1a). Though the maternal aphids were not tested for infectivity it could be assumed that aphid B was infective. It therefore appears that *H. lactucae* is capable of transmitting LNYV transovarially through at least two generations.

An experiment to confirm this was carried out by taking twenty apterous aphids from a viruliferous colony, caging them individually on healthy sowthistle seedlings and transferring them at daily intervals to healthy seedlings for four days. Sets of progeny produced during the 24 hr periods were removed, counted and caged as a set on healthy seedlings. They were transferred at two-day intervals to six sets of healthy seedlings; at each transfer the nymphs in each set of progeny were counted. Both the seedlings on which the maternal aphids dropped progeny and those on which the progeny were given inoculation feeds were indexed. The experiment was carried out in a glasshouse the temperature of which varied between 18° and 28°C.

The twenty maternal aphids produced 73 sets of progeny comprising 379 individuals. The proportions of maternal aphids surviving, reproducing (including the average number of nymphs per set) and transmitting LNYV are shown in Table 3.4. Out of the 20 maternal aphids four (W, X, Y and Z) or 20% transmitted LNYV to five sets of progeny

(Figure 3.1b and Table 3.5). Of the 73 sets of progeny, five sets transmitted the virus (apparently acquiring the virus from their parents) or 6.8% of the groups of progeny were able to transmit LNYV.

Table 3.4 Proportions of apterous maternal *H. lactucae* surviving, reproducing and transmitting LNYV when transferred serially at daily intervals to sowthistle seedlings.

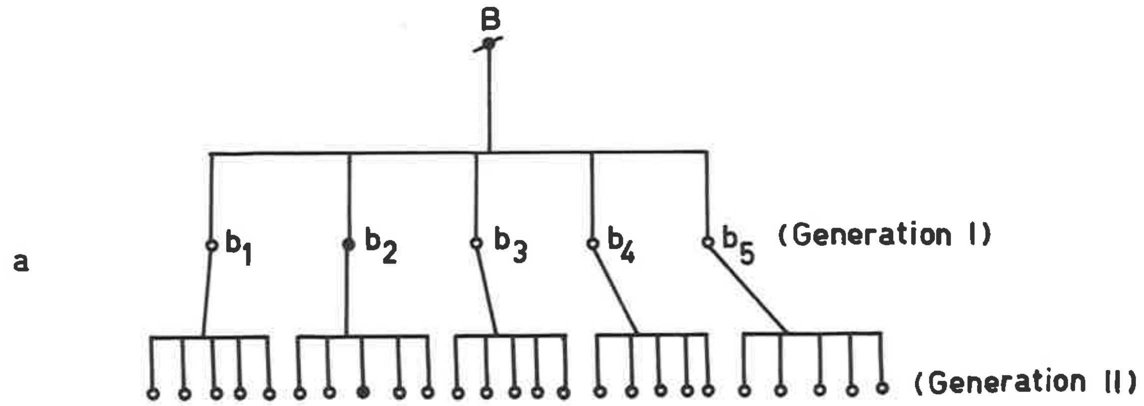
| | Serial transfers at daily intervals | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Proportion of aphids surviving | 20/20 | 20/20 | 20/20 | 17/20[a] |
| " " " reproducing | 19/20 | 20/20 | 18/20 | 16/17[a] |
| " " " transmitting | 13/20 | 18/20 | 17/20 | 14/17 |
| Average progeny/set | 4.8 | 5.9 | 5.3 | 4.5 |

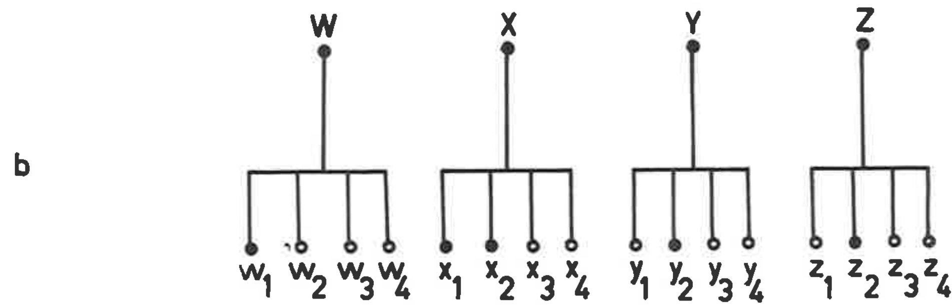
[a] The numerator of row 1 of the last column is the number of surviving maternal aphids at the 4th day of serial transfer so that the denominator of row 2 last column represents the number of aphids present at the final serial transfer.

Three sets of progeny ($w_1$, $x_1$ and $x_2$) transmitted LNYV to the first set of seedlings and transmitted almost consistently to the remaining sets of seedlings to which they were transferred serially. One set of progeny ($y_2$) transmitted only to the second set of seedlings and another ($z_2$) transmitted only to the fourth set of seedlings (Table 3.6). Maternal aphid Y failed to transmit the virus to the first set of seedlings and the set of progeny ($y_1$) dropped on this seedling also failed to acquire the virus (Tables 3.5 and Figure 3.1b). When this maternal

Figure 3.1a Incidence of transovarial transmission of LNYV in *H. lactucae* during the production of the first virus-free colony of aphids.

Figure 3.1b Confirmation of transovarial transmission in *H. lactucae*.




aphid not tested for infectivity

• infective aphid

∘ non-infective aphid

Table 3.5 Infection of sowthistle seedlings with LNYV during daily serial transfers by maternal aphids W, X, Y and Z.

| Maternal aphid | Serial transfers at daily intervals | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| W | + | + | + | + |
| X | + | + | + | + |
| Y | - | + | o | + |
| Z | + | + | + | + |

+ Test seedling infected
- " not infected
o " died

aphid transmitted LNYV to the second set of seedlings, the progeny ($y_2$) also transmitted the virus 3 to 4 days after birth. This suggests that transovarial passage of the virus occurs when the maternal aphid is transmitting the virus (probably after completion of the latent period cf. Sylvester (1969b)). Although maternal aphid Z was infective when it dropped progeny $z_2$ (Table 3.5) this set could only transmit LNYV to the fourth set of sowthistle seedlings (Table 3.6) i.e. 8 days after birth. A similar phenomenon has been reported for transovarial transmission of SYVV by H. lactucae (Sylvester, 1969b). It appears therefore that in some instances when H. lactucae acquires LNYV transovarially the latent period must probably be completed in the aphid before the virus can be transmitted.

Table 3.6 Infection of sowthistle with LNYV by sets of progeny from maternal aphids W, X, Y and Z (Figure 3.1b).

| Set of progeny | Serial transfers at 2-day intervals | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $w_1$ | +(5)[a] | +(5) | +(5) | -(5) | +(5) | o(5) |
| $x_1$ | +(4) | +(4) | +(4) | -(4) | +(4) | +(4) |
| $x_2$ | +(3) | +(3) | +(3) | +(3) | +(3) | +(3) |
| $y_2$ | -(6) | +(6) | -(6) | -(6) | -(6) | -(5) |
| $z_2$ | -(5) | -(4) | -(4) | +(4) | -(4) | -(3) |

\+ Test seedling infected
\- " not infected
o " died

[a] Figures in parentheses represent number of nymphs making up the set; column 1 shows the numbers of nymphs in the set dropped by the maternal aphids during the 24 hr larviposition period.

These results indicate that when transovarial transmission occurred the nymphs usually transmitted within 48 hr of birth (except in the case of $y_2$ and $z_2$ above) suggesting that the latent period must have been completed at or shortly after birth. To show that this was so, an experiment was carried out to determine the latent period in first instar nymphs. A number of apterous H. lactucae were collected from a virus-free colony and caged on a healthy sowthistle seedling for 24 hr. This seedling was later indexed to confirm that the aphids were virus-free.

The aphids were then caged on an infected sowthistle plant (in a plant growth chamber maintained at 28°C and with a 14 hr day-length) and allowed to larviposit for 4 hr. Twenty hours later 12 groups of first instar nymphs (five nymphs in a group) were caged separately on healthy sowthistle seedlings and kept in the plant growth chamber. The aphids were transferred daily to healthy seedlings for 7 days; on the 7th day the aphids were left on the seedlings for 5 days and then killed. The test seedlings were indexed.

Table 3.7 Latent period (in days) of LNYV in groups of first instar *H. lactucae* maintained at 28°C.

| Group No. | Days after acquisition | | | | |
|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7-13 |
| 1 | - | - | - | - | + |
| 2 | - | + | + | + | + |
| 3 | - | + | + | + | + |
| 4 | - | + | + | + | + |
| 5 | - | + | + | + | + |
| 6 | - | - | + | + | + |
| 7 | - | - | + | + | + |
| 8 | - | - | + | + | + |
| 9 | - | + | + | + | + |
| 10 | - | + | + | + | + |

+ Test seedling infected

- " " not infected

Ten out of the 12 groups of nymphs transmitted LNYV and the average latent period was 4.3 ± 0.5 days which is about a day shorter than in the previous experiment (Table 3.1). This difference is probably due to the fact that in the present experiment the aphids were serially transferred to fresh seedlings at daily intervals in contrast to two-day intervals in the previous experiment. Occurrence of latent period in this experiment suggests that the nymphs which transmitted LNYV in the transovarial transmission experiment could not have acquired or transmitted the virus from seedlings on which they were born.

3.5 Inoculation threshold of LNYV in lettuce seedlings

When H. lactucae are transferred directly from sowthistle plants to lettuce they generally fail to probe and walk off the lettuce plant. However, apterous aphids which have been pretreated by starvation in continuous light and a dry environment for 24 hr are more prone to probe (see Chapter 4). Consequently, in transmission studies on lettuce, the aphids were subjected to the above treatment before such studies were made.

A preliminary trial was conducted to find out whether pretreatment (i.e. subjecting aphids to continuous light and dry environment for 24 hr) affected the efficiency of transmission of LNYV by viruliferous H. lactucae. Six groups of apterous aphids (5 to 10 in a group) were collected from a viruliferous colony and were caged separately on healthy sowthistle seedlings for 24 hr. They were pretreated for 24 hr and caged on healthy lettuce seedlings for 2 hr and transferred to a

set of six healthy sowthistle seedlings for a further 2 hr. Because the two sets of sowthistle and the set of lettuce seedlings were all infected with LNYV it appeared that the pretreatment did not affect the ability of viruliferous H. lactucae to transmit LNYV.

Inoculation threshold on lettuce was estimated by allowing pre-treated viruliferous apterous H. lactucae to feed for 30 sec., 1 and 5 min on lettuce seedlings. The aphids (ten for each feeding time) were gently dropped on the test seedlings with a micro-aspirator. The feeding was timed as soon as the aphid's labium (rostrum) was adpressed to the lettuce leaf. The observation was made with a 10x hand lens and feeding was terminated by removing the aphids from the test seedlings. The seedlings were indexed and the inoculation threshold was found to be between 1 and 5 min (Table 3.8).

Table 3.8 Inoculation threshold of LNYV in lettuce seedlings.

| Feeding time (minutes | 0.5 | 1 | 5 |
|---|---|---|---|
| Proportion of seedlings infected | 0/10 | 0/10 | 1/10 |

Another experiment was carried out to determine the effect of duration of inoculation feed on transmissibility of LNYV by H. lactucae to lettuce. To check whether the aphids were infective, 110 apterous aphids from a viruliferous colony were caged singly on healthy sowthistle seedlings for 24 hr. After this period they were pretreated for 24 hr. The aphids were then dropped gently on healthy lettuce seedlings, observed and

timed as previously described. The feeding times were 5, 15 and 30 min, 1 and 2 hr. For the 5, 15 and 30 min feeds the rostrum was observed continuously; in the 1 and 2 hr feeds the observation was continuous for the first 30 min and thereafter at 15 min intervals. The test sowthistle and lettuce seedlings were indexed.

Table 3.9 Proportion of apterous H. lactucae transmitting LNYV to sowthistle after a 24 hr inoculation feed and proportion of lettuce infected at various feeding times.

| | | | | | |
|---|---|---|---|---|---|
| Proportion of aphids infecting sowthistle | 5/13[a] | 5/13 | 10/16 | 8/16 | 6/15 |
| Feeding time on lettuce (in min) | 5 | 15 | 30 | 60 | 120 |
| Proportion of aphids infecting lettuce | 1/5 | 1/5 | 6/10 | 6/9[b] | 3/6 |

[a] The numerator represents number of aphids that transmitted LNYV to sowthistle seedlings out of the number (denominator) which were given an inoculation feed of 24 hr on the seedlings.

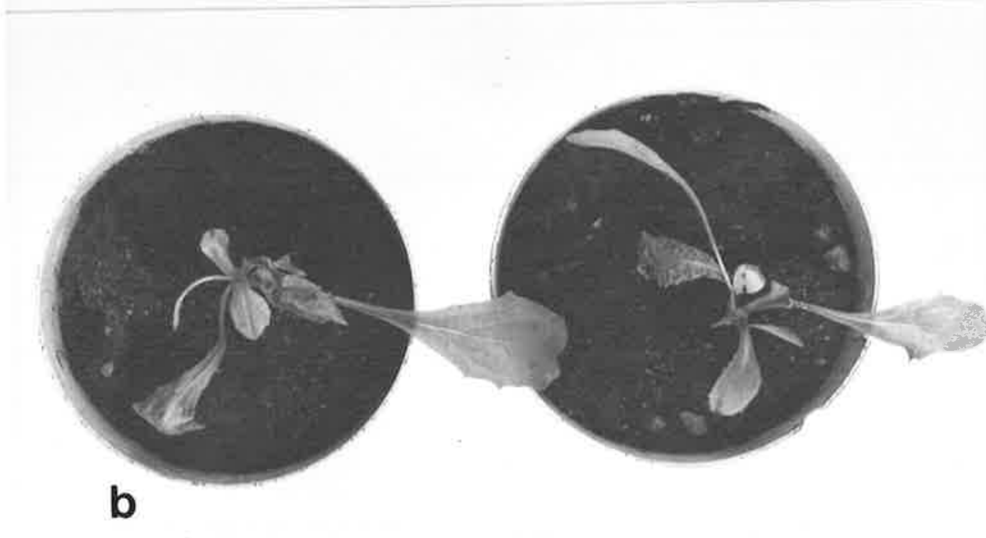
[b] One aphid which failed to transmit the virus to sowthistle seedling transmitted the virus to a lettuce seedling; the numerator represents number of aphids that transmitted LNYV to lettuce out of the number (denominator) of viruliferous aphids.

The results (Table 3.9) show that after the 30 min inoculation feed transmission of LNYV to lettuce reached a plateau. It appears that up to 30 min the longer the feed the greater are the chances for the transmission of LNYV to lettuce. Lettuce seedlings which were

gure 3.2a Symptoms on LNYV-infected lettuce seedlings inoculated by viruliferous H. lactucae (upper seedlings are healthy and the same age as the lower LNYV-infected seedlings).

gure 3.2b A close-up of LNYV-infected lettuce seedlings.

a


b

successfully inoculated during 5 and 15 min feeds showed no symptoms. Symptoms appeared between 8 and 15 days in lettuce seedlings on which inoculation feeds lasted longer than 15 minutes. The symptoms were stunted growth and mottling in the young leaves (Figure 3.2).

### 3.6 Inoculation threshold of LNYV in sowthistle seedlings

An experiment was carried out to assess the effect of duration of inoculation feed on rate of transmission of LNYV to sowthistle seedlings. Sylvester (1969a) stated that in the persistent type of virus-vector relationship there is a positive correlation between the duration of inoculation feed and the probability of virus transmission.

Inoculation feeding periods were 5 and 30 min, 1, 2, 4, 6, 12, 24 and 48 hr. Apterous *H. lactucae* were collected from a viruliferous colony, starved for 4 hr in the laboratory and gently dropped on the test seedlings as described previously (see Section 3.5). Ten aphids were used for each feeding period. The aphids were observed continuously in the 5 and 30 min feeds and periodically for the longer times. On completion of the inoculation feed the aphids were removed.

Table 3.10 Proportion of apterous viruliferous *H. lactucae* transmitting LNYV to sowthistle seedlings after various inoculation feeding times.

| Feeding time | 5[a] | 30[a] | 1 | 2 | 4 | 6 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|---|---|---|
| Prop. of infecting sowthistle | 0/10 | 2/10 | 3/10 | 4/10 | 4/10 | 5/10 | 8/10 | 6/9 | 6/10 |

[a] Time in minutes, rest in hours.

The results (Table 3.10) show that up to 12 hr the longer the inoculation feed the greater the rate of transmission. The inoculation threshold lies between 5 and 30 min.

## 3.7 Discussion

Stubbs and Grogan (1963) suggested that LNYV is transmitted by *H. lactucae* in a persistent manner because they showed the occurrence of a latent period, retention of the virus through a moult and persistence of inoculativity. In addition to confirming the above findings, the present study shows that: (1) most adult apterae, alatae and 4th instar nymphs which have developed on infected sowthistle become infective; (2) transmission of LNYV to lettuce can be achieved through short probes by pretreated viruliferous aphids; (3) duration of the latent period is temperature dependent; (4) the probability of infection of sowthistle and lettuce seedlings by *H. lactucae* is increased by longer inoculation feed; (5) transovarial transmission of LNYV occurs in *H. lactucae*.

Knowledge of the parameters of transmission allows an understanding of the epidemiology of LNYV. Adult apterous and alate *H. lactucae* which have developed on an infected sowthistle plant are capable of transmitting the virus within 24 hr of reaching this stage (see Section 3.3). Thus migrating alatae or dispersive apterae which have developed on infected sowthistle can transmit the disease to lettuce when these plants are encountered. Nymphs of the aphid are unlikely to be important as they will not be infective at least until the 4th instar. Furthermore, the

limited dispersal of the nymphs from sowthistle plants (see Chapter 5) places a further restriction on their ability to spread the disease.

Though short probes by viruliferous H. lactucae are sufficient for the transmission of LNYV to lettuce (see Section 3.5), it appears that the longer the probe the higher the probability of the inoculated plants showing symptoms. Apparently symptom manifestation in inoculated lettuce may be partly influenced by the quantity of inoculum injected into the plant. Under field conditions where multiple infection is more likely, particularly at times of peak flight (Randles and Crowley, 1970), lettuce plants may receive greater amounts of inoculum which may enhance the appearance of symptoms

Black (1959) suggested that the duration of latent period of a virus in its vector is a reflection on the time taken by the ingested virus to be translocated to the salivary glands. Evidence for this has been reported recently by Sinha and Chiykowski (1969) who demonstrated that the time taken (about 8 days) by wheat striate mosaic virus (WSMV) to reach a maximum level in the salivary glands of the leafhopper Endria inimica (Say), coincided approximately with the maximum transmission obtained with insects which acquired the virus from infected plants. Dependence of the duration of latent period on ambient temperature has been reported for other aphid vectors of plant viruses. Within the range of tolerance, high temperatures shorten the duration of the latent period (Osborn, 1935; Heinze, 1959; Duffus, 1963; Sylvester, 1965). The occurrence of this phenomenon is generally regarded as evidence for

virus multiplication in its vector (Sylvester, 1969a). Black (1959) has, however, indicated that the most convincing evidence for multiplication can be obtained by serial passage of viruliferous haemolymph from insect to insect by injection until the dilution obtained exceeds the maximum dilution of the inoculum.

Reduced longevity in H. lactucae which transmitted LNYV (see Section 3.2) indicates a possible pathogenic effect of the virus on the aphid. Under the present investigation it is not certain whether the non-transmitting aphids were in fact virus-free. However, there are instances in which other plant viruses are known to have deleterious effects (e.g. reduction in longevity on their vectors (Sylvester and Richardson, 1969)).

In the transmission of aphid-borne plant Rhabdoviruses like SCV (Frazier, 1968), SYVV (Duffus, 1963) and LNYV in the present study (see Section 3.2) there is a decrease in transmission of the viruses by the vectors 1 to 4 days prior to death. Prior to this period, and immediately after the completion of the latent period, the percentage transmission reaches a plateau level. Paliwal (1968) presented evidence that E. inimica which completely failed to transmit WSMV by the 62nd day after virus acquisition (though they were transmitting prior to this period) still contained virus and suggested that failure to transmit was probably due to formation of a 'barrier' as the insect ages. Similarly, Sylvester (1967) reported that the decline in the rate of transmission of the more persistent isolates of pea enation mosaic virus (PEMV) by Acyrthosiphon pisum (Harris) was correlated with a general reduction in

the metabolic activity of the vector as measured by rates of reproduction and excretion. Whether the failure of H. lactucae to transmit LNYV prior to death is due to reluctance to feed, a decrease in or cessation of saliva production, or a barrier with age (Paliwal, 1968) is not known.

Until recently transovarial transmission of aphid-borne persistent viruses was regarded as non-existent. Miyamoto and Miyamoto (1966) were the first to report transovarial passage of PLRV in the aphid, M. persicae. Sylvester (1969b) has also shown that SYVV is transovarially transmitted by H. lactucae. In both cases a low percentage of transovarial passage was reported. In leafhopper and planthopper-borne viruses e.g. rice dwarf (Fukushi, 1933); clover club-leaf (Black, 1950), rugose leaf curl (Grylls, 1954) and WSMV (Slykhuis and Watson, 1958), the percentage transmission of the viruses to progeny is high (40 to 90%). However, Black (1953) reported low transovarial transmission in two other leaf-hopper-borne viruses; female Agalliopsis novella (Say) transmitted wound tumor virus (WTV) to about 1.8% of their progeny and Agallia constricta (Van Duzee) could pass the New Jersey strain of potato yellow dwarf virus (PYDV) to about 0.8% of its progeny.

Leafhopper-borne viruses are passed through successive generations of the vector (Fukushi, 1939). This has been considered as evidence for multiplication of the virus in the vector (Black, 1950) probably on the assumption that the virus is uniformly distributed in the vector.

Many insects including the Homoptera have bacterium-like or yeast-like organisms which are confined to specialized cells or mycetocytes.

The mycetocytes may be aggregated into a mycetome or may be separated and scattered (Wigglesworth, 1939). It is generally believed that these micro-organisms are symbionts and may be metabolically important in their host because of (1) their constant occurrence in a given species; (2) the existence of elaborate mechanisms for their hereditary transmission; (3) the fact that mycetomes develop in readiness before they become invaded (Wigglesworth, 1939).

In the parthenogenetic phase of the reproductive cycle of aphids there is a single maturation division (Blochmann, 1887 cited in Hagan, 1951). Symbionts are incorporated into the egg after the maturation division. During cleavage, nuclei which eventually develop into the blastoderm move to the surface of the cytoplasm (periplasm) which surrounds the yolk to form a syncytium (Uichanco, 1924; Toth, 1933). It is generally believed that the mycetoblasts, which later develop into mycetomes, are formed by the internal migration of daughter nuclei from the periplasm (Hagan, 1951). As cleavage progresses the periplasm of the posterior pole of the egg becomes progressively thinner until it finally disappears and forms a blastopore (Uichanco, 1924; Tóth, 1933). Mycetocytes of maternal origin invade the egg through the blastopore at the posterior pole and pour their contents into the egg (Tóth, 1933). Thus the maternal symbionts become incorporated in the developing mycetocytes.

It would appear that transovarial transmission of viruses involves association of virus particles with mycetomes which migrate into the

developing egg. Nasu (1965) presented evidence in support of this in his electron microscopic studies of the transovarial transmission of rice dwarf virus by the leafhopper, Nephotettix cincticeps Uhler. He reported the presence of virus particles in the surface membrane of one type of symbiont (L-symbiote) in the mycetocytes of oocytes and in various organs and concluded that the virus entered the egg through the migration of symbionts which carried virus particles. A similar mechanism possibly occurs in the transovarial passage of LNYV in H. lactucae. O'Loughlin and Chambers (1967) demonstrated the occurrence of LNYV particles in several organs including the mycetomes of viruliferous H. lactucae but not in the developing embryo. Possible explanations of the low rate of transovarial transmission of LNYV may be (1) poor association between LNYV particles and mycetomes or (2) inefficient migration of mycetocytes to the developing embryos of H. lactucae. Though transovarial passage of LNYV through two generations has been demonstrated in H. lactucae, this may play a minor role in perpetuation of the virus because of the low rate of transovarial passage; nevertheless it provides evidence for virus-vector specificity (Oman, 1969).

The transmission characteristics of LNYV and SYVV are remarkably similar (Table 3.11). After a 24 and 48 hour acquisition feed (LNYV and SYVV respectively) the viruses can be retained in the vector for a long period except a few days prior to death when some of the aphids (higher in SYVV than in LNYV) fail to transmit the viruses. The rate of

Table 3.11 Comparison of some transmission characteristics of SYVV and LNYV by *H. lactucae*.

| Transmission characteristics | SYVV | Author | LNYV present study |
|---|---|---|---|
| Retention of virus in days after 24 and 48 hr acquisition feed (LNYV and SYVV respectively) | 52 (5°C) | Duffus (1963) | 53 (15°C) |
| Latent period range (days) | 8 - 46 (25° & 5°C) | " | 5 - 27 (28° & 15°C) |
| Days before death when vector ceases to transmit virus | 1.2 | " | 2.0 |
| % aphids failing to transmit virus prior to death | 62 | " | 38 |
| % Transovarial transmission to sets of progeny | 5.1[a] | Sylvester (1969b) | 6.8 |
| Proportions of inoculative sets of progeny transmitting virus within 48 hr of birth | 6/7[b] | " | 3/5 |

[a] Based on data from two separate experiments.

[b] Denominator represents the numbers of sets of progeny which acquired the virus transovarially.

transovarial passage of virus to progeny is low for both viruses. In SYVV (Sylvester, 1969b) as in LNYV, it appears that the nymphs are inoculative at birth if the latent period has been completed in the maternal aphid at the time of larviposition.

In summary it may be stated that the similar efficiency of alate and apterous _H. lactucae_ in the transmission of LNYV suggests that both forms of the aphid may be important in the spread of the disease. The long retention of LNYV in the aphid, the short probe required for transmission of the virus to lettuce and the fact that lettuce is an aphid non-host plant (which means that the aphid is likely to probe more plants) make _H. lactucae_ favourably disposed for the efficient transmission of the virus to a large number of plants. That LNYV multiplies in its vector (O'Loughlin and Chambers, 1967) has been confirmed with further evidence.

Other aspects of aphid behaviour, dispersal and field transmission of LNYV will be presented in subsequent chapters.

## STUDIES ON THE PROBING BEHAVIOUR OF H. LACTUCAE

### 4.1 Investigations into the settling behaviour of H. lactucae on lettuce

The use of lettuce as a test plant for LNYV transmission studies is difficult because of the reluctance with which H. lactucae probe lettuce plants. Investigations were therefore carried out to determine some of the factors which might predispose H. lactucae to probe on lettuce plants.

Apterae of H. lactucae were subjected to the following treatments for 6, 12 and 24 hr at 25°C in a constant temperature cabinet.

A. Darkness and 'humid' environment (RH 95-100%)

B. Darkness and 'dry' environment (RH 65-70%)

C. Continuous light and humid environment

D. Continuous light and dry environment

The method of subjecting aphids to the above treatments, humidity control (for the dry environment) and estimation of relative humidity are described in Chapter 2. For complete darkness the outside of the clear plastic container and lid (see Chapter 2) was painted black; and for the humid environment the NaOH solution was replaced with cotton wool soaked in distilled water. After the aphids had been pretreated for the various periods they were allowed a 15 min exposure to light (2150 lux) in a 25°C constant temperature room where the observations were made. The observations were made on the settling behaviour of individual aphids on either sowthistle or lettuce seedling by gently dropping the aphid on a leaf of the test plant with a micro-aspirator. A fresh seedling was used for each aphid. An aphid was regarded as

settled when it remained motionless and its labium (rostrum) was adpressed to the leaf. In this condition the antennae were usually held backwards horizontally on the back of the aphid. Each observation lasted for 15 min and five aphids were observed per treatment. The settling period was timed, and an aphid was regarded as settled when it remained in the motionless position with the rostrum adpressed to the leaf up to the end of the observation period. Apterous aphids which had been freshly removed from a sowthistle seedling were also exposed to light for 15 min and observed individually on the leaf of either a sowthistle or lettuce seedling.

Although the sample size was small the results (Table 4.1) show that when apterous H. lactucae are starved in continuous light in either humid or dry environment for 12 hr or more their settling behaviour is improved. On sowthistle seedlings the settling of the aphid appears to be unaffected by the treatments. None of the aphids which were freshly removed from sowthistle seedling settled on either sowthistle or lettuce during the period of observation suggesting that starvation was necessary to induce the aphids to settle on sowthistle. Another experiment was carried out using larger numbers of aphids to determine whether there was a real trend in the settling behaviour of H. lactucae which had been subjected to treatment D for the three periods of pretreatment. Apterous aphids were subjected to the four treatments as described previously. Twenty pretreated aphids were placed simultaneously on a single lettuce leaf and the numbers of aphids which settled, as judged

Table 4.1 Numbers out of 5 treated apterous *H. lactucae* which settled on sowthistle (S) and lettuce (L) seedlings up to the end of a 15 min observation period.

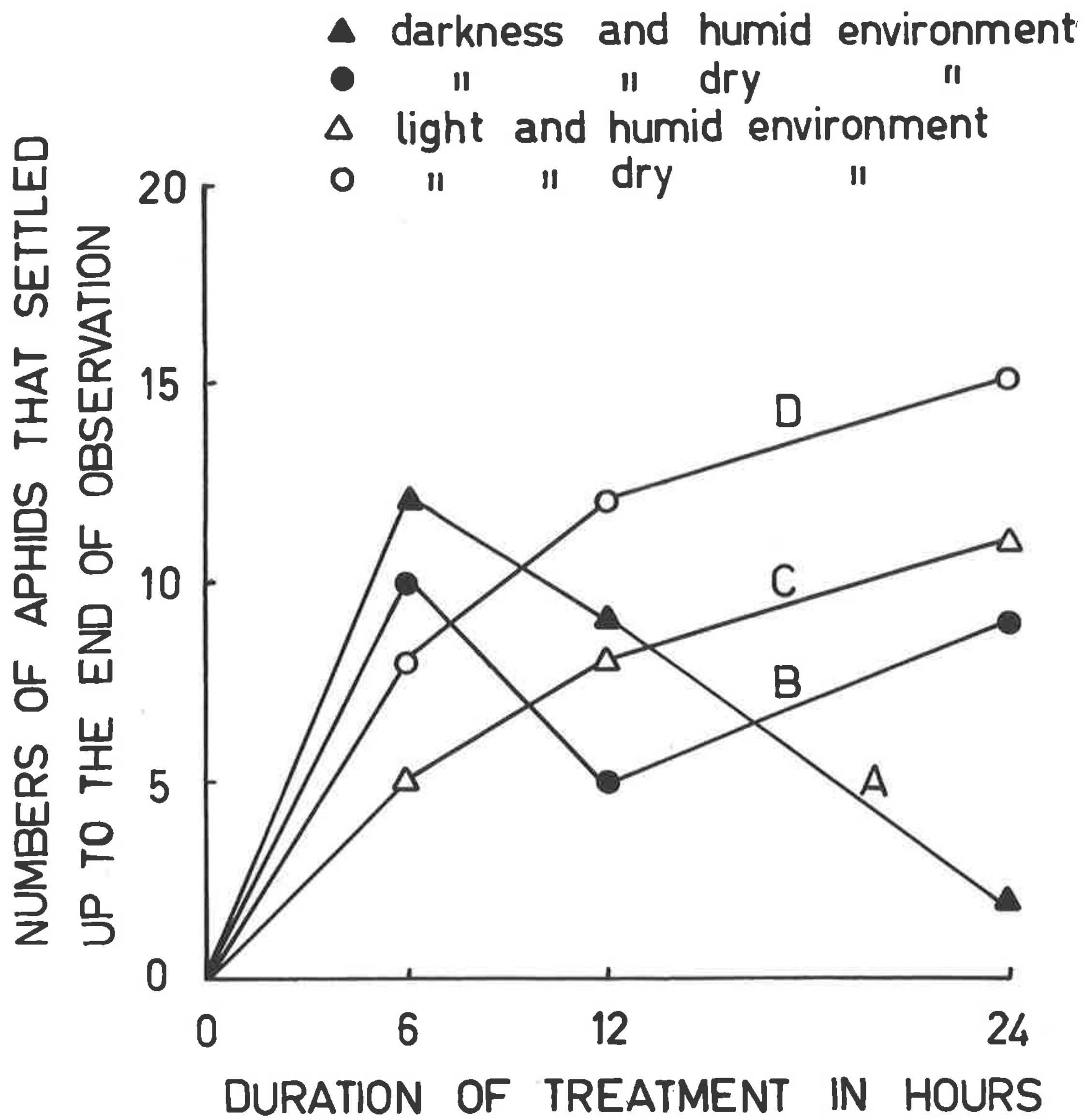
| Treatment No. | Type of Treatment | Duration of treatment in hours | | | | | |
|---|---|---|---|---|---|---|---|
| | | 6 | | 12 | | 24 | |
| | | S | L | S | L | S | L |
| A | Darkness and humid environment | 1<br>(6)[a] | 0<br>- | 1<br>(12) | 0<br>- | 5<br>(7-11.5) | 0<br>- |
| B | Darkness and dry environment | 2<br>(7-8) | 0<br>- | 2<br>(6.5-10) | 0<br>- | 5<br>(7-11.5) | 0<br>- |
| C | Continuous light and humid environment | 4<br>(6-13) | 0<br>- | 5<br>(9-14) | 2<br>(11-14) | 5<br>(10-14) | 1<br>(3.5) |
| D | Continuous light and dry environment | 3<br>(11.5-12) | 0<br>- | 3<br>(8-13.5) | 1<br>(8) | 5<br>(9-15) | 3<br>(10-14) |

[a] Figures in parentheses are the range of settling times (min).

by being motionless and with antennae held backwards and horizontally over the body, up to the end of the 15 min observation period were recorded. Apterous aphids which had been freshly removed from a sowthistle seedling were tested as before.

The results are illustrated in Figure 4.1. There is a tendency towards increased numbers of aphids that settled with increasing duration of treatment (treatments C and D). Treatment B does not show any general trend and though there is a trend in treatment A it is the reverse of

gure 4.1 Numbers of apterous _H._ _lactucae_ (out of 20) which settled on lettuce seedlings up to 15 min after being subjected to four treatments for various periods.



▲ darkness and humid environment
● " " dry "
△ light and humid environment
○ " " dry "
NUMBERS OF APHIDS THAT SETTLED UP TO THE END OF OBSERVATION
20
15
10
5
0
D
C
B
A
0
6
12
24
DURATION OF TREATMENT IN HOURS

treatments C and D.

For LNYV transmission studies on lettuce (see Chapter 3) and other studies to be described later in this chapter, *H. lactucae* were routinely pretreated by starving them in continuous light and dry environment at 25°C for 24 hr.

## 4.2 Saliva production by probing *H. lactucae*

O'Loughlin and Chambers (1967) observed the presence of LNYV particles in the salivary glands of viruliferous *H. lactucae*. This suggests that transfer of virus to lettuce and sowthistle plants is probably via salivary secretions (Day and Iryzkiewicz, 1954).

An experiment was carried out to find whether probing *H. lactucae* deposited saliva on both lettuce and sowthistle leaves when confined on them. A number of apterous *H. lactucae* were pretreated (as previously described) and groups of five aphids were caged on four leaves each of sowthistle and lettuce seedlings with leaf cages. Similar numbers of apterous *H. lactucae*, freshly collected from sowthistle plants, were also caged on sowthistle and lettuce leaves. The aphids were left on the seedlings for 30 min. For a control, leaf cages without aphids were put on the leaves of sowthistle and lettuce seedlings for a similar period.

Salivary secretions were demonstrated by Naito's (1965) technique. After removal of aphids from the leaves the seedlings were kept in the dark for 2 hr for the stomata to close. Pieces of leaf material (to

include areas enclosed by the leaf cages) were cut and immersed in 70% ethanol for 5 min and stained for 5 min with 1% erythrosine. The stained leaf pieces were thoroughly washed under tap water and each was held between two microscope slides and allowed to dry in the laboratory for two days.

The leaves were examined under a binocular microscope. Pink spots of salivary material (Figure 4.2a and b) were observed on both sow-thistle and lettuce leaves on which aphids were caged (irrespective of whether the aphids were pretreated or not). No pink spots were observed on the control leaves. Thus saliva is deposited on the leaf when *H. lactucae* are given access to either lettuce or sowthistle. Whether this deposition indicates 'probing', that is whether the saliva was injected into the leaf tissue or not could not be demonstrated.

## 4.3 Depth of stylet penetration of *H. lactucae* on lettuce and sowthistle seedlings

Depth of penetration of leaf tissue by stylets of *H. lactucae* was estimated by allowing 15-20 pretreated apterous aphids to probe on leaves of sowthistle and lettuce seedlings for 5, 15, 30 and 60 min. Probing was terminated by simultaneously killing and fixing aphids by dropping the leaf with the aphids attached into lactic acid and ethanol fixative (one volume 75% w/w lactic acid and 2 volumes 95% ethanol) previously cooled to -20°C by addition of dry ice. The aphids were retrieved by teasing the leaf tissue with a needle. Lengths of stylet protruding

gure 4.2a Deposits of salivary material (stained pink) by probing apterous *H. lactucae* on sowthistle leaf.

gure 4.2b Deposits of salivary material (stained pink) by probing apterous *H. lactucae* on lettuce leaf.

a

b

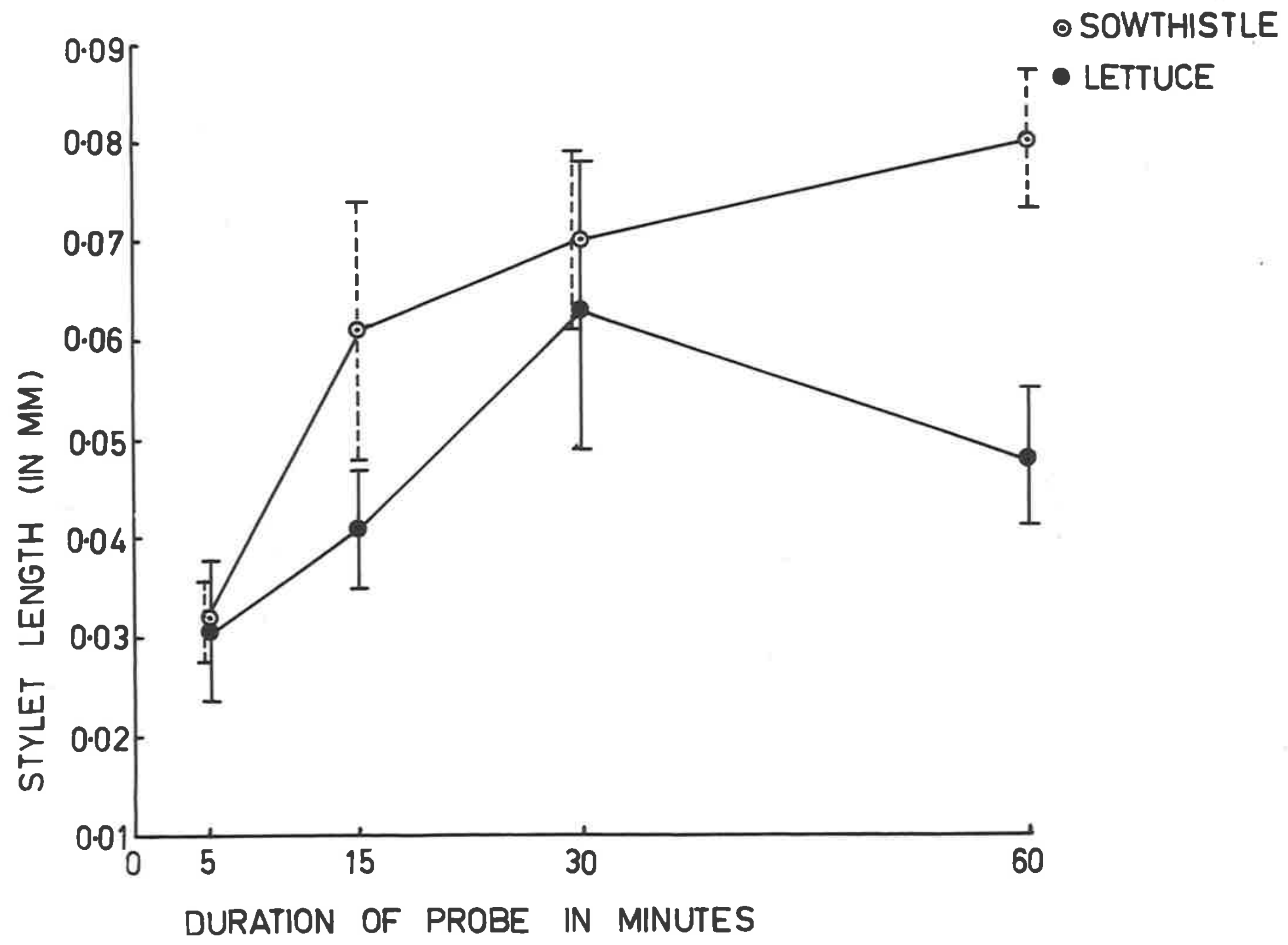
beyond the labium were measured with a binocular microscope fitted with a micrometer. Only those aphids attached to leaf tissue by their stylets were examined.

Mean stylet length ($\pm$ S.E.) of aphids probing on either sowthistle or lettuce plotted against duration of probe is illustrated in Figure 4.3. The results indicate that on sowthistle there is an increase in stylet length with increased duration of probe. A similar situation occurs on lettuce except that beyond the 30 min probe there is a decrease in stylet length. Apparently there is no difference between lengths of extruded stylet on both sowthistle and lettuce for probes lasting up to 30 min but there is a difference when probes lasted for 60 min.

To estimate the tissues reached by probing H. lactucae, leaf sections of sowthistle and lettuce seedlings were cut by hand and distances between leaf surface (cuticle) and the closest vascular tissue of midrib and primary veins in 20 sections were measured from the middle of either the midrib or vein. The depth of epidermal cells (i.e. from outer to inner cell walls) of the leaf lamina was also measured.

The measurements (Table 4.2) show that H. lactucae which probe for 60 min may not have reached the vascular tissue of either the midrib or vein in both sowthistle and lettuce seedlings. The epidermal cells of the leaf lamina would have been pierced through within 5 min from commencement of probing.

Figure 4.3 Comparison of stylet length of pretreated apterous *H. lactucae* which were allowed to probe lettuce and sowthistle leaves for various periods and killed *in situ* with dry ice (solid $CO_2$).



SOWTHISTLE
LETTUCE
0·09
0·08
0·07
0·06
0·05
0·04
0·03
0·02
0·01
STYLET LENGTH (IN MM)
0
5
15
30
60
DURATION OF PROBE IN MINUTES

Table 4.2 Mean measurements (in mm ± S.E.) of distances of vascular tissue of midrib and primary veins from the cuticle and the depth of epidermal cells in leaves of sowthistle and lettuce seedlings.

| Plant Type | Type of plant tissue | | |
|---|---|---|---|
| | Midrib | Vein | Epidermal cell |
| Sowthistle | 0.37 ± 0.01 | 0.20 ± 0.01 | 0.030 ± 0.001 |
| Lettuce | 0.51 ± 0.01 | 0.20 ± 0.01 | 0.033 ± 0.003 |

4.4 Uptake of plant sap by probing *H. lactucae*

To determine whether *H. lactucae* imbibe sap when they probe lettuce seedlings, $^{32}P$ uptake by *H. lactucae* and *Macrosiphum euphorbiae* (Thomas) were compared on labelled sowthistle and lettuce seedlings (see Section 2.52 for method of labelling). *M. euphorbiae* were used for comparison with *H. lactucae* because their size is identical to that of *H. lactucae* and they breed on both sowthistle and lettuce. The labelled seedlings were held in glass vials measuring 3/8" in diameter and 3" high. Cotton wool was wound around the mouth of the vial to completely surround the basal ½" of the stems of seedlings to prevent contamination of aphids. The vial was held in a wooden rack. On the top of the wooden rack and surrounding the glass vial was a 4" x 4" x 1/16" plastic sheet with a central ½" hole through which the glass vial was passed. The top of the plastic sheet was coated with tanglefoot to trap aphids which walked

off the seedlings.

Twenty pretreated apterous aphids of each species were put simultaneously on a leaf of the particular seedling and left for 2 hr. Aphids which remained on the leaf at the end of the period were collected. To estimate the amount of sap taken up by the aphids, known fresh weight of discs from leaves of labelled seedlings was ground in one ml of distilled water and centrifuged. Aliquots of the supernatant were counted to determine the specific radioactivity of sap in the leaf (see Section 2.52). Individual weights of 20 apterous H. lactucae and M. euphorbiae were determined and the mean fresh weight (1.11 $\pm$ 0.06 and 1.19 $\pm$ 0.04 mg for M. euphorbiae and H. lactucae respectively) was used to estimate the volume of sap per mg of body weight imbibed by the aphids.

Table 4.3 shows the amounts of sap imbibed by H. lactucae and M. euphorbiae from either sowthistle or lettuce leaves. The results show that sap uptake by H. lactucae probing on sowthistle was 1.5 times higher than that of M. euphorbiae. On lettuce M. euphorbiae took in 30 times more sap that H. lactucae. Sap uptake by H. lactucae on sow-thistle was 17 times greater than on lettuce. The results also indicate that H. lactucae apparently imbibes very little or no sap when it probes on lettuce. The amounts of sap imbibed by the aphids were highly variable. A probable differential distribution of $^{32}P$ in the leaves (Wright and Barton, 1955) together with a possible different sites of probing by individual aphids may have accounted for the variability.

Table 4.3 Sap uptake (in µl/mg body weight) by pretreated H. lactucae and M. euphorbiae which were allowed to probe on the leaves of $^{32}P$ labelled sowthistle and lettuce seedlings for 2 hr.

| Type of seedling | Aphid species | |
|---|---|---|
| | M. euphorbiae | H. lactucae |
| Sowthistle | $9.8 \times 10^{-2}$* <br> $(0.5 - 18.0 \times 10^{-2})$* | $15.1 \times 10^{-2}$ <br> $(0.4 - 31.8 \times 10^{-2})$ |
| Lettuce | $28.3 \times 10^{-2}$ <br> $(0.9 - 67.9 \times 10^{-2})$ | $0.9 \times 10^{-2}$ <br> $(0.3 - 2.3 \times 10^{-2})$ |

* Values are means, with range in parentheses.

## 4.5 Discussion

Results of experiments described in this chapter indicate that (1) the stylets of pretreated H. lactucae are likely to pierce through leaf tissues of lettuce and sowthistle during probes; (2) the depth of penetration increases with the duration of probe; (3) when H. lactucae are confined on the leaves of sowthistle and lettuce seedlings saliva is deposited on the leaf surface of both plants; (4) when H. lactucae probe lettuce they imbibe little or no sap from the plant but they imbibe sap when they probe on sowthistle.

Ibbotson and Kennedy (1959) observed that Aphis fabae Scop. walked on wax-coated rim of petri dish in continuous light over-night without stopping. Lamb (1963) indicated that loss of water by aphids was unaffected by low humidity probably as a result of water being replenished

in the form of metabolic water. Hunger in insects (as in other animals) may make insects more prone to feeding to replenish deficits arising from energy losses and synthesis of tissue (Dadd, 1970). Though H. lactucae which were subjected to continuous light were not observed at regular intervals they were found to be active on most occasions. It is possible that aphids which were put in continuous light and dry environment might not have lost significant amounts of water (cf. Lamb, 1963) but their activity was likely to have reduced the amount of metabolizable materials present and hence induce hunger. This condition probably accounted for the improvement of the settling behaviour on lettuce of H. lactucae which were subjected to treatments C and D (Figure 4.1). The settling behaviour of aphids in treatments A and B suggests that other unknown behavioural factors may be involved in the settling response of H. lactucae on lettuce.

Estimation of feeding sites of aphids by length of stylet protruding beyond the labium has been used by several workers (e.g. Day and Irzykiewicz, 1954; Gibson, 1972) but in situ observation of stylet or salivary sheath tracks in sections of plant material is a more direct but much more laborious method (Roberts, 1940; Esau et al., 1961; O'Loughlin and Chambers, 1969; Pollard, 1971). Day and Irzykiewicz (1954) have pointed out that both methods are comparable. Increase in depth of penetration of aphids' stylet with duration of probe has been demonstrated in several species of aphids (Roberts, 1940; Esau et al., 1961) and this is supported by the amounts of radioactive material taken up by aphids feeding on labelled plants for various intervals (Watson and

Nixon, 1953; Ehrhardt, 1961).

Up to 30 min the depth of stylet penetration of H. lactucae increases with duration of probe on both lettuce and sowthistle. Beyond 30 min probe, however, there is a decrease in stylet length when the aphid probed on lettuce which suggests a gradual withdrawal of stylet beyond this period. Wensler (1962) showed that Brevicoryne brassicae (L.) could discriminate quickly and clearly between host and non-host leaves. On non-host leaf (Vicia faba L.) B. brassicae probed for a short period and walked off within 3 min of probing. A similar situation occurs when non-treated H. lactucae are allowed to probe on lettuce. It appears that the threshold of probing response of pretreated H. lactucae is lowered considerably at least within the first 30 min of probing. After this period the threshold level probably rises and this may explain the apparent withdrawal of stylets that occurred after 30 min of probing.

It is generally accepted that aphids eject saliva during cell penetration (Bradley, 1952; Kloft 1960; Lamb et al., 1967) but it is also possible that they may secrete saliva onto the cuticle without penetration (van Hoof, 1961). Aphids are known to secrete two types of saliva, that is a fluid, water soluble saliva and a viscous saliva which gels to form the salivary sheath (Miles, 1959) and it is probable that most aphids secrete the two types of saliva (Miles, 1959; Lamb et al., 1967). Secretion of saliva may be intermittent or continuous (Kloft, 1960; Lamb et al., 1967). Lamb et al., (1967) also observed that fluid saliva may be continuously secreted and suggested that this may play an important role in the transmission of aphid-borne persistent viruses.

Negligible amount of sap intake when H. lactucae probe lettuce is unimportant as far as LNYV transmission is concerned; nevertheless it explains why the aphid does not breed on lettuce. The secretion of saliva by H. lactucae when they are confined on both sowthistle and lettuce suggests that introduction of LNYV to these plants by viruliferous aphids is probably via salivary secretions (O'Loughlin and Chambers, 1967). Viruliferous H. lactucae can successfully transmit LNYV to lettuce during a 5 min probe (see Chapter 3) and the fact that the aphid's stylet only penetrates the epidermal layer within 5 min of probing suggests that the site of LNYV introduction into lettuce may be outside the vascular tissue.

# 5. BIOLOGICAL STUDIES ON *H. LACTUCAE*

## 5.1 Comparison of rates of development of *H. lactucae* on seedlings and leaf discs of sowthistle plants

Though leaf discs have been used in biological studies of mites (Rodriguez, 1953 and 1958) and aphids (Johnson and Birks, 1960; Hughes and Woolcock, 1965), whole plants are more likely to resemble the natural environment. So an experiment was carried out to determine whether rates of development of *H. lactucae* on seedlings differed from those on leaf discs of sowthistle plants.

Groups of twenty apterous *H. lactucae* (from a glasshouse colony) were allowed to larviposit by confining them on excised sowthistle leaves in petri dishes for 6 hr and their progeny were pooled. The nymphs were either caged individually on sowthistle seedlings or were confined on leaf discs (measuring 21 mm in diameter) floating on nutrient solution (Hughes and Woolcock, 1965) contained in clear plastic vials which were held in trays. The tray measuring 7" x 3" could hold ten vials. One group of ten aphids (on leaf discs) was kept in a constant temperature room (20$^{\circ}$C) with a light intensity of 9,400 lux at the level of the vials (treatment A). Ten aphids on potted seedlings were put in a plant growth chamber also maintained at 20$^{\circ}$C and with a light intensity of 16,000 lux (treatment B). Another group of ten aphids confined on leaf discs was also kept in the plant growth chamber (treatment C). The light intensity above the vials was maintained as in treatment A by shading the vials with a nylon gauze.

The aphids were observed at 12 hr intervals until the final moult. Aphids were regarded to have moulted when they were completely out of their cast skins; the cast skins were removed during an observation. Mean period between two consecutive observations during which a moult occurred was taken as the time of moulting. Table 5.1 shows the developmental periods of the life stages of the aphid for the three treatments.

Table 5.1 Comparisons of the developmental periods of *H. lactucae* reared on sowthistle seedlings in plant growth chamber and on leaf discs in plant growth chamber and constant temperature room respectively.

| Treatment No. | Type of Treatment | Developmental period to adult (in days) ± S.E. |
|---|---|---|
| A | Leaf discs in constant temperature room | 7.2 ± 0.12 (10)[a] |
| B | Seedlings in plant growth chamber | 7.9 ± 0.14 (9) |
| C | Leaf discs in plant growth chamber | 7.9 ± 0.15 (10) |

[a] Figures in parentheses represent the sample size.

Analysis of variance (after correcting for missing data in treatment B (Snedecor and Cochran,1937)) was applied to the data for complete development; there was no significant difference ($P > 0.05$) among the treatments. This indicates that the rates of development of *H. lactucae*

on seedlings and leaf discs of sowthistle plants are similar.

## 5.2 Effect of temperature on the development of *H. lactucae*

Because of the lack of difference between the rates of development of the aphid on leaf discs and sowthistle seedlings, leaf discs were used to study the effect of temperature on the development of *H. lactucae*. Use of leaf discs is advantageous in that (1) the trays of ten vials required little space (2) by careful selection of leaves it was possible to use leaf discs of uniform texture and from the same leaves (it was possible to cut at least two discs from the same leaf) thus reducing variability due to plant material (3) it was easier to observe the cast skins and finally (4) the temperature above the discs was also uniform. The disadvantage of leaf discs was the occasional trapping of aphids in the nutrient solution.

Nymphs of *H. lactucae* were obtained as described in section 5.1. Groups of twenty first instar aphids were confined individually on leaf discs floating on nutrient solution and were put under a bank of fluorescent light in constant temperature rooms maintained at 15°, 20°, 25°, or 28°C. All aphids were examined for moulting and first time of larviposition at 12 hr intervals. The times for moulting or larviposition were determined as previously described.

## 5.3 Results

### 5.31 Developmental periods of the life stages of the aphid

Developmental periods, with their standard errors, for the various

stages (including total development) of the aphid at 15°, 20°, 25°, and 28°C are shown in Table 5.2. In calculating the development period, data for aphids which drowned at some stage of the experiment were excluded. Except at 15°C there was a slight but progressive increase in the duration of development from the first to the fourth instar stages. The results suggest that the pre-larviposition period in *H. lactucae* is comparatively short at the various temperatures except at 28°C where its duration is significantly lengthened.

Table 5.2 The developmental periods (in days) of the life stages of *H. lactucae* reared on leaf discs of sowthistle at 15°, 20°, 25° and 28°C.

| Stage | Temperature °C | | | |
|---|---|---|---|---|
| | 15 | 20 | 25 | 28 |
| First instar | 2.6 ± 0.3 | 1.7 ± 0.4 | 1.1 ± 0.3 | 0.9 ± 0.3 |
| Second instar | 2.1 ± 0.2 | 2.0 ± 0.4 | 1.4 ± 0.2 | 1.4 ± 0.2 |
| Third instar | 2.7 ± 0.4 | 1.9 ± 0.3 | 1.4 ± 0.2 | 1.6 ± 0.2 |
| Fourth instar | 3.5 ± 0.6 | 2.1 ± 0.2 | 1.7 ± 0.2 | 1.7 ± 0.2 |
| Pre-larviposition period | 0.8 ± 0.2 | 0.8 ± 0.4 | 0.7 ± 0.2 | 1.7 ± 0.5 |
| Total developmental time to reproduction | 11.7 ± 0.6 | 8.3 ± 1.1 | 6.3 ± 0.5 | 7.4 ± 0.6 |

### 5.32 Developmental zero

The effect of temperature on the rate of development of insects has been studied by several workers (Howe, 1967). Based on such studies

numerous mathematical expressions have been proposed to describe the relationship between temperature and the rate of development (Peairs, 1927; Davidson, 1942 and 1944; Andrewartha and Birch, 1954). Although these descriptions of mathematical relationships are precise for some species of insects, attempts to fit them to curves have not been altogether successful. The failure is due to the sigmoid and asymmetrical shape of the curves as well as the fact that statistics used for testing the 'goodness of fit' are so sensitive that slight experimental errors, for example slight fluctuations of temperature, make small differences highly significant (Browning, 1952; Howe, 1967). Over a restricted portion of temperature range the relationship between temperature and rate of development is approximately a straight line (Shelford, 1927). The point of interception after extrapolating the straight line to the temperature axis gives the so-called developmental zero, the validity of which has been questioned (Andrewartha and Birch, 1954). Others however consider the developmental zero as a biological reality (Hunter-Jones, 1968). Thermal summation and developmental zero have some practical use provided the temperature does not remain outside the linear zone; they have been used for the prediction of pest outbreaks (Bean, 1961) and the calculation of the number of generations of insects (Bodenheimer and Swirski, 1957).

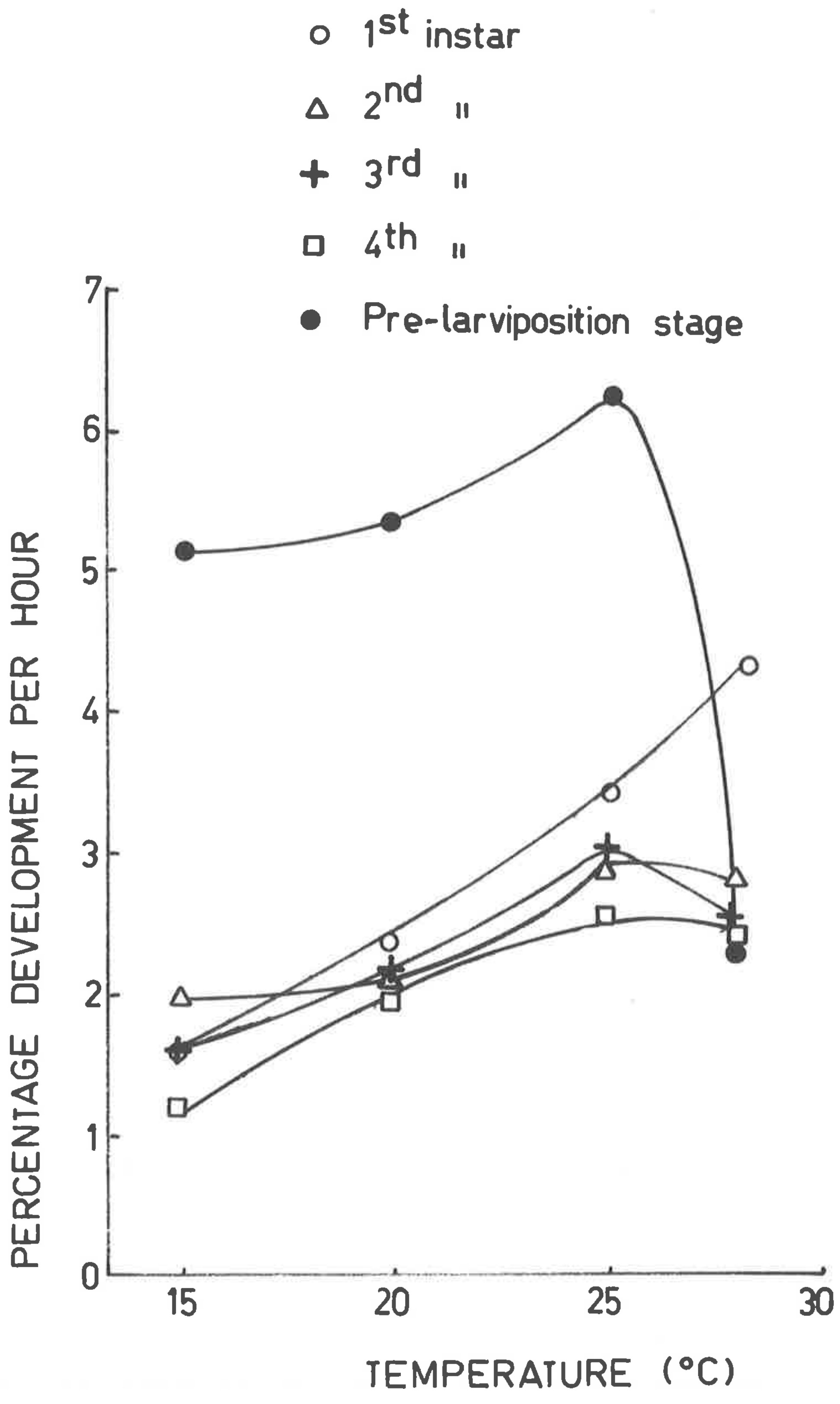
To obtain more precise curves for rate of development (i.e. reciprocal of the developmental period) against temperature requires at least ten intervals not greater than 2.5° and in addition to this more points should be included on either side of the optimum and limits of

temperature (Howe, 1967). These conditions could not be met in this experiment. Curves (fitted by eye) for the rate of development for each of the four instars (including the pre-larviposition period) of the aphid are shown in Figure 5.1. At 28°C the curves for the second, third and fourth instars begin to drop; a more precipitous drop in the curve for the pre-larviposition stage occurs at this temperature (28°C). Apparently this temperature is beyond the upper limit of development (Davidson, 1944). The curves also suggest that the rate of development of the various stages differ and will therefore have different values for the developmental zero. Consequently data for the total developmental period (excluding that for 28°C) were used in calculating the regression line, $y = a + bx$ (where y, a and x are the rate of development, a constant and temperature respectively). However to obtain a relation which applies to the whole range, including 28°C, the following formula can be used, $y = a + bx - ce^x$ or $y + ce^x = a + bx$ (where c is a constant and $e = 2.7182$) and the point $(x, y + ce^x)$ at which $x = 28$ can be determined (Lipka, 1918). The results (Figure 5.2) indicate that the developmental zero for *H. lactucae* is 10°C, and this was used to determine the number of generations in the experiment to be described in the next section.

## 5.4 Influence of population density on dispersal of *H. lactucae*

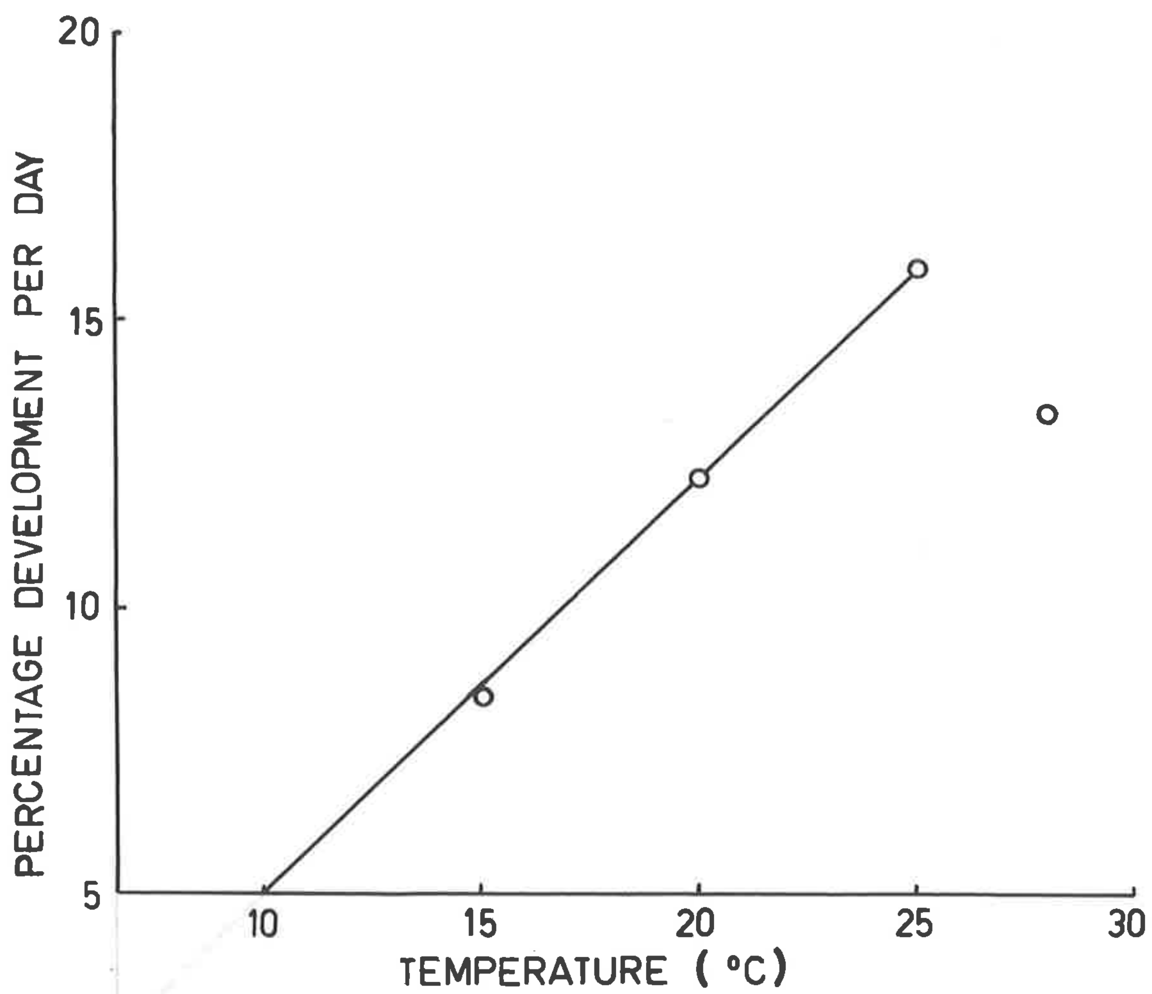
Departure of insects from their host plants or breeding sites has been interpreted as a mechanism by which insects respond to currently adverse factors in the environment such as shortage of food (as a result

gure 5.1 Curves for rates of development of the life stages of *H. lactucae* at various temperatures.



1st instar
2nd "
3rd "
4th "
Pre-larviposition stage
PERCENTAGE DEVELOPMENT PER HOUR
TEMPERATURE (°C)
0
1
2
3
4
5
6
7
15
20
25
30

gure 5.2 Graphical determination of temperature threshold for the total development of *H. lactucae*.



20
15
10
5
PERCENTAGE DEVELOPMENT PER DAY
10
15
20
25
30
TEMPERATURE ( °C)

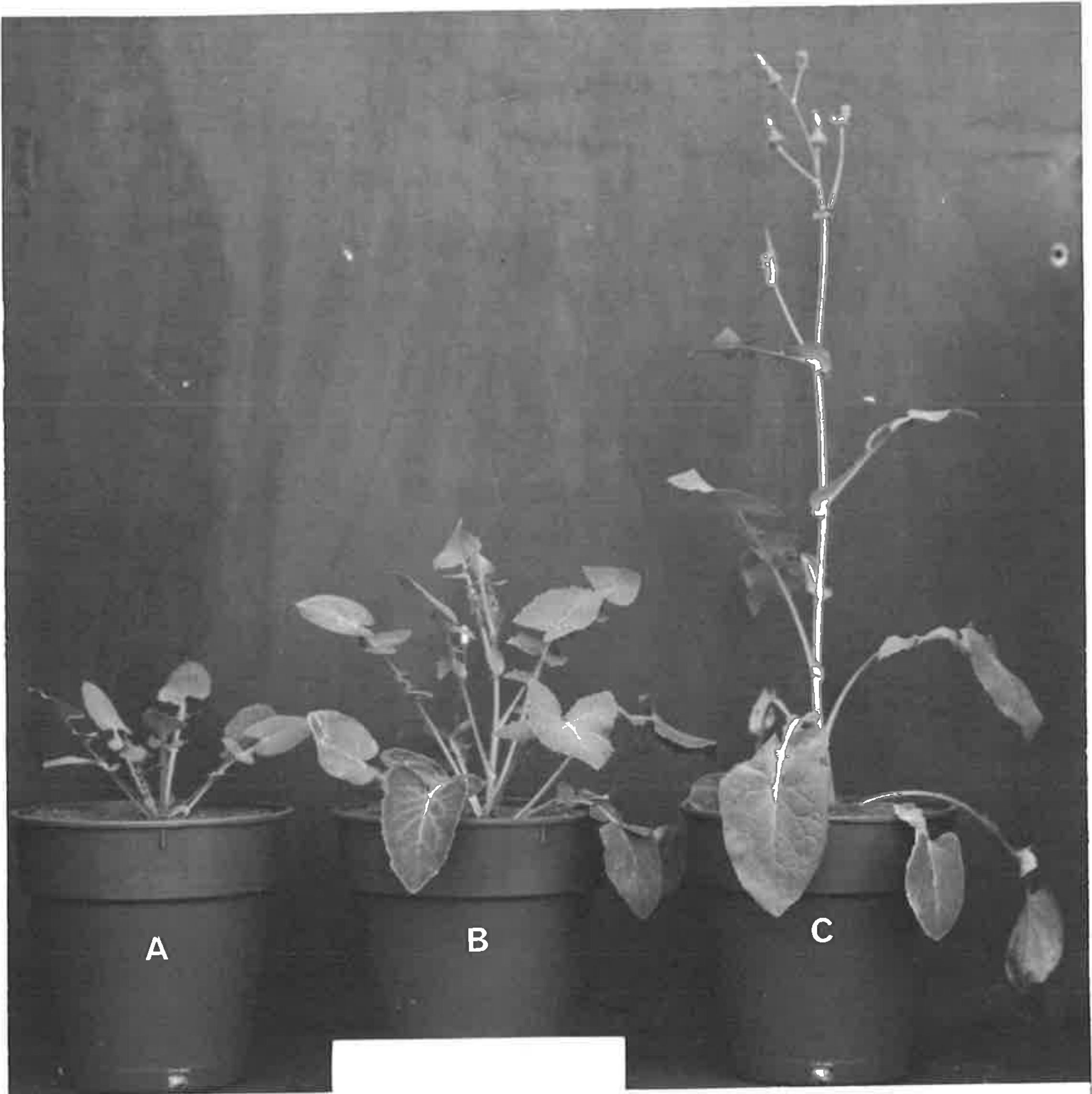
of over-crowding) or a means of seeking shelter for the purposes of hibernation (Prokopy and Gyrisco, 1965 a, b). Although this may be true for some species of insects (Johnson, 1969) there may be other factors involved other than currently adverse conditions of the environment (Kennedy, 1956; Johnson, 1960). Johnson (1969) proposed that dispersal or migration is a behavioural activity which is controlled by an environmental-endocrine system that produces morphometric phases in insects

Hyperomyzus lactucae does not breed on lettuce to which it transmits LNYV. To transmit the virus to lettuce plants the aphid must necessarily leave the host plant (i.e. sowthistle). The experiment described in this section was aimed at determining some of the factors which influence the dispersal of H. lactucae from sowthistle plants.

The design of the experiment, which was conducted in the field, was a completely randomized factorial design comprising two factors (plant size and population density). Three sizes of potted sowthistle plants designated 'small', 'medium' and 'large' (Figure 5.3) were used for breeding four initial densities (2, 4, 8 and 16) of fourth instar apterous H. lactucae. The twelve treatments were replicated three times.

Two sizes of cage, (measuring 12" x 12" x 16" high for the small plants and 18" x 18" x 24" high for medium and large plants) with sides covered with fibre-glass net and roofs of thin Polythene sheet, were used for enclosing the plants. The cages were stood on pieces of white opaque plastic sheets and builders' sand was spread beneath the plastic pieces thus enabling the edges of the cages to fit tightly. This

gure 5.3 Typical examples of small (A), medium (B) and large (C) sowthistle plants used in the population studies of *H. lactucae*. Beneath B is a 6" ruler.


A
B
C

arrangement made it possible to confine crawling aphids in the cages.

Potted sowthistle plants which had been growing in a glasshouse were caged in Alverstoke orchard for a week to aclimmatize. Apterous progeny of viviparae apterae of H. lactucae was established by putting 4 - 8 field collected apterous adults on the plants to larviposit for 24 hr after which period the maternal aphids were removed. Three days later the progeny were reduced to within 2-3 of the numbers required on each plant. After 7 days the aphids were thinned to leave the required number of fourth instar aphids. One and a half weeks later the potted plants were sunk into the soil (at sunrise at which time the aphids were virtually not disturbed) to provide more room for the growing plants. The plants were watered when necessary. Temperature was recorded in the two sizes of cage and in the shade with thermo-couples connected to a 'Honeywell' chart recorder immediately the aphids started to reproduce (i.e. after the completion of the first generation). The experiment was deemed to have started on the day the maternal aphids were confined on the sowthistle plants.

The cages were examined daily for aphids that had left the plants. Apterous aphids were found to have left some of the plants 17 days after the start of the experiment. After this period all 'emigrant' aphids were collected (shortly before sunrise to avoid flight by alates) daily from the tops, sides and floor of the cages with an aspirator and counted. The plants were harvested 32 days after the start of the experiment and all aphids were retrieved by shaking individual plants in water to which

a few drops of detergent had been added. All the aphids were counted to determine the numbers of the various forms.

## 5.5 Results

The sowthistle plants were in good condition for most of the experiment except towards the end (3 to 4 days) when some of them, particularly the small plants with initial populations of 8 and 16 aphids, began to show signs of deterioration.

### 5.51 Temperature in the cages

To determine whether the differences between densities were confounded with differences in temperature between cages, temperature within each cage was summated for the course of the experiment. Maelzer (personal communication) found that temperatures recorded at midnight, 5.30, 8 a.m., noon, 5 and 8 p.m. give a good estimate of the overall field temperature conditions at the Waite Institute. For temperature summation the mean temperatures between the times of observation are calculated and expressed as values above the threshold of development. Accumulative day-degrees is obtained by multiplying the mean temperature (above the threshold of development) for each interval by the fraction of day that the interval constitutes and adding on the products. Maelzer's computer programme was used for the analysis in the present experiment. Linear summation was possible in this experiment because the temperatures were not outside the linear zone.

Because two types of cage were used in the experiment a 't - test' was applied to the temperatures recorded at the six periods (see above) for each of the 22 days to find whether there was difference in the temperatures recorded; there was no significant difference between the temperatures recorded in the two cages. This is also reflected in the calculated day-degrees for temperatures recorded in the small and large cages (187.6 and 190.7 respectively); that for the shade was 179.0. There appears to be no difference in temperatures recorded at the three sites indicating that the temperatures in the shade and cages were apparently similar.

From these summed temperatures the number of generations during the course of the experiment was estimated. The estimated number of generations during the 22 days (period during which temperature was recorded) was 2.4. Thus the aphid populations completed 3.4 generations during the experimental period.

5.52 Influence of population density on reproduction of *H. lactucae*

The totals of aphids that emigrated from the plants and those collected at the end of the experiment were pooled for each plant. The mean numbers of aphid which originated from a single initial apterous *H. lactucae* were calculated. When the means of the treatments were plotted against their standard deviations the curve obtained showed a proportionate relationship between the two statistics. So a logarithmic transformation was applied to the data to reduce the heterogeneity of

variance (Snedecor and Cochran, 1937). Because Bartlett's test for heterogeneity of variance is inadequate for sample sizes of less than 5, the ratio $s^2$ max./$s^2$ min. was used for the test of heterogeneity of variance of the transformed data (Pearson and Hartley, 1954). The variance was found to be homogenized by the transformation ($s^2$ max./$s^2$ min. = 13.9, $P > 0.05$). The analysis of variance (Table 5.3) indicates that the effect of density on the rate of reproduction was highly significant ($P < 0.001$) and the effect of plant size was also significant ($P < 0.05$). The interaction between plant size and aphid population density on the rate of reproduction was non-significant. Comparisons between treatments were made by the method of least significant difference. The results (Table 5.4) show that differences between densities for a particular

Table 5.3 Analysis of variance (on transformed data) for comparison of the effect initial population densities and plant size on the rate of reproduction of apterous *H. lactucae*.

| Source of variation | DF | SS | MS | F |
|---|---|---|---|---|
| Treatment | 11 | 3.67565 | 0.33415 | 9.3130 |
| Population density | 3 | 3.18679 | 1.06226 | 29.6059*** |
| Plant size | 2 | 0.35770 | 0.17885 | 4.9847* |
| Density x size | 6 | 0.13116 | 0.02186 | 0.6092 |
| Error | 24 | 0.86116 | 0.03588 | - |

* $P < 0.05$

*** $P < 0.001$

plant size were significant at the 1% level. Though differences existed in the effect of plant size and rate of reproduction the differences were not consistent. For instance (1) at a density of 2 initial apterous aphids there is no difference between medium and large plants but they differ significantly from the small plants; (2) at a density of 4 there is no difference among the three plant sizes; (3) at density 8 there is no difference between large and small plants but these differ significantly from the medium plants; a similar situation occurs at density 16.

Table 5.4 Mean numbers of *H. lactucae* produced per single apterous aphid at various initial population densities on three plant sizes.

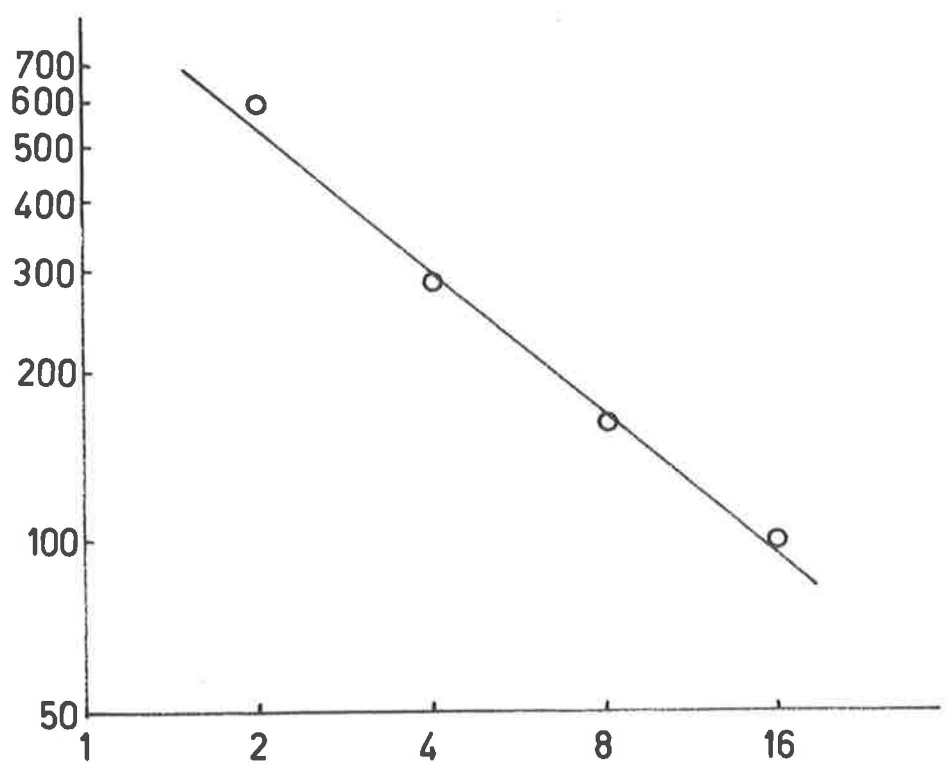
| Plant size | | Initial population density | | | |
|---|---|---|---|---|---|
| | | 16 | 8 | 4 | 2 |
| Small | (a)* | 140 | 188 | 335 | 927 |
| | (b)* | 2.07347 | 2.25514 | 2.50651 | 2.94834 |
| Medium | (a) | 66 | 109 | 245 | 462 |
| | (b) | 1.79568 | 2.00240 | 2.35315 | 2.65627 |
| Large | (a) | 93 | 196 | 287 | 409 |
| | (b) | 1.94651 | 2.26461 | 2.44582 | 2.60690 |

* (a) untransformed data
(b) transformed data

LSD (population density) at 5% : 0.01645
1% : 0.02229

LSD (plant size) at 5% : 0.15960

gure 5.4 Relationship between mean numbers of aphids produced per plant (means of the three plant sizes) by initial single apterous *H. lactucae* at various population densities.


MEAN NUMBERS OF APHIDS PRODUCED
PER SINGLE INITIAL APTEROUS APHID
700
600
500
400
300
200
100
50
1
2
4
8
16
INITIAL POPULATION DENSITY

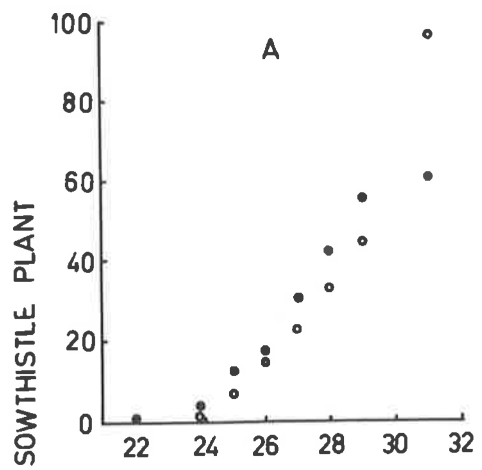
In addition the data of Table 5.4 indicates that the initial population density of apterous aphids has a profound effect on the rate of reproduction of H. lactucae. The rate is at its maximum when the population density is low and it decreases with increasing density. This effect occurs irrespective of plant size. The relationship between the mean numbers of aphids produced (P) per single aphid per plant and the initial density (I) of apterous aphids is given by the general equation $\log P = K - b \log I$, where K is a constant (Figure 5.4). At a particular density the effect of plant size (Table 5.4) does not show a clear pattern; it appears however that the aphids reproduce better on small and large plants than on medium size plants.

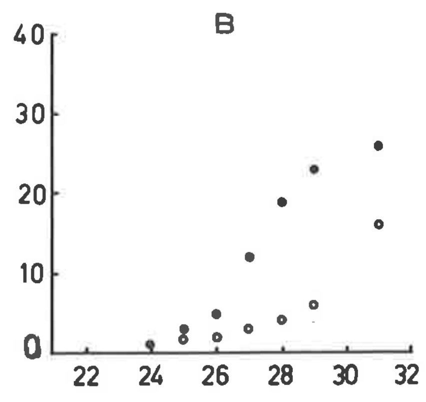
### 5.53 Influence of population density on emigration of H. lactucae from sowthistle plants

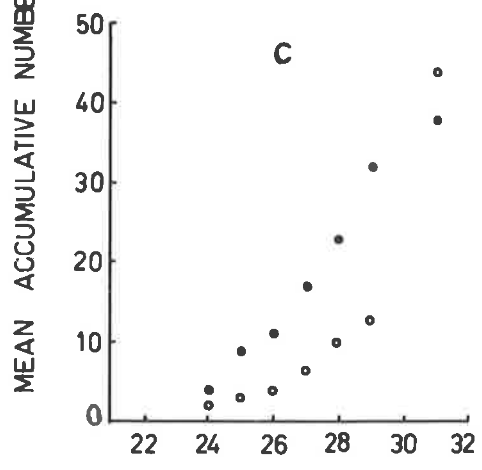
Daily numbers of emigrant aphids per plant (at a particular density) were plotted against time but this proved unsatisfactory so the accumulative numbers were used instead. Emigration curves for apterae and alatae are illustrated in Figures 5.5, 5.6, 5.7 and 5.8. The curves indicate that at certain levels of density more apterous than alate aphids emigrate from sowthistle plants. As the population grows, more emigrant alates leave the plants (Figure 5.8 a, b and c). Further population increase leads to a decrease in numbers of emigrant apterae until a stage is reached when emigration virtually ceases (e.g. Figure 5.8 a, b). Percentage alatae produced on the three plant sizes, including 3rd and 4th instar alatiform nymphs which were collected from harvested sow-

gure 5.5 Mean accumulative numbers of adult apterae and alatae emigrating from small (A), medium (B) and large (C) sowthistle plants infested with 2 initial apterous *H. lactucae* per plant.

• apterae
∘ alatae



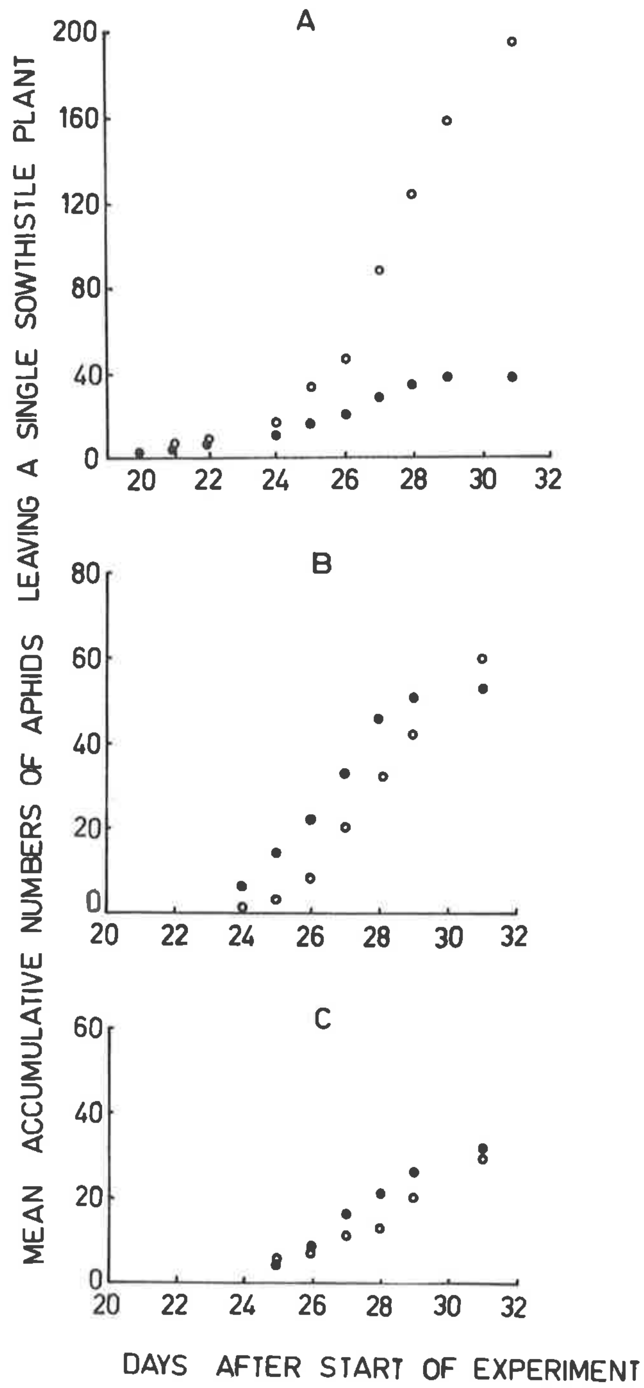




MEAN ACCUMULATIVE NUMBERS OF APHIDS LEAVING A SINGLE SOWTHISTLE PLANT

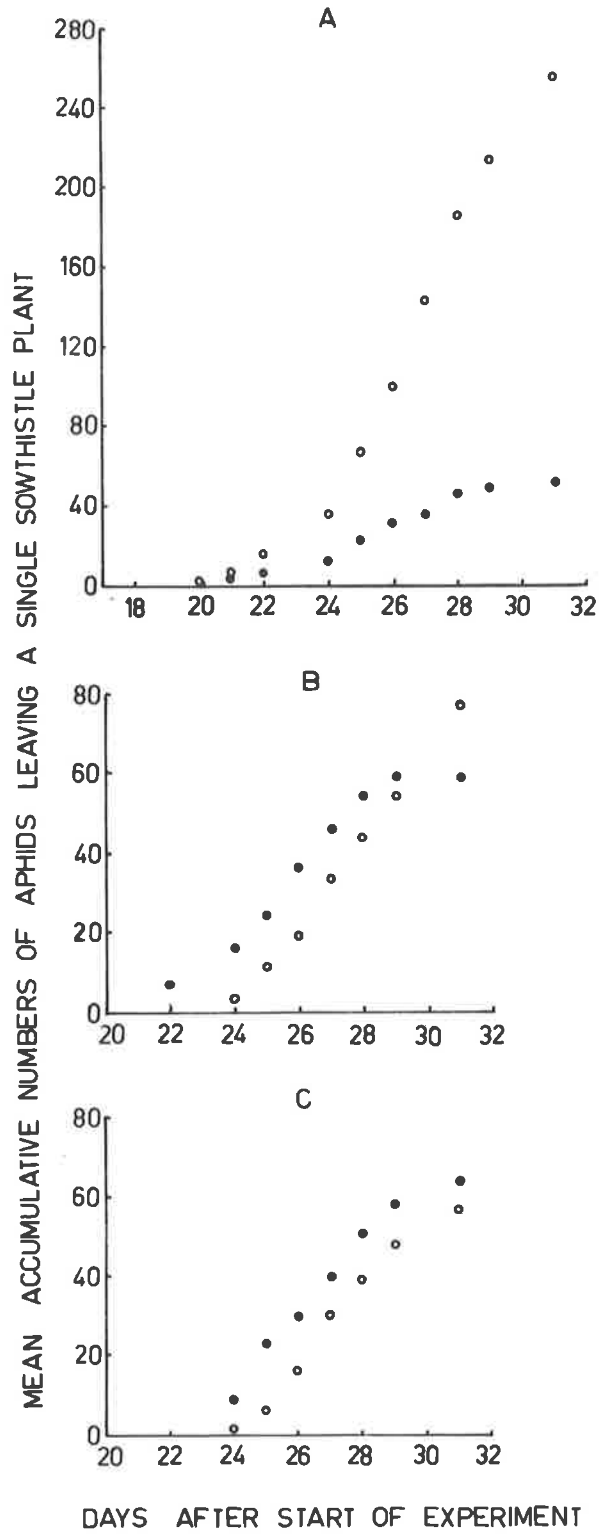
DAYS AFTER START OF EXPERIMENT

;ure 5.6 Mean accumulative numbers of adult apterae and alatae emigrating from small (A), medium (B) and large (C) sowthistle plants infested with 4 initial apterous H. lactucae per plant.



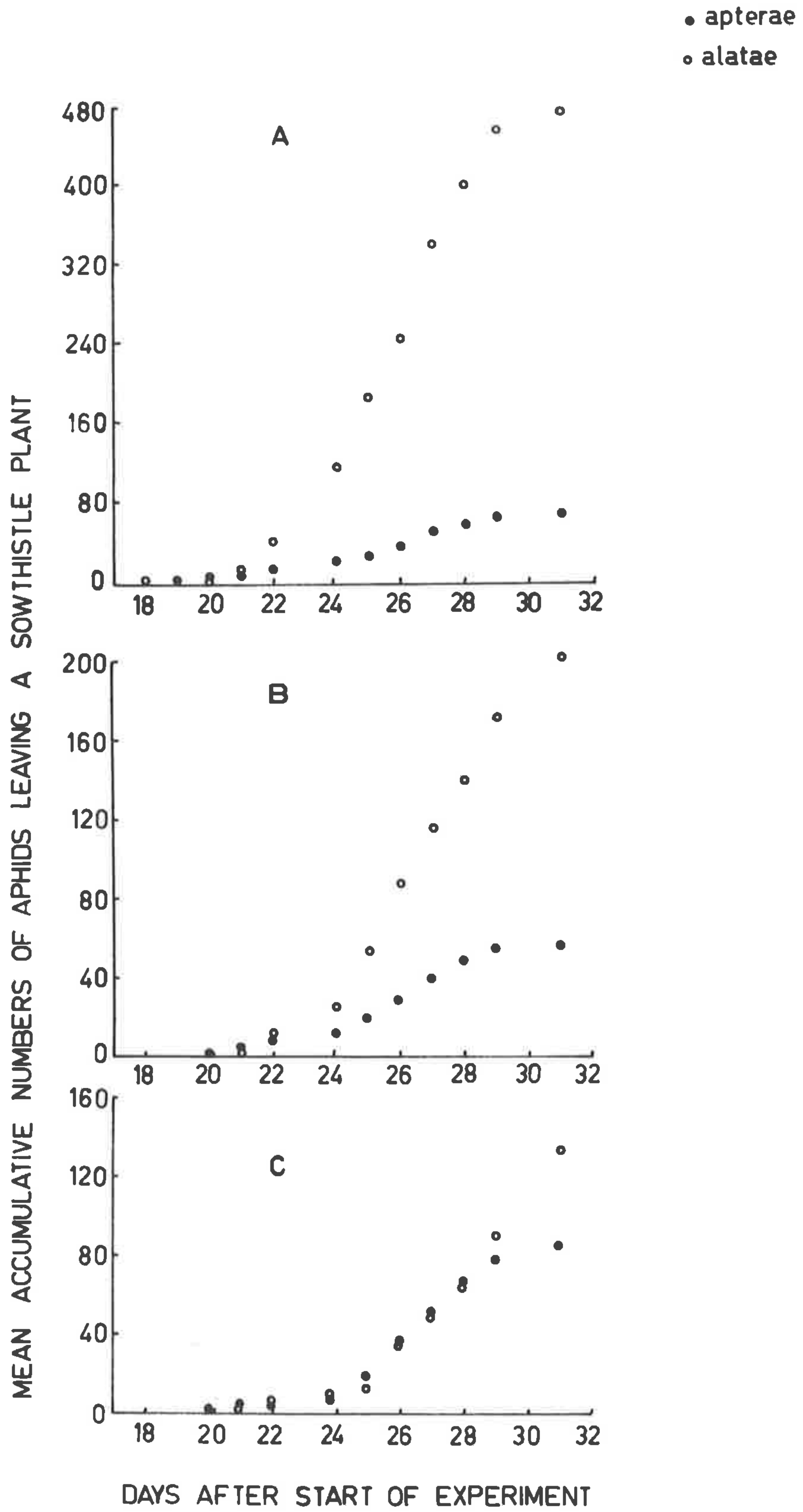
• apterae
o alatae
A
B
C
MEAN ACCUMULATIVE NUMBERS OF APHIDS LEAVING A SINGLE SOWTHISTLE PLANT
200
160
120
80
40
0
60
20
20 22 24 26 28 30 32
DAYS AFTER START OF EXPERIMENT

ure 5.7 Mean accumulative numbers of adult apterae and alatae emigrating from small (A), medium (B) and large (C) sowthistle plants infested with 8 initial apterous *H. lactucae* per plant.



• apterae
o alatae
A
B
C
MEAN ACCUMULATIVE NUMBERS OF APHIDS LEAVING A SINGLE SOWTHISTLE PLANT
DAYS AFTER START OF EXPERIMENT

ure 5.8 Mean accumulative numbers of adult apterae and alatae emigrating from small (A), medium (B) and large (C) sowthistle plants infested with 16 initial apterous *H. lactucae* per plant.



• apterae
∘ alatae
A
B
C
MEAN ACCUMULATIVE NUMBERS OF APHIDS LEAVING A SOWTHISTLE PLANT
DAYS AFTER START OF EXPERIMENT

thistle plants (see Section 5.4), are plotted against initial population densities (Figure 5.9). There is an increase in the percentage of alatae produced with increasing initial population densities.

To determine the effect of population density and plant size on numbers of aphids (apterae and alatae) emigrating per plant, analysis of variance was computed on the logarithmically transformed data (reasons as mentioned in section 5.52). The transformation homogenized the variance ($s^2$ max./$s^2$ min. = 59.1, P > 0.05). The results (Table 5.5) show that population density and plant size significantly (P < 0.001) influenced the numbers of aphids that emigrated. The interaction between population density and plant size was not significant.

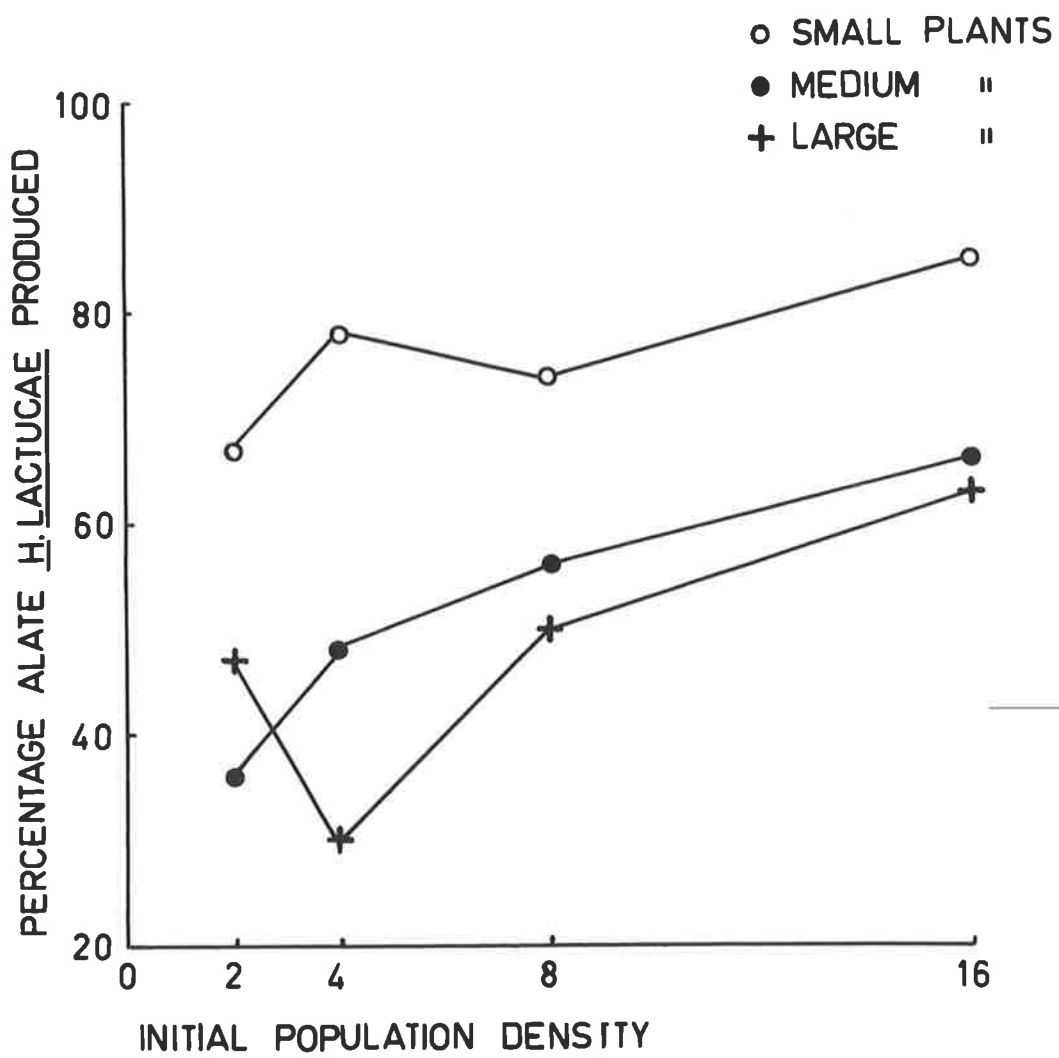
Table 5.5 Analysis of variance (on transformed data) of the effect of initial population density and plant size on numbers of *H. lactucae* that emigrated from a single sowthistle plant.

| Source of variation | DF | SS | MS | F |
|---|---|---|---|---|
| Treatment | 11 | 3.24871 | 0.29534 | 4.2933 |
| Population density | 3 | 1.77737 | 0.59246 | 8.6126*** |
| Plant size | 2 | 1.23008 | 0.61504 | 8.9408*** |
| Density x plant size | 6 | 0.24126 | 0.04021 | 0.5845 |
| Error | 24 | 1.65101 | 0.06879 | - |

*** P < 0.001

Differences between the effects of population density and plant size on emigration were determined by the method of least significant

ure 5.9 Percentage alate aphids produced by the various initial population densities of apterous *H. lactucae* on small, medium and large sowthistle plants during the experimental period.


o SMALL PLANTS
● MEDIUM "
+ LARGE "
100
80
60
40
20
0
2
4
8
16
PERCENTAGE ALATE H. LACTUCAE PRODUCED
INITIAL POPULATION DENSITY

difference. Differences between plant size at a particular population density were significant at the 1% level (Table 5.6). With the exception of population density 2 in which more aphids emigrated from the large than medium plants the trend was as follows: small > medium > large. On small plants there was no difference in the numbers of aphids that emigrated at densities 2 and 4; similarly there was no difference at densities 8 and 16. Differences between densities 4 and 8 were not significant on the medium plants. On large plants densities 2 and 4 were not significantly different; a similar situation occurred at densities 8 and 16.

Table 5.6 Mean numbers of *H. lactucae* that emigrated per sowthistle plant at various initial population densities.

| Plant size | | Initial population density | | | |
|---|---|---|---|---|---|
| | | 2 | 4 | 8 | 16 |
| Small | (a)* | 158 | 232 | 308 | 543 |
| | (b)* | 2.18691 | 2.30595 | 2.47116 | 2.62261 |
| Medium | (a) | 42 | 113 | 169 | 258 |
| | (b) | 1.62594 | 2.01440 | 2.1780 | 2.41046 |
| Large | (a) | 82 | 58 | 143 | 218 |
| | (b) | 1.79445 | 1.69371 | 2.06353 | 2.31719 |

* (a) untransformed data
(b) transformed data

LSD for population density (5%): 0.25518
(1%): 0.34582

LSD for plant size (5%): 0.02366
(1%): 0.03205

Comparison between mean numbers of adults and nymphs of H. lactucae that emigrated (per plant) in the course of the experiment is shown in Table 5.7. Numbers of nymphs departing from the plants when compared with adults are obviously smaller.

Table 5.7 Mean numbers of H. lactucae (adult and nymph) that emigrated from a single sowthistle plant.

| Plant size | Initial population density | Adult | Nymph |
|---|---|---|---|
| Small | 2 | 158 | 9 |
| | 4 | 232 | 4 |
| | 8 | 308 | 2 |
| | 16 | 543 | 4 |
| Medium | 2 | 42 | 1 |
| | 4 | 113 | 3 |
| | 8 | 169 | 2 |
| | 16 | 258 | 1 |
| Large | 2 | 82 | 2 |
| | 4 | 58 | 0 |
| | 8 | 143 | 3 |
| | 16 | 218 | 3 |

## 5.5 Discussion

The results of the experiment described in this section indicate that population density influences both the rate of reproduction and the numbers of adult aphids that emigrate from sowthistle plants. At

low population density the rate of reproduction increases but the rate slows down at high densities (Figure 5.4). Conversely, more adult aphids leave sowthistle plants at high than at low population densities; there appears to be virtually no effect of density on the emigration of nymphs (at least during the period of experimentation). Population density affects the relative numbers of alatae and apterae that emigrate; as the population grows probably more alatae are produced (Figure 5.9) and so more alatae than apterae leave the sowthistle plants (Figure 5.8 a, b and c).

That population density has a profound effect on the rate of reproduction of aphids has been demonstrated in other species of aphids for example Drepanosiphum platanoides (Schr.) (Dixon, 1963), A. fabae (Way and Banks, 1967) and B. brassicae (Way, 1968). In his studies on the sycamore aphid, D. platanoides, in which a reproductive diapause occurs, Dixon (1963) showed that at initial high densities there is a reversible drop in the reproductive rate of the aphid but a more lasting effect is induced during prolonged crowding. On observing the corpus allatum of D. platanoides he found that the size of this organ was much larger in actively reproducing than in sluggishly reproducing or diapausing aphids; he suggested that when crowding reached a certain threshold the brain could inhibit the activity of the corpus allatum thereby reducing the rate of reproduction. In addition to a decrease in the rate of reproduction in A. fabae as population density increases, Way and Banks (1967) also found that the size of aphids decreased. They

indicated that changes in population of A. fabae were a means of checking the over-exploitation of the growing plant by adjusting to changing food and space provided by the plant.

In the context of Way and Banks (1967) one would expect that the rate of reproduction of H. lactucae would be far greater on the large sowthistle plants than on the small ones. Though circulation of nutrients in plants is upwards (in the xylem) from roots to leaves and downwards (in the phloem)from leaves to roots, there are circumstances when translocation in these two systems is unidirectional, for example into developing flowers and growing shoot tips (Zimmermann, 1969). Field observations show that H. lactucae are found mostly on the flower stalk and terminal stems of sowthistle plants (cf. Hille Ris Lambers, 1949). The aphids are found in these sites probably as a result of the concentration of nutrients in these parts of the plant. Though the plant may be large, in reality the aphids may be more crowded on these plants than perhaps on young plants. This may account for the lower rate of reproduction of H. lactucae observed on the large sowthistle plants.

The results also show that numbers of the aphid emigrating from sowthistle is dependent upon the population density of the aphid; the rate of emigration increases with increasing density (Tables 5.5 and 5.6). The results also indicate that more apterous than alate H. lactucae depart from the plants during the initial stages of population growth; as the population reaches a certain level more alatae depart and the rate of emigration of apterae decreases sharply (Figures 5.4 to

5.8) probably as a result of greater production of alates (Figure 5.9). Although there is no evidence for proportions of alatae and apterae remaining on the plants to those that emigrate at any one time it may be assumed that numbers of aphids that left the plants at a particular time reflected on the numbers that were present. Crowding of aphids on host plants may (Way and Banks, 1967) or may not (Murdie, 1969) lead to a reduction in food quality; but crowding would promote restlessness which in turn increases the encounters between aphids (Johnson, 1965; Murdie, 1969).

Importance of tactile contacts between aphids as a result of crowding or 'effet de groupe' (Bonnemaison, 1951) with the concomitant production of alatae has been recognised in several species of aphids (Johnson, 1965; Toba et al., 1966; Lees, 1967; Sutherland, 1969). Food quality may also play a significant role in alary polymorphism in aphids. For example Johnson (1966) observed that food quality and crowding can affect the production of alatae in Aphis craccivora Koch. He found that on old leaves of broad bean (Vicia faba L.) crowding favoured alate production but on young seedlings crowding produced the opposite effect. He therefore suggested that the two factors can be either supplementary or antagonistic to one another. Similarly Sutherland (1967 and 1969) observed that when the green strain of A. pisum was crowded soon after the onset of parturition the production of alate aphids on mature leaves of V. faba was double that of seedlings. It appears from the foregoing that inadequate nutrition can directly

induce the production of alates in A. craccivora and A. pisum. This gains support from studies on M. persicae in which it was demonstrated that single chemical differences in the diet of the aphid could effect changes in the proportions of alates formed (Mittler and Dadd, 1966; Sutherland and Mittler, 1971). Lees (1966) has suggested that the effect of crowding on alate production has a humoral basis.

It may be concluded that dispersal of H. lactucae from sowthistle is influenced by crowding which may also affect the quality of nutrients in the plant. Under these circumstances proportions of the two morphological forms appear to be affected differently (as reflected by numbers of apterous and alate aphids that emigrate). One can therefore speculate that dispersal of H. lactucae from sowthistle may be a response to conditions of the environment (i.e. possible reduction of food quality resulting from crowding). The crowding is likely to have an effect on the central nervous system which may lead to the production of more alates ('migrants').

The next chapter describes investigations into the form of H. lactucae (alatae or apterae) which is more likely to play a significant role in the spread of LNYV in the field.

# 6. FIELD TRANSMISSION OF LETTUCE NECROTIC YELLOWS VIRUS BY H. LACTUCAE

## 6.1 Introduction

Flights of infective 'migrant' *H. lactucae* are probably responsible for the introduction of LNYV into lettuce crops. In the absence of knowledge on the flight behaviour of *H. lactucae* it is perhaps pertinent, for the understanding of some aspects of the field spread of LNYV, to draw on the information obtained from studies on other species of aphids although Bodenheimer and Swirski (1957) have cautioned against generalizations in aphid studies.

Moericke (1955) categorized various 'moods' through which alate aphids passed before they finally settle on host plants. He suggested that at the teneral stage the aphid was in a resting mood (Ruhestimmung) and at the end of this stage it passes into the flight mood (Flugstimmung) when it makes long distance flight. This stage is followed by the attack mood (Befallsstimmung) and finally the alighting mood (Ansiedlungsstimmung) in which the aphid settles and starts to larviposit. Moericke's theory triggered off interest in studies on flight behaviour of aphids by numerous workers with the resultant proposal of new theories.

Taylor (1957) and Woodford (1969) have suggested that the teneral stage has both developmental and behavioural components. Changes in numbers of flight-mature aphids depend on the diurnal periodicity of moulting and the temperature-dependent teneral stage (Johnson *et al.*, 1957; Taylor, 1957). During take-off (depending upon the aphid species),

aphids first seek the highest available point on the plant (Johnson, 1958) or "move to the lower surface of leaves and pick themselves up in flight" (Heine, 1955). Take-off or first flight is influenced by temperature and light intensity (Taylor, 1957; Heathcote and Cockbain, 1966) and this is reflected in the diurnal periodicity of flight of aphids in the field (Taylor, 1958). Thus numbers of air-borne aphids are controlled by rates of moulting, teneral development and by temperature and light thresholds for take-off (Johnson, 1969).

Johnson (1958) showed that the strength of settling response in *A. fabae* depended upon the duration of previous flight and suggested that this was probably due to fatigue as a result of the accumulation of metabolic products. In addition to confirming this finding Kennedy and Booth (1963a, 1963b and 1964) and Kennedy (1965 and 1966) in a series of experiments on the effects of flight duration on the settling response of *A. fabae* on non-host leaf, host leaf and on a card observed that the after-effect of landing was a typically 'boosting' or 'rebound' flight which was sometimes accompanied by seconds of flight depression. Through a series of one minute flights they observed that the two effects were greater after landings on a leaf than on card, where the settling response was weak. The settling response was, however, strongest on host than on non-host leaf. They therefore concluded that the two after-effects of settling on flight were antagonistic to each other. Consequently, Kennedy (1965 and 1966) suggested that the apparent reciprocal effects were co-ordinated primarily by the central nervous system. Recently,

Shaw (1970 a, b and c) demonstrated that when A. fabae are crowded at different population densities, three types of alates (i.e. migrants, flyers and non-flyers) are produced and suggested that the relative concentration of juvenile hormones probably governs the type of alate produced.

Theories about alighting and probing behaviour of aphids are particularly relevant to the transmission of LNYV by H. lactucae because lettuce is a non-host plant of the aphid and yet the aphid can probe on lettuce sufficiently long to transmit LNYV (see Chapter 3). The question of how far or how long alates of H. lactucae must fly before they will alight on lettuce plants is crucial to an understanding of the epidemiology of the disease.

Stubbs et al., (1963) investigated the incidence of LNYV in lettuce crops in relation to distance from a presumed virus source (aphid-infested sowthistle plants). Of the two lettuce plots studied (Stubbs et al., 1963), one plot was completely free of sowthistle plants and was 700 yd away from the nearest possible virus source; the other plot was 'adjacent' (the exact distance was not indicated) to aphid-infested sowthistle plants. Their observations indicated that LNYV incidence was much higher (76.5% infection) in the plot which was adjacent to the sowthistle plants than in the distant plot that contained no sowthistle (6% infection). They further observed that LNYV infection in the sow-thistle-free plot was randomly distributed whereas in the other plot there was a progressive increase in infection towards the virus source.

They concluded that the LNYV was transmitted to lettuce at "close range" and suggested eradication of sowthistle plants within or near lettuce crops as a means of controlling the disease.

The above observations provide no insight into the way in which H. lactucae introduce LNYV into crops as well as the morphological forms (apterae or alatae) involved in the close range transmission of the disease. Close range transmission of aphid-borne plant viruses may be effected by wandering apterous aphids (Ribbands, 1963), or by alates which are likely to make short flights when the environmental temperature is not sufficiently above the flight threshold temperature of the aphid (Heathcote and Cockbain, 1966). The aim of the experiment described in this chapter was to determine which form of H. lactucae is involved in the field transmission of LNYV. Consequently the following hypotheses were thought to be worth testing:

1. Alate H. lactucae which occur on nearby old infected sowthistle plants are important in transmitting LNYV to lettuce.

It is generally conceded that on the first migratory flight aphids are carried high in the air which suggests that they would be carried perhaps several miles away from their source (Johnson, 1969); however it is also known that migratory species may be carried only a short distance as a result of unfavourable environmental condition (Heathcote and Cockbain, 1966). In a field experiment conducted in the spring, Heathcote and Cockbain (1966) observed that there was a progressive decrease in the number of sugar beet plants infected with beet mild yellows virus (BMYV) with increasing distance from mangold clamps which

liberated the vectors, M. persicae and Rhopalosiphoninus staphyleae tulipaellus (Theob.). Percentage of beet plants infected within a distance of 0-20 yd was 90%, but only 6% for plants that were 440-880 yd away from the virus source. It was further observed that during the spring temperatures rose only a little above the flight threshold of M. persicae and Rhopalosiphoninus s. tulipaellus. They therefore concluded that at such temperatures, when flights did occur they were probably short and this must have contributed to the close range transmission of the disease.

2. Apterous aphids which occur on nearby old infected sowthistle plants may play an important role in the spread of LNYV.

Crowding influences the dispersal of H. lactucae from sowthistle plants; during the early stage of population growth more apterous than alate aphids leave the host plant (see Chapter 5). Apterous aphids are known to spread plant viruses in the field (Ribbands, 1963). Ribbands (1963) studied the spread of beet yellows virus (BYV) and beet mild yellows virus (BMYV) by placing apterous M. persicae infected with these viruses on single sugar beet plants which were 50 yd apart in a field of sugar beet. Nearby plants were examined at weekly intervals for three weeks and it was observed that 66, 92 and 96% of aphids found had moved to a new plant during the 1st, 2nd and 3rd counts respectively. No alates were observed within a month although patches of sugar beet more than 11 ft in diameter had become infected and the diameter extended to about 20 ft two months later. Ribbands (1963) concluded from these observations that disease spread was effected predominantly by wandering

apterous *M. persicae*. In his studies on inter-plant movement of apterous *M. persicae*, Ferrar (1967) showed that this morphological form was proficient in walking across soil. When walking aphids encountered a host plant they settled but on non-host plants they moved after making several probes. Ferrar (1967) suggested that under some conditions apterous aphids are likely to be significantly involved in the spread of plant viruses by walking across soil from plant to plant.

3. Instead of dispersing alatae or apterae spreading disease from old sowthistle plants as in (1) and (2) above, infective alates may inoculate and start colonies on virus-free sowthistle seedlings germinating in lettuce crops, and migrants from these colonies may be primarily responsible for the spread of the disease. Because of cultural practices the sowthistle seedlings would have germinated at or after the time of germination of the lettuce plants.

This possible mode of disease transmission was postulated by Watson (1942) who recognised two types of infection of sugar beet viruses in the field. The first consisted of patches of varying size in which practically all plants were infected; the second consisted of a 'peppery' distribution of infection. She observed that the first type of infection occurred fairly early in the season and attributed this pattern of infection to outward movement of progeny of colonizing alates. The peppery distribution was interpreted to be probably due to aphid infestations containing high numbers of viruliferous alates which caused a number of scattered infections on their entry into the field. The above situation (i.e. type one infection) may occur in LNYV spread if

migrant viruliferous *H. lactucae* raise colonies on sowthistle seedlings growing in a lettuce crop.

## 6.2 Layout of experimental plots

The experiment involved treatments in which the morphological form (alatae and apterae) of the aphids on sowthistles were manipulated. Ideally plots for each treatment in such an experiment should be large and well separated from each other. However, the restrictions of land availability and of labour limited the experiment to only two separate sites, ½ mile apart and the plots to 64 plants each. Consequently the treatments involving apterous aphids were located at one of these sites (Claremont orchard) and the treatment involving alate aphids was located in the other (Alverstoke orchard).

At each site eight rows of lettuce seeds were planted, rows 1, 2, 3 and 4, and 5, 6, 7 and 8 being 21" apart. Rows 4 and 5 were 42" apart to allow the placing of infected and aphid infested sowthistle plants. After germination, the seedlings were thinned to 12" spacing and plots 8 plants long (64 plants per plot) were marked, allowing guard rows of four plants wide between them (Figures 6.1 and 6.2 a, b).

## 6.3 Treatments

The treatments which consisted of two replicates were as follows:

Treatment A: old infected sowthistle plants infested mostly with alates and in which apterae were prevented from moving onto the lettuce plants by means of a water barrier (see Section 2.62 and Figures 6.1, 6.2a).

Figure 6.1 Plan of field plots for the experiment on field spread of LNYV by *E. lactucae* (a, Alverstoke; b, Claremont).

A, old sowthistle infested with alate aphids.

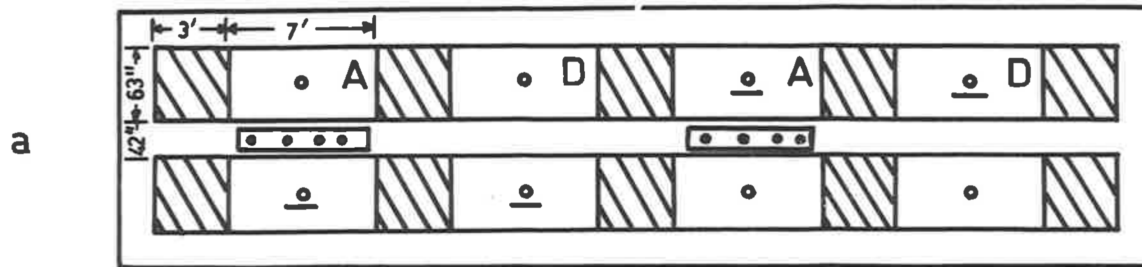
B, old sowthistle infested with apterous aphids.

C, sowthistle seedlings infested with apterous progeny of infected alate aphids.

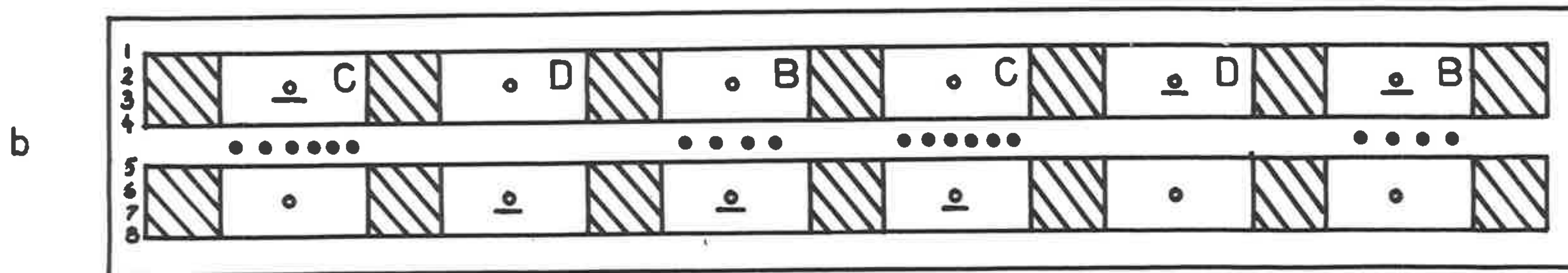
D, plot with neither sowthistle nor aphids.

1-8, rows of lettuce plants.

a




b




WATER TRAP ON A BRICK

WATER TRAP ON SOIL

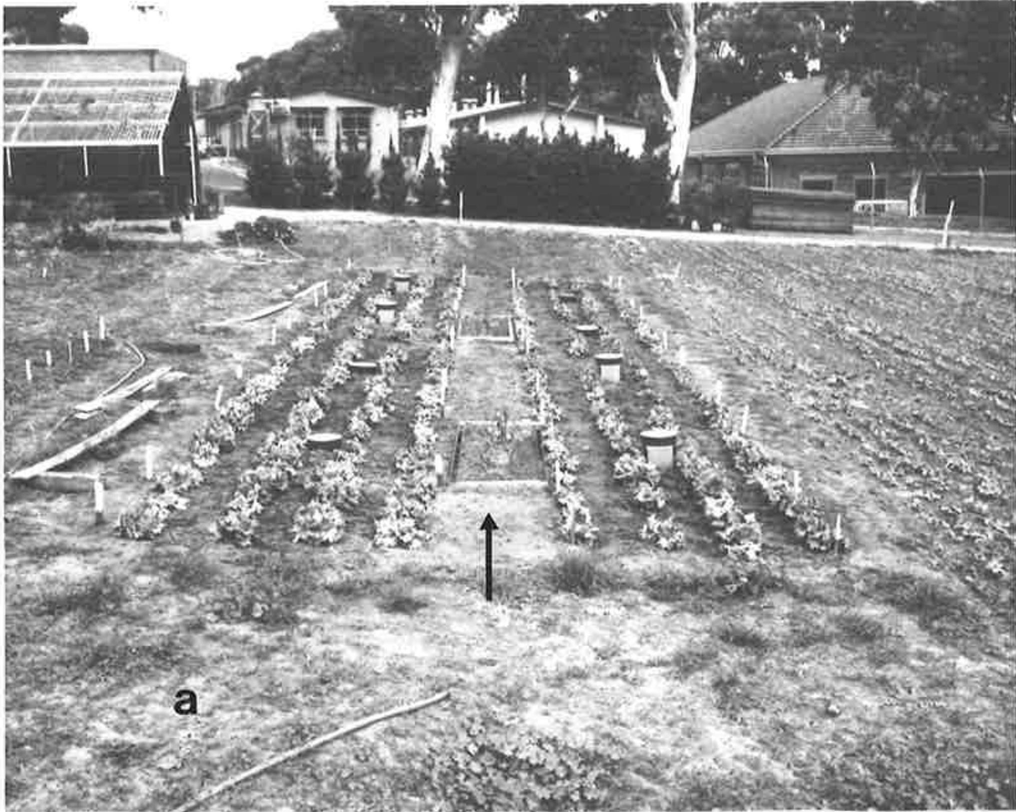
SOWTHISTLE PLANTS

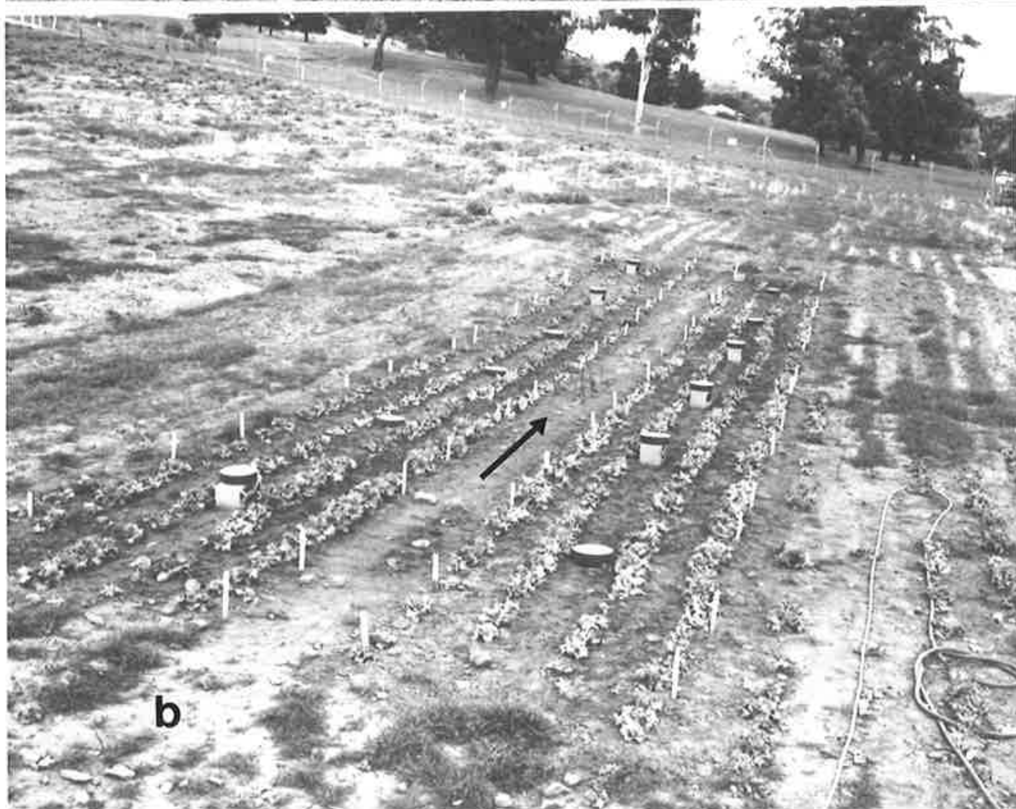
WATER BARRIER

GUARD ROW

gure 6.2a View of field plots for the experiment on field spread of LNYV by H. *lactucae* (Alverstoke orchard).
Arrow shows the metal gutter barrier used to prevent movement of apterous H. *lactucae* onto lettuce.

gure 6.2b View of field plots for the experiment on field spread of LNYV by H. *lactucae* (Claremont orchard).
Note LNYV-infected sowthistle plants (arrowed) which were infested with H. *lactucae* before commencement of the experiment.


a


b

Treatment B: old infected sowthistle plants infested mostly with apterae (see Section 2.62). All newly moulted alates and some alatiform nymphs were collected daily from the sowthistle plants at sunrise at which time the aphids were virtually not disturbed so that these aphid populations produced no alates in the experimental plots.

Treatment C: sowthistle seedlings which had been inoculated with LNYV by viruliferous alate H. lactucae and were infested with apterous progeny of these aphids (see Section 2.63). Removal of newly moulted alates and alatiform nymphs was the same as in Treatment B.

Control plots: lettuce plants alone.

In treatments A, B and C the appropriate potted aphid-infested sowthistle plants were sunk in the soil so that the rims of pots were at soil level. The potted plants were placed midway between rows 4 and 5. Four potted plants were used in treatments A and B and six in treatment C. The potted plants were left in the field for six weeks. Two water traps (Figures 6.1 and 6.2 a, b) were put in each plot and trapped H. lactucae were collected at three-day intervals and counted.

## 6.4 Measurement of effects of treatment

To interpret the effect of treatment C it was necessary to determine the period after which LNYV became available to the progeny of the original viruliferous alate aphids on sowthistle seedlings. This was done by allowing virus-free apterous H. lactucae to larviposit on caged sowthistle seedlings which had been inoculated with LNYV (at the same time as in treatment C) by viruliferous alates 24 hr previously (see

Section 2.64). The maternal apterous aphids were left on these seedlings for 24 hr and were removed. A number of progeny were removed from the inoculated seedlings periodically and tested for infectivity by caging them on healthy sowthistle seedlings which were kept in a glasshouse. These seedlings as well as those inoculated by viruliferous alates were indexed. The latter seedlings were all positive for LNYV. Table 6.1 shows that in the field LNYV becomes available to _H._ _lactucae_ which develop on inoculated seedlings between 9 and 12 days after the seedlings have been inoculated.

Table 6.1 Availability of LNYV to aphids when virus-free _H._ _lactucae_ are allowed to breed on sowthistle which have been inoculated for 24 hr and aphids tested periodically for infectivity.

| Days after inoculation | 5 | 6 | 7 | 8 | 9 | 10 | 12 | 14 | 16 | 18 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Infectivity | - | - | - | - | o | o | + | + | + | + | + |

- Test seedlings not infected
o Test seedling died
+ Test seedling positive for LNYV

The effects of the treatments were measured in terms of the number of lettuce plants showing symptoms of disease at various times after the start of the experiment. The experiment was considered to have started on the day on which treatments were set up by planting out the relevant sowthistle plants. The first census to record lettuce plants

showing LNYV symptoms was taken 8 days after the aphid-infested sow-thistle plants had been put in the field (i.e. 4 weeks after the germination of lettuce seedlings) and thereafter censuses were taken at weekly intervals for ten weeks. All plants were examined during a census.

Diseased plants were arbitrarily scored as a, b and c depending upon the severity of symptoms. Plants which were assigned the symbol 'a' had leaves which showed mottling and a dark green coloration, these plants were usually stunted; 'b' had yellowish-green leaves; 'c' had yellowish leaves and often showed signs of imminent death.

Because of the similarity between symptoms of lettuce plants attacked by LNYV and TSWV (Stubbs and Grogan, 1963) it was necessary to confirm the presence of LNYV in diseased lettuce plants in the experimental plots. Twenty diseased lettuce plants were therefore selected at random from the plots and the extract from each plant (see Section 2.67) was mechanically inoculated onto *N. glutinosa* and White Burley tobacoo.

## 6.5 Results

### 6.51 Confirmation of LNYV in diseased lettuce plants

The twenty diseased lettuce plants which were indexed on *N. glutinosa* and White Burley tobacco were all positive for LNYV but not for TSWV. This suggests that the diseased lettuce plants in the experimental plots were infected with LNYV. This, together with the absence of any plants with TSWV-like symptoms,confirmed that LNYV was probably the only virus present.

6.52 Availability of LNYV to *H. lactucae* in treatment C

The sowthistle seedlings which were inoculated and put in the lettuce plots (treatment C) were indexed at the end of the experiment. They were all positive for LNYV indicating that they were successfully inoculated by the viruliferous alates placed on them 24 hr before they were put in the field (see Section 2.63). The presence of LNYV in these seedlings could also have been effected by migrant alate *H. lactucae* in the field. That the first virus inoculation was not probably due to migrant aphids is supported by the evidence that identically inoculated seedlings in which visits by migrant aphids were prevented (see Section 2.64) were also all positive for LNYV. The evidence that LNYV became available to *H. lactucae* which developed on these seedlings between 9 and 12 days (Table 6.1) suggests that the apterous aphids on the inoculated seedlings (treatment C) probably acquired LNYV at about the same period.

6.53 Alate *H. lactucae* caught in water traps

Regardless of position (Figure 6.1), water traps, which were placed on bricks, caught less alate aphids than those placed on the soil (Table 6.2). This may be attributed to a propable higher wind turbulence around the traps on bricks than around traps on soil. This may have blown some of the alighting aphids away from the traps. Total numbers of aphids caught in Alverstoke orchard were greater than in Claremont orchard probably as a result of alate *H. lactucae* being present on the sowthistle plants which were put in the Alverstoke plots (treatment A).

Table 6.2  Comparison of total numbers of alate *H. lactucae* caught in water traps which were either placed on bare soil or on brick in the lettuce plots.

| Treatment No. | Type of treatment | Location | Position of water trap | |
|---|---|---|---|---|
| | | | Brick | Soil |
| A | Old sowthistle, alate aphids | Alverstoke | 110 | 207 |
| Control | No sowthistle, no aphids | " | 104 | 175 |
| B | Old sowthistle, apterous aphids | Claremont | 90 | 116 |
| C | Sowthistle seedlings, apterous progeny of infected alates | " | 66 | 116 |
| Control | No sowthistle, no aphids | " | 85 | 113 |

### 6.54 The effects of treatments

Effects of the treatments can be measured in many ways. The simplest and crudest method is to record the total percentage of diseased plants in each treatment at the end of the experiment (i.e. 50 days after the start of the experiment) as in Table 6.3. The data in Table 6.3 show that even the control plots had a high incidence of disease, indicating a high background effect. Against this background effect, simple differences in the total percentage of diseased plants between treatments are obviously meaningless.

A number of methods were tried for 'correcting' for the high

Table 6.3 Percentage of LNYV infected lettuce plants in various treatments on the last day of disease census (50 days after start of experiment).

| Treatment No. | Type of treatment | Location | % diseased plants[a] |
|---|---|---|---|
| A | Old sowthistle, alate aphids | Alverstoke | 48 |
| Control | No sowthistle, no aphids | " | 40 |
| B | Old sowthistle, apterous aphids | Claremont | 79 |
| C | Sowthistle seedlings, apterous progeny of infected alates | " | 46 |
| Control | No sowthistle, no aphids | " | 40 |

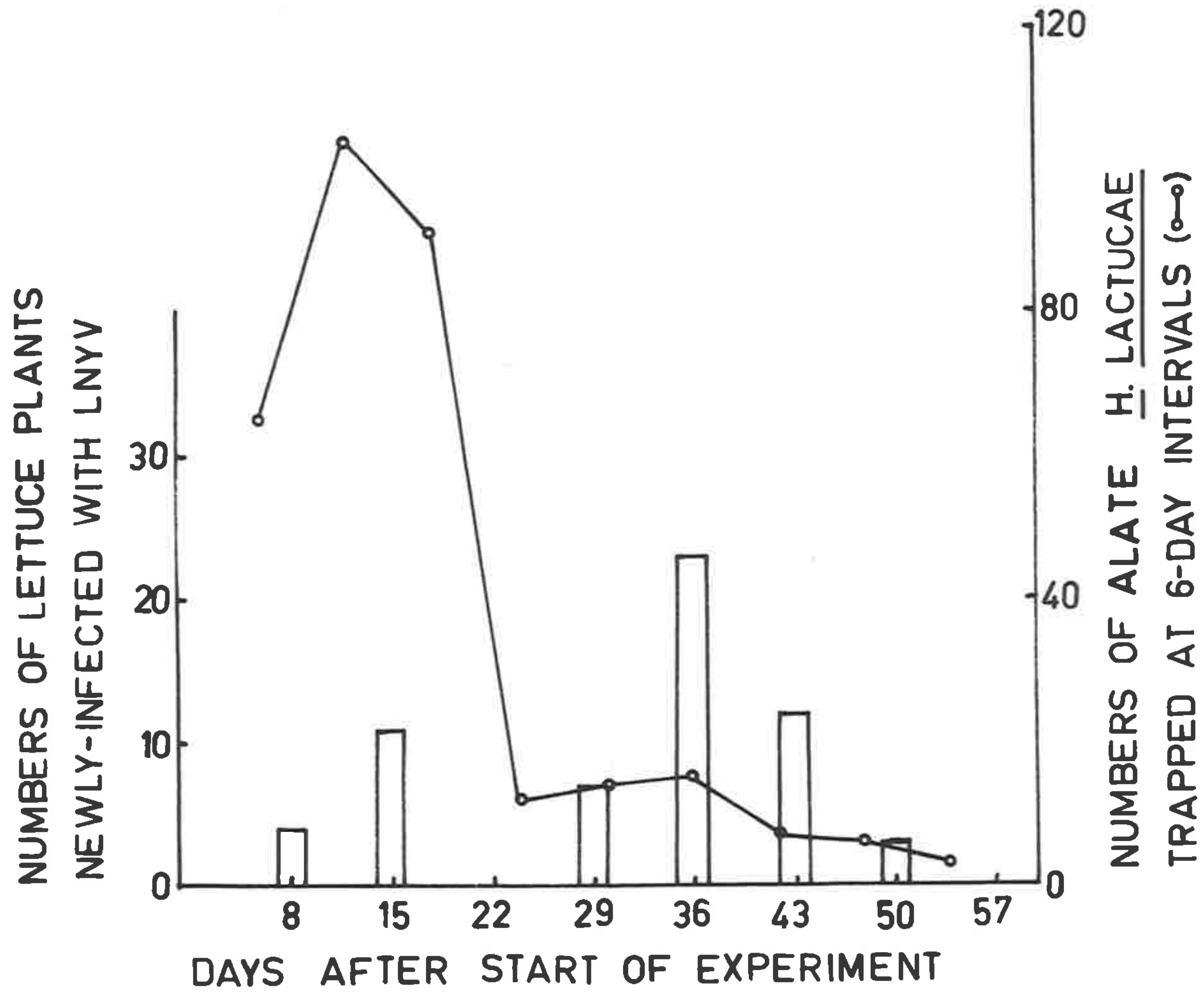
[a] based on total number of plants for the two replicates i.e. 128 plants.

background effect by weighting the results of the treatment plots with the results of the controls, but none of the transformed data thus obtained could be used with confidence. The only other form of data treatment that could be used for analysis was that of numbers of diseased plants at different times after the start of the experiment. A plant was regarded as being diseased if it showed symptoms on that date as well as on the following two census dates. To confirm infection in plants that showed symptoms for the first time on the day of the last (8th) census, two further examinations of plants were made at weekly intervals.

The increments in the numbers of diseased plants (totals of the two replicates) at each census date for each treatment are shown in Figure 6.3a, b, c, d and e; and since Randles and Crowley (1970) had suggested an association between numbers of alate _H. lactucae_ trapped and the peak incidence of LNYV, the numbers of alate _H. lactucae_ caught in water traps in the plots of each treatment are also shown in Figure 6.3 a - e. The data in all the figures suggest an association between peak trap catches of alate _H. lactucae_ and peak disease incidence with the high disease incidence occurring 24 to 33 days after the peak of aphid catches. The data of treatment A, illustrated in Figure 6.3a, when compared with those of the control (Figure 6.3b) indicate that the alate _H. lactucae_ which were added to the plot on the old sowthistle plants made no significant contribution to the occurrence of the disease in that plot. Similarly there was no significant difference in the numbers of trapped alate _H. lactucae_ between treatment A and the control. The data of treatment B (Figure 6.3c) when compared with the control (Figure 6.3e) indicate that the apterous aphids which were added to the plot on the old sowthistle plants made no significant contribution to disease incidence in that plot. Similarly the progeny of infected alate _H. lactucae_ which were added to the plot on sowthistle seedlings (treatment C, Figure 6.3d) made an insignificant contribution to incidence of LNYV in that plot.

Finally, to test the influence of the dispersing apterae in treatments B and C on the incidence of disease, a regression analysis was applied to the data to find whether there was a gradient in symptom appearance in relation to distance of lettuce plants from the source of

Figure 6.3a The incidence of LNYV in lettuce and the numbers of alate *H. lactucae* trapped in treatment A (old sowthistle with alate aphids). Alverstoke.



NUMBERS OF LETTUCE PLANTS NEWLY-INFECTED WITH LNYV
NUMBERS OF ALATE H. LACTUCAE TRAPPED AT 6-DAY INTERVALS
DAYS AFTER START OF EXPERIMENT
0
10
20
30
8
15
22
29
36
43
50
57
0
40
80
120

Figure 6.3b The incidence of LNYV in lettuce and the numbers of alate *H. lactucae* trapped in the control plot (no sowthistle nor aphids), Alverstoke.

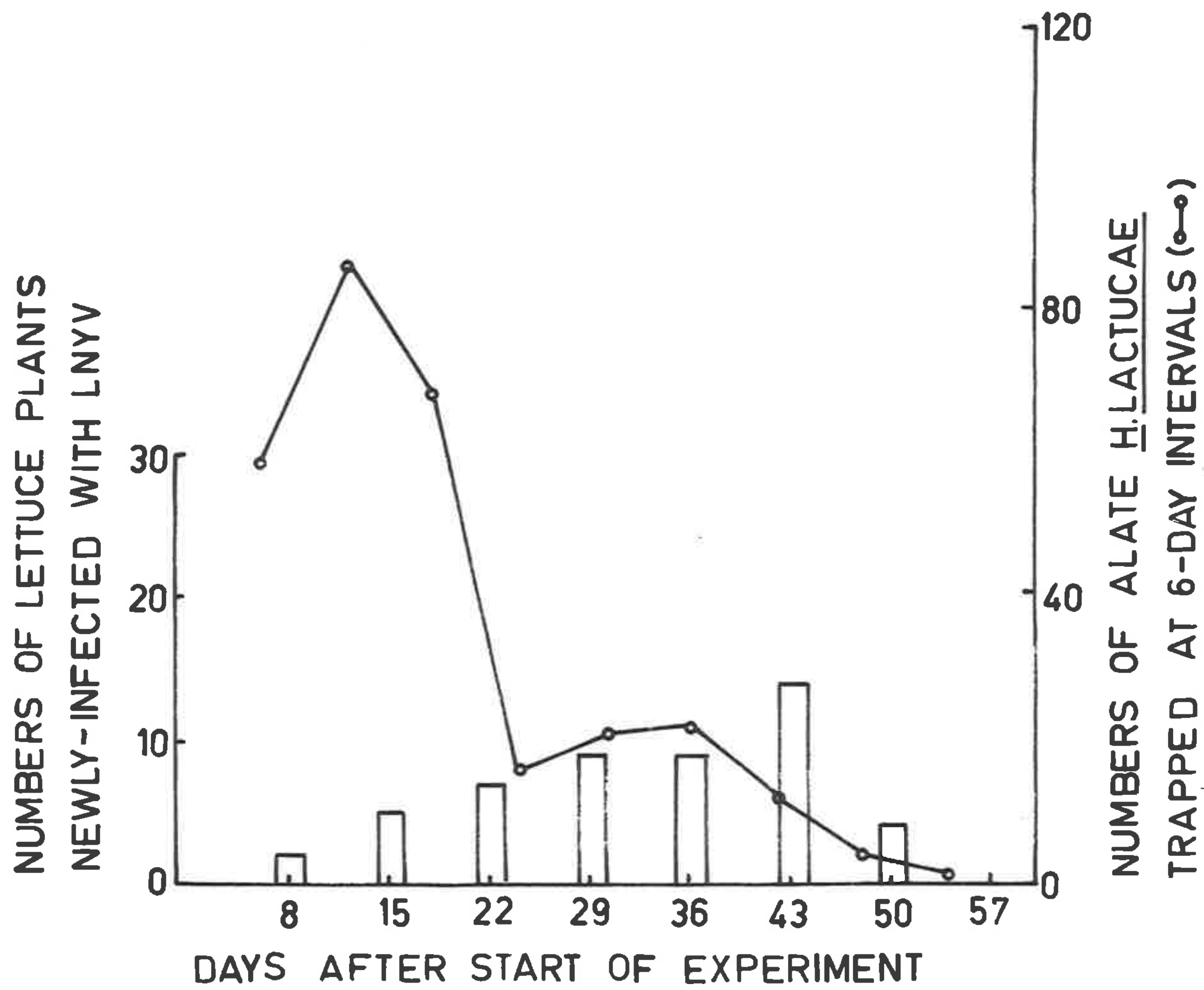
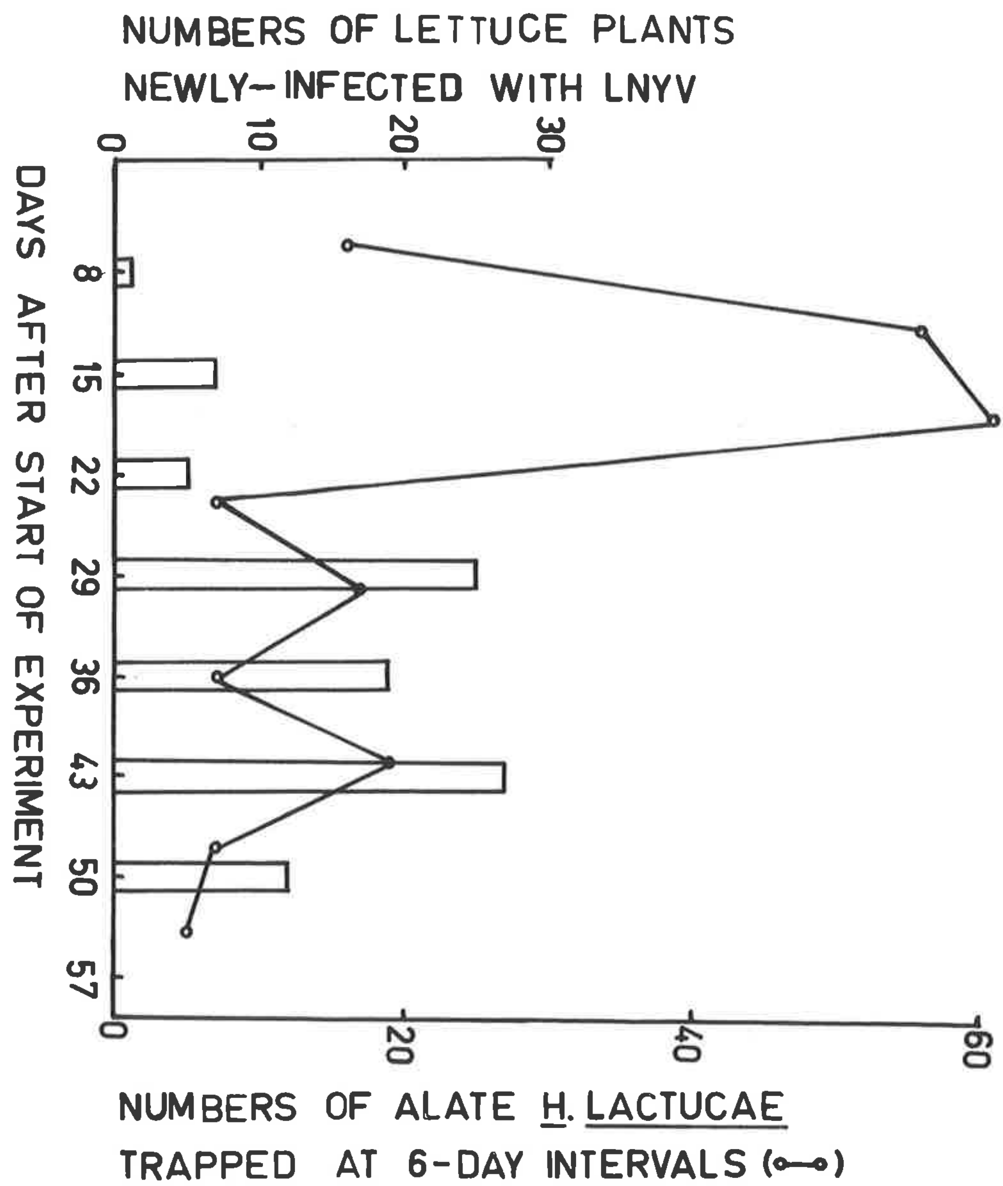
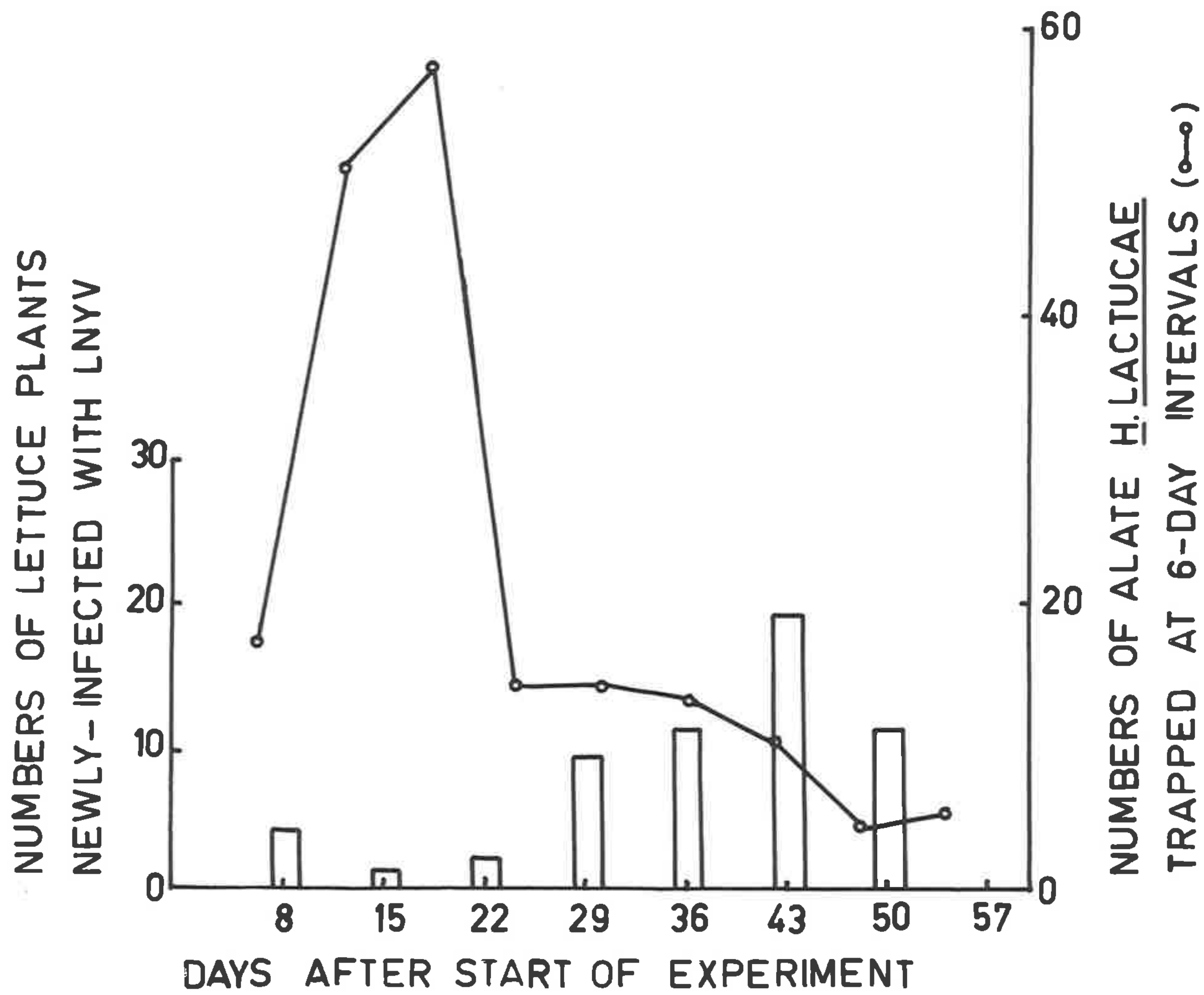

NUMBERS OF LETTUCE PLANTS
NEWLY-INFECTED WITH LNYV
30
20
10
0
8
15
22
29
36
43
50
57
DAYS AFTER START OF EXPERIMENT
120
80
40
0
NUMBERS OF ALATE H.LACTUCAE
TRAPPED AT 6-DAY INTERVALS

Figure 6.3c The incidence of LNYV in lettuce and the numbers of alate *H. lactucae* trapped in treatment B (old sowthistle with apterous aphids), Claremont.



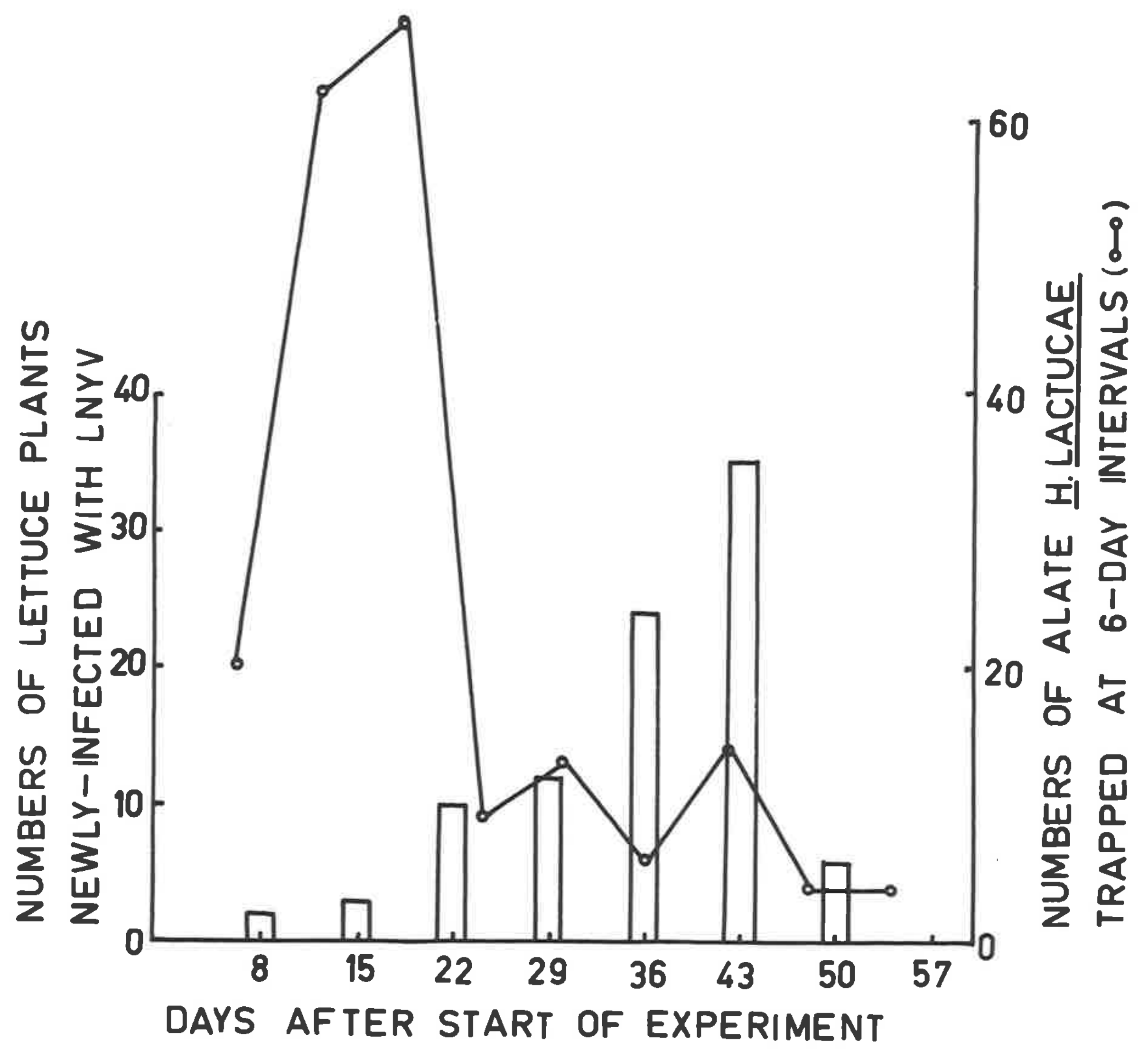
NUMBERS OF LETTUCE PLANTS
NEWLY-INFECTED WITH LNYV
0
10
20
30
DAYS AFTER START OF EXPERIMENT
8
15
22
29
36
43
50
57
0
20
40
60
NUMBERS OF ALATE H. LACTUCAE
TRAPPED AT 6-DAY INTERVALS (o—o)

Figure 6.3d    The incidence of LNYV in lettuce and the numbers of alate *H. lactucae* trapped in treatment C (sowthistle seedlings with apterous aphids), Claremont.



NUMBERS OF LETTUCE PLANTS
NEWLY- INFECTED WITH LNYV
30
20
10
0
8
15
22
29
36
43
50
57
DAYS AFTER START OF EXPERIMENT
60
40
20
0
NUMBERS OF ALATE H. LACTUCAE
TRAPPED AT 6-DAY INTERVALS

Figure 6.3e The incidence of LNYV in lettuce and the numbers of alate *H. lactucae* trapped in the control plot (no sowthistle nor aphids), Claremont.


NUMBERS OF LETTUCE PLANTS
NEWLY-INFECTED WITH LNYV
40
30
20
10
0
8
15
22
29
36
43
50
57
DAYS AFTER START OF EXPERIMENT
60
40
20
0
NUMBERS OF ALATE H. LACTUCAE
TRAPPED AT 6-DAY INTERVALS

infective aphids. The analysis had to allow for some lettuce plants that had died from causes other than disease due to LNYV, and the missing plants were corrected for as follows: Suppose W1 represents the number of lettuce plants present in a plot at any one time and W2 the maximum that should have been present then the inverse ratio W2/W1 was used as the weighting factor. Periods of first symptom appearance in a plant were then ranked from 1 to 7 (corresponding to 1st and 7th disease censuses) and 8 for those plants which had not shown visible disease symptoms by the 7th census. A regression analysis was carried out on the mean period of symptom appearance (by combining data of the two replicates of a treatment) on the order of the row. The results (Table 6.4) show that the coefficient of regression 'b' is significant ($P < 0.05$) in treatment B but not in treatment A or C. These results suggest that the apterous aphids in treatment B (old sowthistle plants) played a role in the spread of LNYV, whereas the apterous aphids in treatment C (on young, newly-infested sowthistle plants) apparently made no contribution to the incidence of LNYV. The data for treatment B are plotted in Figure 6.4.

Table 6.5 shows numbers of apterous _H. lactucae_ trapped at 6-day intervals in treatments B, C and the control. The maximum distance of a trap from a source of apterous aphids was approximately 9 ft. Though the number of apterous aphids trapped is small it at least gives an indication that apterous _H. lactucae_ are capable of walking across the soil and up into a trap.

ure 6.4 Regression of mean period of symptom appearance in lettuce on order of row.

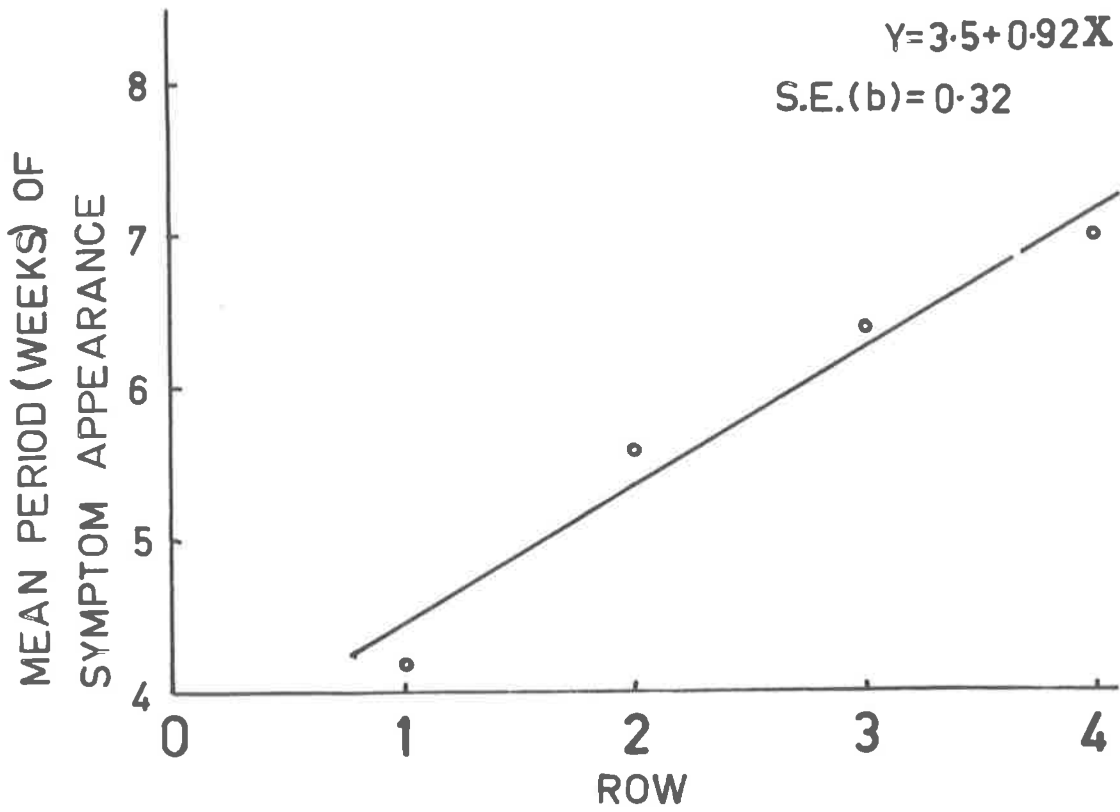

Y=3·5+0·92X
S.E.(b)=0·32
MEAN PERIOD (WEEKS) OF SYMPTOM APPEARANCE
8
7
6
5
4
0
1
2
3
4
ROW

Table 6.4 Regression coefficients (with their standard errors) for the regression of the mean period of symptom appearance and order of row of lettuce plants in relation to LNYV source.

| Treatment No. | Type of Treatment | Location | b | S.E. |
|---|---|---|---|---|
| A | Old sowthistle, alate aphids | Alverstoke | 0.37 | 0.88 |
| Control | No sowthistle, no aphids | " | 0.05 | 0.56 |
| B | Old sowthistle, apterous aphids | Claremont | 0.92* | 0.32 |
| C | Sowthistle seedlings apterous progeny of infected alates | " | -0.08 | 0.40 |
| Control | No sowthistle, no aphids | " | 0.21 | 0.32 |

* $P < 0.05$

## 6.6 Discussion

The high background infection (Table 6.3) was probably due to the unusually dry and warm autumn of 1972 which must have favoured the production of high aphid populations; and because of the small plot size visits by aphids (both alatae and apterae) from nearby treatment plots into the control plots could not be prevented. If these limitations had not been present the effects of the treatments may have been more clearly demonstrated. Despite these limitations it can be inferred from the results that:

1. Alate *H. lactucae* are important in the spread of LNYV (probably from outside the treatment plots) for the following reasons:

(a) association between peak disease incidence and flight activity (as indicated by trap catches); (b) apparent non-significant contribution

Table 6.5 Numbers of apterous H. lactucae trapped in water traps at 6-day intervals in lettuce plots (Claremont).

| Treatment No. | Type of Treatment | Days after start of experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 |
| B | Old sowthistle, apterous aphids | 8 | 3 | 2 | 1 | - | - | - | - |
| C | Sowthistle seedlings apterous progeny of infected alates | - | 1 | 1 | 2 | - | - | - | - |
| Control | No sowthistle, no aphids | 1 | 3 | - | 1 | - | 1 | - | - |

by alates put in the crop to numbers of lettuce plants infected;
(c) absence of a gradient in symptom appearance in all treatments except that containing old sowthistle plants infested with apterous aphids;
(d) the high background infection.

2. Apterous H. lactucae which develop on infected sowthistle plants may play an important role in the close range spread of LNYV because of (a) apterous aphids trapped in the water traps and (b) presence of a gradient in symptom appearance in lettuce plants in relation to virus source (treatment B). Spread of disease is effected by wandering apterous aphids.

3. Virus-free sowthistle seedlings which occur in a lettuce crop and which become colonized by infected migrant H. lactucae are unlikely to be an important source of infected aphids for within field spread of LNYV because of the absence of a gradient in symptom appearance in lettuce plants in treatment C.

It is generally agreed that aphid-borne plant viruses must spread from field to field by alate aphids (Ribbands, 1965; Broadbent, 1965); but the form of aphid responsible for within field spread of viruses has been controversial(Ossiannilsson, 1966). The importance attached to the spread of plant viruses by alate aphids is dependent primarily on (a) correlations between the numbers of alates trapped and disease incidence (Broadbent, 1950; Broadbent *et al.*, 1950; Broadbent and Tinsley, 1951; Hollings, 1955; Heitzel, 1962); (b) lack of correlation between numbers of apterae and virus spread (Watson and Healy, 1953) and (c) the use of insecticides to control virus spread (Hille Ris Lambers *et al.*, 1953; Heitzel and Müller, 1959; Burt *et al.*, 1964).

The most compelling evidence in favour of disease spread by alates can be obtained from the use of insecticides which prevent the development of apterous aphid populations but not visits by alates. Several investigations have been made into the use of insecticides to control the spread of potato viruses by aphids (e.g. Emilsson and Castberg, 1952; Schepers *et al.*, 1955). Emilsson and Castberg (1952) controlled aphids with parathion but not the spread of potato virus Y (PVY). Schepers *et al.*, (1955) sprayed potato plants twice weekly with nicotine from emergence to harvest. No apterous aphids were allowed to develop yet there was a considerable spread of PLRV and PVY and the distribution of infected plants in both treated and control plots was similar. They concluded that the spread of both viruses within and in to the field was caused by alates arriving from outside the field.

It is probable, as indicated by Broadbent (1965), that both alatae and apterae spread viruses within crops, and that the relative importance varies with crop, season, aphid species present and the size of population.

In the field, LNYV in inoculated sowthistle seedlings apparently becomes available to H. lactucae between 9 and 12 days after inoculation. On acquiring the virus the aphids require from 5 to 18 days (depending on temperature, see Chapter 3) for the completion of the latent period. Assuming that fluctuating temperatures in the field do not afffect the duration of latent period of LNYV in H. lactucae, it would therefore appear that the offspring of viruliferous migrant alate H. lactucae infesting sowthistle seedlings within the crop would require from 2 to 4 weeks to make any significant contribution to LNYV spread in a lettuce crop. Within this period viruliferous migrant alates would have already transmitted the disease to the lettuce crop. The absence of a gradient in the plot containing inoculated sowthistle seedlings (treatment C, Table 6.4) suggests that LNYV incidence was effected predominantly by migrating alate H. lactucae.

Despite the high background infection there was a significant relationship between period of symptom appearance and position of lettuce plants in relation to infective apterous H. lactucae (Table 6.4 and Figure 6.4). This effect must have been produced by apterae which walked off the infected sowthistle plants. It may be that the lettuce plants which were closer to the infective source of apterous aphids received more probes from the dispersing aphids and thus received greater amounts of LNYV inoculum than plants further away. The apparently high inoculum

injected into the plants could then have hastened the appearance of symptoms. Though there is no report about the dependence of the period of symptom appearance on the concentration of virus injected into plants by insect vectors, Raymer and Diener (1969) have demonstrated this phenomenon in tomato plants mechanically inoculated with potato spindle tuber virus (PSTV). Apterous H. lactucae which develop on old infected sowthistle plants close to a lettuce crop may therefore contribute to LNYV spread though their role may be eclipsed by the activity of infective migrant alates.

Association between peak numbers of alate H. lactucae and peak incidence of LNYV (Figures 6.3 a - e) confirms earlier findings by Randles and Crowley (1970) and suggests that alate H. lactucae may make the most significant contribution to LNYV spread in lettuce. The apparent non-significant contribution by alates put in the plots to numbers of lettuce plants infected indicates that the spread of LNYV in lettuce must come from outside sources but the distance is indeterminate (Johnson, 1969). The lapse of 24 to 33 days between peak alate catches and highest disease incidence may represent the incubation period in LNYV in lettuce in the field (cf. Randles and Crowley, 1970).

Alate aphids are known to alight and probe indiscriminately on host and non-host plants (Kennedy et al., 1959) and the most important characteristics affecting the efficiency of virus spread are the activity and ability of alate aphids to transmit a virus rather than the aphids' potential as crop pests (Kennedy, 1950). Spread of non-persistent viruses by transient aphid populations is known to occur. For instance

onion yellow dwarf (Drake et al., 1933), cantaloupe mosaic (Dickson et al., 1949), bean yellow mosaic (Swenson, 1957), potato viruses Y and B (Edwards, 1963) are transmitted to plants which are not colonized by the aphids. Transmission of SYVV (Duffus et al., 1970) and LNYV to lettuce are probably the only known examples of aphid transmission of persistent viruses to non-host plants of the aphid.

Ability of alate H. lactucae to transmit LNYV within 24 hr of reaching the adult stage, the long retention of the virus in the vector, the short probes by H. lactucae on lettuce which can effect LNYV transmission (see Chapter 3) and a likely indiscriminate alighting and probing on lettuce would particularly enhance the spread of LNYV by alate H. lactucae to a large number of plants.

## 7. EPIDEMIOLOGY OF LNYV - A GENERAL DISCUSSION

In modern agricultural practices monoculture is the rule rather than the exception and spread of viruses is much easier than in plants in a natural vegetation. Many of these viruses are known to spread between wild host plants as well as to crops and their spread is effected mostly by insects. It is therefore not surprising that most viruses have become associated with highly mobile insects such as aphids.

The spread of LNYV depends on the movements of infective *H. lactucae* into lettuce crops. The virus is acquired mainly from LNYV-infected sowthistle, the principal host plant of the aphid and the virus (Stubbs and Grogan, 1963), and to a lesser extent from *S. hydrophilus* and *E. megalocarpa* (Randles and Carver, 1971). Although sowthistle is an annual it occurs throughout the year and therefore contributes to the perpetuation of the virus; this may be supplemented by the perennials, *S. hydrophilus* and *E. megalocarpa* both of which can be infected by the virus and support populations of *H. lactucae* (Randles and Carver, 1971). Because of the occurrence of these LNYV reservoir plants throughout the year, it is unlikely that transovarial transmission of the virus in the vector would play a significant role in the perpetuation of LNYV (see Chapter 3).

As stated earlier, mobility or dispersal of a vector encourages the spread of plant viruses which infect crop plants. Emigration of insects from host plants or breeding sites is influenced both by currently adverse conditions of the environment (e.g. food shortage and

lack of space) and an endogenously controlled mechanism which results in the production of migrant forms of the insect (Johnson, 1969). The high rate of reproduction of _H. lactucae_ which occurs in small initial populations (see Chapter 5) is undoubtedly associated with the need to exploit the ephemerally suitable parts of the host plant. Over-exploitation of sowthistle is, however, curbed by the departure of the aphids (adult apterae and alatae) as the population grows. Furthermore, population increase leads to the production and emigration of high proportions of alatae (migrants). It is these migrant alates of _H. lactucae_ which are responsible for the spread of LNYV in lettuce crops (see Chapter 6).

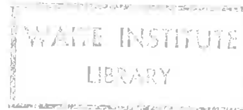
Within 24 hr of reaching the adult stage, apterous and alate _H. lactucae_ which had developed on LNYV-infected sowthistle are able to transmit the virus (see Chapter 3). This suggests that alatae and apterae which emigrate from infected sowthistle will be capable of infecting lettuce plants when these plants are encountered and probed by the aphids. Evidence for this possibility was obtained from the field experiment (see Chapter 6) in which it was demonstrated that apterous _H. lactucae_, which dispersed from infected sowthistle that were put in a lettuce crop, made a contribution to the spread of LNYV. Similarly, the correlation between peak catches of alate _H. lactucae_ and high disease incidence in lettuce (which confirms the study by Randles and Crowley (1970)) demonstrated that this morph is important in the spread of the disease. These alates acquired the virus from infected plants on which they developed and were infective by the time they arrived in

the lettuce crop. Although apterous *H. lactucae* which occur on LNYV-infected sowthistle near lettuce crops contribute to the spread of the virus their role is eclipsed by the activity of migrant and transient alate aphids (see Chapter 6).

Short probes by infective *H. lactucae* on lettuce can effect transmission of LNYV (see Chapter 3). This characteristic together with, (1) the fact that lettuce is a non-host plant of the vector (which suggests that the aphid is likely to test probe a lot of lettuce plants (Ferrar, 1967)), and (2) the persistence of inoculativity; will greatly increase the chances of each aphid spreading the disease to several plants. This may contribute to the high incidence of LNYV reported in cultivated lettuce crops (but see page 102).

The control of LNYV in lettuce crops depends on (1) the elimination or reduction of populations of *H. lactucae*; (2) eradication of the source of virus i.e. sowthistle, the principal host plant of the virus (Stubbs *et al.*, 1963); and (3) prevention of infection of lettuce by changes in cultural practices or by changing the areas of lettuce production.

Elimination of *H. lactucae* is not practicable because of the sparse but widespread distribution of sowthistle, the major aphid host plant. Reduction of numbers of the aphid by parasites and/or predators is a possible approach to disease control. It has been shown that population size is one of the factors which influence the production and dispersal of alate *H. lactucae* (see Chapter 5). Although parasites of *H. lactucae* are absent in Australia they are known to be present in Europe and the


WAITE INSTITUTE
LIBRARY



Far East Asian countries (Starý, 1966; Starý and Schlinger, 1967). Their introduction into Australia and manipulation could be a practical approach to the lowering of population levels of *H. lactucae* which in turn would lead to a decrease in the numbers of alates and hence LNYV spread.

Eradication or reduction of sowthistle populations would be another approach towards control of LNYV incidence. The use of herbicides or biological agents such as insects to eradicate sowthistle is unlikely to be successful because of the widespread distribution of the plant. However, elimination of sowthistle in the vicinity of lettuce crops may reduce close range transmission of the disease (Stubbs *et al.*, 1963).

Because of the difficulties which may arise if control measures of LNYV are geared to the elimination of the vector or total eradication of sowthistle, an alternative approach would be the prevention of virus introduction into lettuce crops by viruliferous *H. lactucae*. Use of several methods can be considered.

Kennedy (1965) suggested that in aphids there is a two-way interaction between flight and settling and each has a positive and negative after-effect on the other. Thus the less flight is excited prior to landing on a plant the greater the inhibitory effect on flight and vice versa. On non-host plants flight is usually less depressed and hence the aphids resort to rebound flight when they land on such plants. This characteristic rebound flight has been utilized for reducing the incidence of virus diseases by cultivating non-host barrier crops within crop plants. For instance, Heathcote (1968) was able to reduce the incidence

of BMYV, a persistent virus, in sugar beet stecklings by using barley, a non-host plant of the vector (*M. persicae*) and the virus, and mustard as cover crops. He observed that although barley made a poor screen (in contrast to mustard) it protected the beet against alate *M. persicae* and the virus. He further observed that the alate aphids did not stay long on barley and when they took off again few moved to the beet below; most of them flew upwards and out of the crop. The growing of barrier crops within lettuce may be useful in reducing the incidence of LNYV.

The repellent effect of certain colours to aphids is another possible approach to reducing LNYV incidence in lettuce. Many aphids distinguish between the two parts of the spectrum and they alight and probe in response to wave-lengths greater than 500 mμ (Moericke, 1950; Kennedy *et al.*, 1961). Both leaves and soil emit long-wave energy in contrast to light from the sky whose peak energy emission lies below 500 mμ; hence aphids' colour vision permits discrimination between the sky and the ground. It appears, however, that the change in aphids' behaviour from upper level to low level flights does not result from reversal of response to short-wave sky light (i.e. from positive to negative) but to a relative increase in response to long-wave light from the ground (Kennedy *et al.*, 1961). The response of flying aphids to colour and light has been utilized to devise methods that prevent aphids from alighting on crop plants. The use of aluminium foil to repel aphids was suggested by Kring (1964) and the repellent effect is probably due to the reflection of short-wave light from the aluminium surface (which also reflects long-wave light) (Kring, 1972). Aluminium foil has been

used to reduce the incidence of virus diseases introduced into crops by transient aphids (Johnson et al., 1967). Depending upon the economic feasibility, aluminium foil may be useful for the control of LNYV.

It has been demonstrated that in South Australia lettuce crops sown in mid-September and between early February and late March stand the greatest risk of LNYV infection. Maximum infection occurs in crops sown between six and ten weeks before peak numbers of alate H. lactucae are trapped (Randles and Crowley, 1970). Timing of planting dates of lettuce appears to be one of the methods which may lead to a decrease of LNYV incidence in a crop. It would also seem worthwhile to seek areas where the H. lactucae population is low; if such areas can be found they could be used for lettuce cultivation. For instance Fisken (1959) indicated that in summer, infestation of potato crops by virus-bearing aphids particularly M. persicae was high in the Fife and Angus area of Scotland and infestation decreased sharply further inland. He suggested that this condition was due probably to the liberation of alate M. persicae (which coincided with high infestations) from brassica crops in the Edinburgh area which were transported by southerly winds prevalent during the summer. Fisken (1959) further suggested that the low infestation in potato crops further inland could be attributed to the weakened condition of the air-borne aphids which prevented them from landing on potato crops in the area.

The present work has confirmed that alatae but not apterae of H. lactucae contribute significantly to the spread of LNYV in the field. It is therefore pertinent that investigations are made into the flight

behaviour of the aphid on which no information is at present available. In this regard it is suggested that future work in the following areas could be useful.

1. Because spread of LNYV in lettuce crops is effected by alate *H. lactucae* from outside the lettuce crop the questions that need to be answered are (a) How long does *H. lactucae* fly? and (b) How long can *H. lactucae* fly? Consequently it would be worthwhile if investigations are made into the direction and distance of trivial and migratory flights of the aphid. This would indicate the pattern of *H. lactucae* infestation state-wide and thus give an insight into the type of control measures that can be adopted. For example the infestation pattern could give an indication of areas of South Australia which have low or negligible population levels of the aphid and where LNYV incidence is likely to be low. Such areas could be used for cultivating lettuce.

2. Studies should be made into the factors which influence the landing and probing of alate aphids on sowthistle and lettuce. In this regard, studies on the effect of flight on landing and probing behaviour of the aphid would be worth investigating. In addition it may be useful to study the mechanism of host selection in *H. lactucae*. The suggestion that alate *H. lactucae* may indiscriminately alight and probe on lettuce plants is conjectural. The results of such studies would perhaps permit the use of appropriate barrier crops or reflective surfaces within lettuce to reduce the incidence of LNYV.

BIBLIOGRAPHY

ANDREWARTHA, H.G. and BIRCH, L.C. (1954). The distribution and abundance of animals.
University of Chicago Press, Chicago, 782 pp.

BALD, J.G. and SAMUEL, G. (1931). Investigations on "Spotted Wilt" of tomatoes II.
C.S.I.R.O. Australia Bull. No. 54. 24 pp.

BEAN, J.L. (1961). Predicting emergence of second-instar spruce budworm larvae from hibernation under field conditions in Minnesota.
Ann. ent. Soc. Am. 54: 175-177.

BEST, R.J. (1968). Tomato spotted wilt virus.
Adv. Virus Res. 13: 65-146.

BLACK, L.M. (1950). A plant virus that multiplies in its insect vector.
Nature, Lond. 166: 852-853.

BLACK, L.M. (1953). Occasional transmission of some plant viruses through the eggs of their insect vectors.
Phytopathology 43: 9-10.

BLACK, L.M. (1959). Biological cycles of plant viruses in insect vectors.
In The Viruses 2: 157-185 (Ed. Burnet, F.M. and Stanley, W.M.) Academic Press, New York.

BJÖRLING, K. and OSSIANNILSSON, F. (1958). Investigations on individual variations in the virus-transmitting ability of different aphid species.
Socker 14: 1-13.

BODENHEIMER, F.S. and SWIRSKI, E. (1957). The Aphidoidea of the Middle East.
Weizmann Science Press, Jerusalem, 378 pp.

BONNEMAISON, L. (1951). Contribution à l'etude des facteurs provoquant l'apparition des formes ailées et sexuées chez les Aphidinae.
Annls Epiphyt. 2: 1-380.

BRADLEY, R.H.E. (1952). Studies on the aphid transmission of a strain of henbane mosaic virus.
Ann. appl. Biol. 39: 78-97.

BROADBENT, L. (1950). The correlation of aphid numbers with the spread of leaf roll and rugose mosaic in potato crops.
Ann. appl. Biol. 37: 58-65.

BROADBENT, L. (1965). The importance of alate aphids in virus spread within crops.
Proc. XII int. Congr. Ent. (London, 1964), pp 523-524.

BROADBENT, L., CHAUDHURI, R.P. and KAPICA, L. (1950). The spread of virus diseases to single potato plants by winged aphids.
Ann. appl. Biol. 37: 355-362.

BROADBENT, L. and TINSLEY, T.W. (1951). Experiments on the colonization of potato plants by apterous and alate aphids in relation to the spread of virus diseases.
Ann. appl. Biol. 38: 411-424.

BROWNING, T.O. (1952). The influence of temperature on the rate of development of insects, with special reference to the eggs of Gryllulus commodus Walker.
Aust. J. scient. Res. (B) 5: 96-111.

BURT, P.E., HEATHCOTE, G.D. and BROADBENT, L. (1964). The use of insecticides to find when leaf roll and Y viruses spread within potato crops.
Ann. appl. Biol. 54: 13-22.

COTTIER, W. (1953). Aphids of New Zealand.
D.S.I.R. New Zealand Bull. No. 106. 382 pp.

CROWLE, A.J. (1961). Immunodiffusion.
Academic Press, New York, 333 pp.

DADD, R.H. (1970). Arthropod nutrition.
In Chemical Zoology 5: 35-95 (Ed. Florkin, M. and Scheer, B.T.). Academic Press, New York.

DAVIDSON, J. (1942). On the speed of development of insect eggs at constant temperatures.
Aust. J. exp. Biol. med. Sci. 20: 233-239.

DAVIDSON, J. (1944). On the relationship between temperature and the rate of development of insects at constant temperatures.
J. Anim. Ecol. 13: 26-38.

DAY, M.F. and IRZYKIEWICZ, H. (1954). On the mechanism of transmission of non-persistent phytopathogenic viruses by aphids.
Aust. J. biol. Sci. 7: 251-273.

DICKSON, R.C., SWIFT, J.E., ANDERSON, L.D. and MIDDLETON, J.T. (1949). Insect vectors of cantaloupe mosaic in California's desert valleys.
J. econ. Ent. 42: 770-774.

DIXON, A.F.G. (1963). Reproductive activity of the sycamore aphid, Drepanosiphum platanoides (Schr.) (Hemiptera, Aphididae).
J. Anim. Ecol. 32: 33-48.

DRAKE, C.J., TATE, H.D. and HARRIS, H.M. (1933). The relationship of aphids to the transmission of yellow dwarf of onions.
J. econ. Ent. 26: 841-846.

DUFFUS, J.E. (1963). Possible multiplication in the aphid vector of sowthistle yellow vein virus, a virus with an extremely long insect latent period.
Virology 21: 194-202.

DUFFUS, J.E. and RUSSELL, G.E. (1969). Sowthistle yellow vein virus in England.
Pl. Path. 18: 144.

DUFFUS, J.E., ZINK, F.W. and BARDIN, R. (1970). Natural occurrence of sowthistle yellow vein virus on lettuce.
Phytopathology 60: 1383-1384.

EASTOP, V.F. (1958). A study of the Aphididae (Homoptera) of East Africa.
H.M. Stationery Office: London, 126 pp.

EASTOP, V.F. (1961). A study of the Aphididae (Homoptera) of West Africa.
British Museum, London, 93 pp.

EDWARDS, J.S. (1963). A non-colonizing aphid vector of potato virus diseases.
Nature, Lond. 200: 1233-1234.

EHRHARDT, P. (1961). Zur Nahrungsaufnahme von *Megoura viciae* Buckt., einer phloemsaugenden Aphide (Homoptera, Rhynchota).
Experientia 17: 461-462.

EMILSSON, B. and CASTBERG, C. (1952). Control of potato aphids by spraying with parathion and the effect on spread of virus Y.
Acta Agric. scand. 2: 247-257.

ESAU, K., NAMBA, R. and RASA, E.A. (1961). Studies on penetration of sugar beet leaves by stylets of *Myzus persicae*.
Hilgardia 30: 517-529.

FERRAR, P. (1967). Interplant movement of apterous aphids with special reference to *Myzus persicae* (Sulz.) (Hem., Aphididae)
Bull. ent. Res. 58: 653-660.

FISKEN, A.G. (1959). Factors affecting the spread of aphid-borne viruses in potato in Eastern Scotland II. Infestation of the potato crop by potato aphids, particularly *Myzus persicae* (Sulzer).
Ann. appl. Biol. 47: 264-273.

FRANCKI, R.I.B. (1973). Plant Rhabdoviruses.
Adv. Virus Res. 18: (in press).

FRANCKI, R.I.B. and RANDLES, J.W. (1972). RNA-dependent RNA polymerase associated with particles of lettuce necrotic yellows virus.
Virology 47: 270-275.

FRAZIER, N.W. (1968). Transmission of strawberry crinkle virus by the dark strawberry aphid Chaetosiphon jacobi.
Phytopathology 58: 165-172.

FUKUSHI, T. (1933). Transmission of the virus through the eggs of an insect vector.
Proc. imp. Acad. Japan 9: 457-460.

FUKUSHI, T. (1939). Retention of virus by its insect vectors through several generations.
Proc. imp. Acad. Japan 15: 142-145.

GARRETT, R.G. (1971). The mechanism of transmission of non-persistent viruses by aphids.
Ph.D. Thesis, University of Adelaide, pp 111.

GIBSON, R.W. (1972). The distribution of aphids on potato leaves in relation to vein size.
Entomologia exp. Appl. 15: 213-223.

GRYLLS, N.E. (1954). Rugose leaf curl - a new virus disease transovarially transmitted by the leafhopper Austroagallia torrida.
Aust. J. biol. Sci. 7: 47-58.

HAGAN, H.R. (1951). Embryology of the viviparous insects.
Ronald Press Company, New York, 472 pp.

HARRISON, B.D., FINCH, J.T., GIBBS, A.J., HOLLINGS, M., SHEPHERD, R.J., VALENTA, V. and WETTER, C. (1971). Sixteen groups of plant viruses.
Virology 45: 356-363.

HEATHCOTE, G.D. (1968). Protection of sugar beet stecklings against aphids and viruses by cover crops and aluminium foil.
Pl. Path. 17: 158-161.

HEATHCOTE, G.D. and COCKBAIN, A.J. (1966). Aphids from mangold clamps and their importance as vectors of beet viruses. Ann. appl. Biol. 57: 321-336.

HEINE, E. (1955). The flight activity of the sycamore aphid, Drepanosiphum platanoides Schr. (Hemiptera, Aphididae). J. Anim. Ecol. 24: 388-394.

HEINZE, K. (1959). Versusche zur Ermittlung der Haltbarkeit des Blattroll-Virus der Kartoffel und des Virus der Vergilbungskrankheit der Rube im Ubertrager. Arch. Virusforsch. 9: 396-410.

HEROLD, F. and MUNZ, K. (1967). Morphological studies of maize mosaic virus I. J. gen. Virol. 1: 227-234.

HILLE RIS LAMBERS, D. (1949). Contributions to a monograph of the Aphididae of Europe IV. Temminckia 8: 182-323.

HILLE RIS LAMBERS, D., REESTMAN, A.J. and SCHEPERS, A. (1953). Insecticides against aphid vectors of potato viruses. Neth. J. agric. Sci. 1: 188-201.

HILLS, G.J. and CAMPBELL, R.N. (1968). Morphology of broccoli necrotic yellows virus. J. Ultrastruct. Res. 24: 134-144.

HOLLINGS, M. (1955). Aphid movement and virus spread in seed potato areas of England and Wales, 1950-53. Pl. Path. 4: 73-82.

HOOF, H.A. van (1961). Where do virus and aphid meet inside the plant? In Recent Advances in Botany. University of Toronto Press, Toronto, pp. 522-528.

HOWATSON, A.F. (1970). Vesicular stomatitis and related viruses. Adv. Virus Res. 16: 195-256.

HOWE, R.W. (1967). Temperature effects on embryonic development in insects. A. Rev. Ent. 12: 15-42.

HUGHES, R.D. and WOOLCOTT, L.T. (1965). A modification of Johnson's method of rearing aphids, for ecological studies. N.Z. Jl. agric. Res. 8: 728-736.

HULL, R. (1970). Large RNA plant-infecting viruses. In The Biology of large RNA viruses pp 153-163. (Ed. Barry, R.D. and Mahy, B.W.J.). Academic Press, New York.

HUNTER-JONES, P. (1968). The effect of constant temperature on egg development in the desert locust *Schistocerca gregaria* (Forsk.). Bull. ent. Res. 59: 707-718.

IBBOTSON, A. and KENNEDY, J.S. (1959). Interaction between walking and probing in *Aphis fabae* Scop. J. exp. Biol. 36: 377-390.

JOHNSON, B. (1957). Studies on the degeneration of the flight muscles of alate aphids - I. A comparative study of the occurrence of muscle breakdown in relation to reproduction in several species. J. Insect Physiol. 1: 248-256.

JOHNSON, B. (1958). Factors affecting the locomotor and settling responses of alate aphids. Anim. Behav. 6: 9-26.

JOHNSON, B. (1965). Wing polymorphism in aphids II. Interaction between aphids. Entomologia exp. Appl. 8: 49-64.

JOHNSON, B. (1966). Wing polymorphism in aphids III. The influence of the host plant. Entomologia exp. Appl. 9: 213-222.

JOHNSON, B. and BIRKS, P.R. (1960). Studies on wing polymorphism in aphids I. The developmental process involved in the production of the different forms. Entomologia exp. Appl. 3: 327-339.

JOHNSON, C.G. (1960). A basis for a general system of insect migration and dispersal by flight.
Nature, Lond. 186: 348-350.

JOHNSON, C.G. (1969). Migration and dispersal of insects by flight.
Methuen and Co. Ltd., London, 763 pp.

JOHNSON, C.G., HAINE, E., COCKBAIN, A.J. and TAYLOR, L.R. (1957). Moulting rhythm in the alienicolae of Aphis fabae Scop. (Hemiptera: Aphididae) in the field.
Ann. appl. Biol. 45: 702-708.

JOHNSON, C.G. and TAYLOR, L.R. (1957). Periodism and energy summation with special reference to flight rhythm in aphids.
J. exp. Biol. 34: 209-221.

JOHNSON, G.V., BING, A. and SMITH, F.F. (1967). Reflective surfaces to repel dispersing aphids and reduce spread of aphid-born cucumber mosaic virus in gladiolus plantings.
J. econ. Ent. 60: 16-18.

KENNEDY, J.S. (1950). Aphid migration and the spread of plant viruses.
Nature, Lond. 165: 1024-1025.

KENNEDY, J.S. (1956). Phase transformation in locust biology.
Biol. Rev. 31: 349-370.

KENNEDY, J.S. (1965). Co-ordination of successive activities in an aphid. Reciprocal effects of settling on flight.
J. exp. Biol. 43: 489-509.

KENNEDY, J.S. (1966). The balance between antagonistic induction and depression of flight activity in Aphis fabae Scopoli.
J. exp. Biol. 45: 215-228.

KENNEDY, J.S., BOOTH, C.O. (1963a). Free flight of aphids in the laboratory.
J. exp. Biol. 40: 67-85.

KENNEDY, J.S. and BOOTH, C.O. (1963b). Co-ordination of successive activities in an aphid. The effect of flight on settling responses.
J. exp. Biol. 40: 351-369.

KENNEDY, J.S. and BOOTH, C.O. (1964). Co-ordination of successive activities in an aphid. Depression of settling after flight.
J. exp. Biol. 41: 805-824.

KENNEDY, J.S., BOOTH, C.O. and KERSHAW, W.J.S. (1959). Host finding by aphids in the field II. Aphis fabae Scop. (Gynoparae) and Brevicoryne brassicae L.; with a re-appraisal of the role of host-finding behaviour in virus spread.
Ann. appl. Biol. 47: 424-444.

KENNEDY, J.S., BOOTH, C.O. and KERSHAW, W.J.S. (1961). Host finding by aphids in the field III. Visual attraction
Ann. appl. Biol. 49: 1-21.

KENNEDY, J.S., DAY, M.F. and EASTOP, V.F. (1962). A conspectus of aphids as vectors of plant viruses.
Commonw. Inst. Ent. London, 114 pp.

KITAJIMA, E.W. and COSTA, A.S. (1966). Morphology and developmental stages of Gomphrena virus.
Virology 29: 523-539.

KLOFT, W. (1960). Wechselwirkungen zwischen pflanzensaugenden Insekten und den von ihnen besogenen pflanzengeweben.
Z. angew. Ent. 45: 337-381.

KRING, J.B. (1964). New ways to repel aphids.
Front. Plant Sci. 17: 6-7.

KRING, J.B. (1972). Flight behaviour of aphids.
A. Rev. Ent. 17: 461-492.

LAMB, K.P. (1963). Water loss from aphids at moderate temperatures.
J. Insect Physiol. 9: 703-711.

LAMB, K.P., EHRHARDT, P. and MOERICKE, V. (1967). Labelling of aphid saliva with Rubidium - 86.
Nature, Lond. 214: 602-603.

LEE, P.E. (1967). Morphology of wheat striate mosaic virus and its localization in infected cells.
Virology 33: 84-94.

LEES, A.D. (1966). The control of polymorphism in aphids.
Adv. Insect Physiol. 3: 207-277.

LEES, A.D. (1967). The production of the apterous and alate forms in the aphid Megoura viciae Buckton, with special reference to the role of crowding.
J. Insect Physiol. 13: 289-318.

LIPKA, J. (1918). Graphical and mechanical computation.
John Wiley and Sons Inc., New York, 259 pp.

MADGE, D.S. (1961). The control of relative humidity with aqueous solutions of sodium hydroxide.
Entomologia exp. Appl. 4: 143-147.

McLEAN, G.D. and FRANCKI, R.I.B. (1967). Purification of lettuce necrotic yellows virus by column chromatography on calcium phosphate gel.
Virology 31: 585-591.

McLEAN, G.D., WOLANSKI, B.S. and FRANCKI, R.I.B. (1971). Serological analysis of lettuce necrotic yellows virus preparations by immunodiffusion.
Virology 43: 480-487.

MACLEOD, R., BLACK, L.M. and MOYER, F.H. (1966). The fine structure and intracellular localization of potato yellow dwarf virus.
Virology 29: 540-552.

MARAMOROSCH, K. (1963). Arthropod transmission of plant viruses.
A. Rev. Ent. 8: 369-414.

MATTHEWS, R.E.F. (1960). Properties of nucleoprotein fractions isolated from turnip yellow mosaic virus preparations.
Virology 12: 521-539.

MILES, P.W. (1959). Secretion of two types of saliva by an aphid.
Nature, Lond. 183: 756.

MITTLER, T.E. and DADD, R.H. (1966). Food and wing determination in *Myzus persicae* (Homoptera:Aphididae).
Ann. ent. Soc. Am. 59: 1162-1166.

MIYAMOTO, S. and MIYAMOTO, Y. (1966). Notes on aphid-transmission of potato leafroll virus.
Sci Rep. Hyogo Univ. Agr. (Series Plant Protection) 7: 51-66.

MOERICKE, V. (1950). Über das Farbsehen der Pfirsichblattlaus (*Myzodes persicae* Sulz.).
Z. Tierpsych. 7: 265-274.

MOERICKE, V. (1951). Eine Farbfalle zur Kontrolle des Fluges von Blattlausen, insbesondere der Pfirsichblattlaus.
NachrBl. dt. PflSchutzdienst (Stuggart) 3: 23-24.

MOERICKE, V. (1955). Über die Lebensgewohnheiten der geflugelten Blattläuse (Aphidina) unter besonderer Berücksichtigung des Verhaltens beim Landen.
Z. angew. Ent. 37: 29-91.

MURDIE, G. (1969). Some causes of size variation in the pea aphid, *Acyrthosiphon pisum* Harris.
Trans. R. ent. Soc. Lond. 121: 423-442.

NAITO, A. (1965). On relation between the feeding behaviour of *Laodelphax striatellus* Fallen (Delphacidae) and the ovarial maturation.
Jap. J. appl. Ent. Zool. 9: 305-306.

NASU, S. (1965). Electron microscopic studies on transovarial passage of rice dwarf virus.
Jap. J. appl. Ent. Zool. 9: 225-237.

NEITZEL, K. (1962). Beziehungen zwischen dem Auftreten von virus übertragenden Blattläusen und Viruskrankheiten bei Kartoffeln in der Deutschen Demokratischen Republik.
NachrBl. dt. PflSchutzdienst (Berlin) 16: 130-134.

NEITZEL, K. and MÜLLER, H.J. (1959). Erhoehter virusbefall in den Randreihen von Kartoffelbestaenden als Folge des Flugverhaltens der Vektoren.
Entomologia exp. Appl. 2: 27-37.

O'LOUGHLIN, G.T. and CHAMBERS, T.C. (1967). The systemic infection of an aphid by a plant virus.
Virology 33: 262-271.

O'LOUGHLIN, G.T. and CHAMBERS, T.C. (1969). The feeding sites in Sonchus oleraceus of Hyperomyzus lactucae, the aphid vector of lettuce necrotic yellows virus.
Aust. J. biol. Sci. 20: 629-637.

OMAN, P. (1969). Criteria of specificity in virus-vector relationships. In Viruses, vectors, and vegetation pp 1-22.
(Ed. Maramorosch, K.).
Interscience Publishers, New York.

OSBORN, H.T. (1935). Incubation period of pea mosaic in the aphid Macrosiphum pisi.
Phytopathology 25: 160-177.

OSSIANNILSSON, F. (1966). Insects in the epidemiology of plant viruses.
A. Rev. Ent. 11: 213-232.

PALIWAL, Y.V. (1968). Changes in relative virus concentration in Endria inimica in relation to its ability to transmit wheat striate mosaic virus.
Phytopathology 58: 386-387.

PEAIRS, L.M. (1927). Some phases of the relation of temperature to the development of insects.
Bull. West Virginia agric. Exp. Stn. No. 208: 1-62.

PEARSON, E.S. and HARTLEY, H.O. (1954). Biometrika tables for statisticians Vol. 1.
Cambridge University Press, Cambridge, 238 pp.

PIRONE, T.P. (1969). Mechanism of transmission of stylet-borne viruses. In Viruses, vectors, and vegetation pp 199-234.
(Ed. Maramorosch, K.).
Interscience Publishers, New York.

POLLARD, D.G. (1971). Some aspects of plant penetration by Myzus persicae (Sulz.) nymphs (Homoptera:Aphididae).
Bull. ent. Res. 61: 315-324.

POSNETTE, A.F. (1960). Some aspects of virus spread among plants by vectors.
Report of 7th Commonw. ent. Conf. London, pp 162-165.

PROKOPY, R.J. and GYRISCO, G.G. (1965a). Summer migration of the alfalfa weevil, Hypera postica (Coleoptera:Curculionidae).
Ann. ent. Soc. Am. 58: 630-641.

PROKOPY, R.J. and GYRISCO, G.G. (1965b). Diel flight activity of migrating alfalfa weevils, Hypera postica (Coleoptera: Curculionidae).
Ann. ent. Soc. Am. 58: 642-647.

RANDLES, J.W. and CARVER, M. (1971). Epidemiology of lettuce necrotic yellows virus in South Australia II. Distribution of virus, host plants, and vectors.
Aust. J. agric. Res. 22: 231-237.

RANDLES, J.W. and COLEMAN, D.F. (1970). Loss of ribosomes in Nicotiana glutinosa L. infected with lettuce necrotic yellows virus.
Virology 41: 459-464.

RANDLES, J.W. and CROWLEY, N.C. (1970). Epidemiology of lettuce necrotic yellows virus in South Australia I. Relationship between disease incidence and activity of Hyperomyzus lactucae (L.).
Aust. J. agric. Res. 21: 447-453.

RAYMER, W.B. and DIENER, T.O. (1969). Potato spindle tuber virus: a plant virus with properties of a free nucleic acid.
Virology 37: 343-350.

RIBBANDS, C.R. (1963). The spread of apterae of Myzus persicae (Sulz.) and of yellows viruses within a sugar-beet crop.
Bull. ent. Res. 54: 267-283.

RIBBANDS, C.R. (1965). The significance of apterous aphids in the spread of viruses within agricultural crops.
Proc. XII int. Congr. Ent. (London, 1964) pp 525-526.

RICHARDSON, J. and SYLVESTER, E.S. (1968). Further evidence of multiplication of sowthistle yellow vein virus in its aphid vector, Hyperomyzus lactucae. Virology 35: 347-355.

ROBERTS, F.M. (1940). Studies on the feeding methods and penetration rates of Myzus persicae Sulz., Myzus circumflexus Buckt., and Macrosiphum gei Koch. Ann. appl. Biol. 27: 348-358.

RODRIGUEZ, J.G. (1953). Detached leaf culture in mite nutrition studies. J. econ. Ent. 46: 713.

RODRIGUEZ, J.G. (1958). The comparative NPK nutrition of Panonychus ulmi (Koch) and Tetranychus telarius (L.) on apple trees. J. econ. Ent. 51: 369-373.

SCHEPERS, A., REESTMAN, A.J. and HILLE RIS LAMBERS, D. (1955). Some experiments with systox. Proc. 2nd Conf. Potato Virus Diseases (Lisse-Wageningen). pp 75-83.

SHAW, M.J.P. (1970a). Effects of population density on alienicolae of Aphis fabae Scop. I. The effect of crowding on the production of alatae in the laboratory. Ann. appl. Biol. 65: 191-196.

SHAW, M.J.P. (1970b). Effects of population density on alienicolae of Aphis fabae Scop. II. The effects of crowding on the expression of a migratory urge among alatae in the laboratory. Ann. appl. Biol. 65: 197-203.

SHAW, M.J.P. (1970c). Effects of population density on alienicolae of Aphis fabae Scop. III. The effect of isolation on the development of form and behaviour of alatae in a laboratory clone. Ann. appl. Biol. 65: 205-212.

SHELFORD, V.E. (1927). An experimental investigation of the relations of the codling moth to weather and climate. Bull. Ill. nat. Hist. Surv. 16: 311-440.

SINHA, R.C. and CHIYKOWSKI, L.N. (1969). Synthesis, distribution and some multiplication sites of wheat striate mosaic virus in a leafhopper. Virology 38: 679-684.

SLYKHUIS, J.T. and WATSON, M.A. (1958). Striate mosaic of cereals in Europe and its transmission by Delphacodes pellucida (Fab.). Ann. appl. Biol. 46: 542-553.

SNEDECOR, G.W. and COCHRAN, W.G. (1967). Statistical methods (6th Ed.). Iowa State University Press, Ames, 593 pp.

SOLOMON, M.E. (1957). Estimation of humidity with cobalt thiocyanate papers and permanent colour standards. Bull. ent. Res. 48: 489-506.

STARÝ, P. (1966). Aphid parasites of Czechoslovakia. W. Junk, The Hague, 242 pp.

STARÝ, P. (1970). Biology of aphid parasites (Hymenoptera:Aphidiidae) with respect to integrated control. W. Junk, The Hague, 643 pp.

STARÝ, P. and SCHLINGER, E.I. (1967). A revision of the Far East Asian Aphidiidae (Hymenoptera). W. Junk, The Hague, 204 pp.

STEGWEE, D. and PONSEN, M.B. (1958). Multiplication of potato leaf roll virus in the aphid Myzus persicae (Sulz.). Entomologia exp. Appl. 1: 291-300.

STUBBS, L.L. and GROGAN, R.G. (1963). Necrotic yellows: a newly recognized virus disease of lettuce. Aust. J. agric. Res. 14: 439-459.

STUBBS, L.L., GUY, J.A.D. and STUBBS, K.J. (1963). Control of lettuce necrotic yellows virus disease by the destruction of common sowthistle (Sonchus oleraceus). Aust. J. exp. Agric. Anim. Husb. 3: 215-218.

SUTHERLAND, O.R.W. (1967). Role of host plant in production of winged forms by a green strain of pea aphid Acyrthosiphon pisum Harris.
Nature, Lond. 216: 387-388.

SUTHERLAND, O.R.W. (1969). The role of the host plant in the production of winged forms by two strains of the pea aphid, Acyrthosiphon pisum.
J. Insect Physiol. 15: 2179-2201.

SUTHERLAND, O.R.W. and MITTLER, T.E. (1971). Influence of diet composition and crowding on wing production by the aphid Myzus persicae.
J. Insect Physiol. 17: 321-328.

SWENSON, K.G. (1957). Transmission of bean yellow mosaic virus by aphids.
J. econ. Ent. 50: 727-731.

SYLVESTER, E.S. (1954). Aphid transmission of non-persistent plant viruses with special reference to Brassica nigra virus.
Hilgardia 23: 53-98.

SYLVESTER, E.S. (1958). Aphid transmission of plant viruses.
Proc. 10th int. Congr. Ent. (Montreal, 1956) 3: 195-200.

SYLVESTER, E.S. (1962). Mechanisms of plant virus transmission by aphids.
In Biological transmission of disease agents pp 11-31.
(Ed. Maramorosch, K.).
Academic Press, New York.

SYLVESTER, E.S. (1965). The latent period of pea enation mosaic virus in the pea aphid, Acyrthosiphon pisum (Harris) - an approach to its estimation.
Virology 25: 62-67.

SYLVESTER, E.S. (1967). Retention of inoculativity in the transmission of pea-enation mosaic virus as associated with virus isolates, aphid reproduction and excretion.
Virology 32: 524-531.

SYLVESTER, E.S. (1969a). Virus transmission by aphids - a viewpoint. In Viruses, vectors, and vegetation pp 159-173. (Ed. Maramorosch, K.). Interscience Publishers, New York.

SYLVESTER, E.S. (1969b). Evidence of transovarial passage of the sowthistle yellow vein virus in the aphid Hyperomyzus lactucae. Virology 38: 440-446.

SYLVESTER, E.S. and RICHARDSON, J. (1966). "Recharging" pea aphids with pea enation mosaic virus. Virology 30: 592-597.

SYLVESTER, E.S. and RICHARDSON, J. (1969). Additional evidence of multiplication of the sowthistle yellow vein virus in an aphid vector - serial passage. Virology 37: 26-31.

TAYLOR, L.R. (1957). Temperature relations of teneral development and behaviour in Aphis fabae Scop. J. exp. Biol. 34: 189-208.

TAYLOR, L.R. (1958). Aphid dispersal and diurnal periodicity. Proc. Linn. Soc. Lond. 129: 67-73.

TOBA, H.H., PASCHKE, J.D. and FRIEDMAN, S. (1967). Crowding as the primary factor in the production of the agamic alate form of Therioaphis maculata (Homoptera:Aphididae). J. Insect Physiol. 13: 381-396.

TOMLINSON, J.A., WEBB, M.J.W. and FAITHFULL, E.M. (1972). Studies on broccoli necrotic yellows virus. Ann. appl. Biol. 71: 127-134.

TÓTH, L. (1933). Über die frühembryonale Entwicklung der viviparen Aphiden. Z. Morph. Ökol. Tiere 27: 692-731.

UICHANCO, L. (1924). Studies on the embryogeny and postnatal development of the Aphididae with special reference to the history of the 'symbiotic organ' or 'mycetom'. Philipp. J. Sci. 24: 143-247.

WANT, J.P.H., van der (1954). Onderzoekingen over Virusziekten van de Boom (Phaseolus vulgaris L.). Doctoral Thesis, Wageningen Agr. Univ. A. Veeman and Zonen, Wageningen.

WATSON, M.A. (1942). Sugar-beet yellows virus a preliminary account of experiments and observations on its effect in the field. Ann. appl. Biol. 29: 358-365.

WATSON, M.A. and HEALY, M.J.R. (1953). The spread of beet yellows and beet mosaic viruses in the sugar-beet root crop II. The effect of aphid numbers on disease incidence. Ann. appl. Biol. 40: 38-59.

WATSON, M.A. and NIXON, H.L. (1953). Studies on the feeding of Myzus persicae (Sulz.) on radioactive plants. Ann. appl. Biol. 40: 537-545.

WATSON, M.A. and PLUMB, R.T. (1972). Transmission of plant-pathogenic viruses by aphids. A. Rev. Ent. 17: 425-452.

WATSON, M.A. and ROBERTS, F.M. (1939). A comparative study of the transmission of Hyoscyamus virus 3, potato virus Y and cucumber virus 1 by the vectors Myzus persicae (Sulz.) M. circumflexus (Buckton), and Macrosiphum gei (Koch). Proc. R. Soc. Lond. (B) 127: 543-575.

WAY, M.J. (1968). Intra-specific mechanisms with special reference to aphid populations. In Insect Abundance. (Ed. Southwood, T.R.E.). R. ent. Soc. Lond. Symp. 4: 18-36.

WAY, M.J. and BANKS, C.J. (1967). Intra-specific mechanisms in relation to the natural regulation of numbers of Aphis fabae Scop. Ann. appl. Biol. 59: 189-205.

WENSLER, R.J.D. (1962). Mode of host selection by an aphid. Nature, Lond. 195: 830-831.

WIGGLESWORTH, V.B. (1939). Principles of insect physiology (6th Edition).
Methuen and Co. Ltd., London 741 pp.

WOLANSKI, B.S. and CHAMBERS, T.C. (1972). Structure of lettuce necrotic yellows virus III. Electron microscopical studies of the viral envelope.
Virology 47: 656-663.

WOLANSKI, B.S., FRANCKI, R.I.B. and CHAMBERS, T.C. (1967). Structure of lettuce necrotic yellows virus I. Electron microscopy of negatively stained preparations.
Virology 33: 287-296.

WOODFORD, J.A.T. (1969). Behavioural and developmental components of teneral stage in *Myzus persicae*.
Entomologia exp. Appl. 12: 337-350.

WRIGHT, K.E. and BARTON, N.L. (1955). Transpiration and the absorption and distribution of radioactive phosphorus in plants.
Pl. Physiol. 30: 386-388.

ZIMMERMANN, M. (1969). Translocation of nutrients.
In *Physiology of plant growth and development* (Ed. Wilkins, M.B.).
McGraw-Hill, London pp 383-417.